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STUDY ON THE SCIENTIFIC EVALUATION OF 12 SUBSTANCES IN THE CONTEXT OF ENDOCRINE DISRUPTER PRIORITY LIST OF ACTIONS

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EXECUTIVE SUMMARY

1. Background

In June 2001, the Commission adopted a follow up Communication to the Council and European Parliament on the implementation of the Community Strategy for Endocrine Disrupters [COM(2001)262]. In this Communication (EC 2001) the Commission proposed a priority list of actions to further evaluate the role of specific "candidate" substances in endocrine disruption. One of these priority actions was the initiation of an in-depth evaluation of a group of 12 candidate substances consisting of 9 industrial substances (2,2'-bis(4-(2,3-epoxypropyl)phenyl)propane, Carbon disulphide, 4-Chloro-3-methylphenol, 2,4-Dichlorophenol, 4-Nitrotoluene, o-Phenylphenol, Resorcinol, 4-*tert* Octylphenol and 2,2',4,4'-Tetrabrominated diphenyl ether or tetra BDE) and three natural/synthetic hormones (Oestrone, Oestradiol and Ethinyloestradiol).

This report comprises a review of the 12 substances with the following objectives:

1. Conduct an in-depth evaluation of nine (9) candidate substances for which scientific evidence of endocrine disruption or potential endocrine disruption was identified in the BKH report and which are neither restricted nor currently being addressed under existing Community legislation;

2. Conduct an in-depth evaluation of three (3) synthetic/natural hormones, oestrone, oestradiol and ethinyloestradiol, with a particular focus on up to date evidence of environmental exposure and related effects.

3. Identify specific cases of consumer or ecosystem exposure to these substances, with particular attention to potentially vulnerable consumer groups such as children.

At a stakeholder meeting on 21-22 Februray 2002 which discussed the strategy to be adopted for the review a representative from Sweden stated that one of the industrial substances tetra-brominated diphenylether (tetra BDE) was a component of Penta BDE which was in the process of being banned. Therefore, in the interests of efficiency, resources were not assigned to a review of tetra BDE

2. Approach adopted

Within the European Commission Strategy for Endocrine Disrupters this report, and the evaluation framework that has been developed, is designed to represent a stage between the identification of potential substances of concern (in a prioritisation) and any potential action following input of these substances into policy discussions. The evaluation framework has been developed to review the nature and extent of endocrine disrupting effects of identified chemicals (and potentially others in the future) and is based on robust datasets. The derived framework is criteria-based (where possible) so that decisions are made in an objective rather than a subjective manner. However, expert judgement is required at each stage and it is important to record the basis of decisions to aid transparency. It also needs to be recognised that the framework does **not** involve carrying out a full Risk Assessment of a substance under the Existing Substances Regulation 793/93, but it is appropriate to use established procedures from the Technical Guidance Document (EC 1994) where these are relevant. The

adoption of this approach is designed to maximise consistency in terms of terminology used and the procedures adopted (for example in the areas of data relevance and study validity).

The developed evaluation framework considers a number of issues most importantly:

1. Does the available data indicate there is evidence that a chemical causes endocrine disrupting effects in target groups of humans and/or wildlife?

2. Do endocrine disrupting effects of the chemical in target groups of humans and/or wildlife occur at lower concentrations than those causing effects on general systemic toxicological endpoints?

3. Are particular target groups of workers, consumers or wildlife organisms in the environment likely to be exposed to concentrations of chemicals which exceed effects thresholds due to current emission patterns.

In the review the International Programme for Chemical Safety (IPCS) definition of an endocrine disrupter has been adopted, namely that it is "*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*". As a result the review considers other mechanisms of action than oestrogenicity and anti-oestrogenicity including androgenicity and anti-androgenicity, effects on thyroid function and effects on hormone secretion and synthesis and steroidogenesis, where relevant data are available.

In the context of the review it is recognised that there are various laboratory-based *in vivo* and *in vitro* methods utilising a range of (eco)toxicological endpoints that are claimed by different sources to be relevant to the assessment of endocrine disruption in humans and/or wildlife. However, since this field is still in an early stage of development there is uncertainty regarding the significance of many of the current findings.

From the numerous recent reviews of potential test methods (such as the Detailed Review Paper prepared by OECD in 1997) there is a clear consensus in terms of the hierarchy of the relevance of test methods. In this hierarchy longer-term *in vivo* studies considering effects on reproduction and/or development (and including mechanistic information) are of greater relevance than short-term *in vivo* screening tests which are of greater relevance than *in vitro* assays. The greater relevance of chronic *in vivo* tests or those assessing effects during critical windows of sensitivity is also evidenced by the fact that these are the key (eco)toxicological methods being developed in the OECD Endocrine Disruption Testing and Assessment (EDTA) Programme. This hierarchy approach to data relevance has been adopted in the review along with a weight of evidence consideration of the available data.

For consideration of the risk of a substance to target groups of humans and/or wildlife a Margin of Safety (MOS) approach has been adopted. The MOS (for consumer use) is calculated by dividing the lowest No Observable Adverse Effect Level (NOAEL) of a compound by its Systemic Exposure Dose (SED) during normal use. If the MOS exceeds 100, the substance is regarded as safe for use. The value of 100 can be modified to account for perceived sensitive target groups (for example children). This approach is based on that given in the Scientific Committee for Consumer Products and Non-Food Products intended for Consumers (SCCNFP) "Notes of Guidance for Testing of Cosmetic Ingredients for their Safety Evaluation" (SCCNFP/0321/00 Final, 2001). A similar approach is applied to workers and also to wildlife groups using appropriate exposure and effects data. It should be recognised that this approach does not provide a as robust a measure of potential risk as a PEC/PNEC

comparison but that it is designed to highlight situations where further effort may be needed to clarify the nature and extent of exposure to a target group.

3. Outcome of the reviews

In considering the data on the potential endocrine disrupting effects of individual natural and synthetic substances on target groups of humans and/or wildlife it needs to be recognised that a vast majority of the available data is from studies conducted using current internationally recognised test guidelines or studies carried out using alternative (possibly non-regulatory) procedures. As such the studies may provide information on potential adverse effects on reproduction and development in the species studied but, because they are not specifically designed to address endocrine disruption, do not usually provide data on changes in endocrine function (for example changes in hormone levels). In the assessment of endocrine disruption in humans and wildlife the question of 'proof' of adverse effects and of underlying causative factors is crucial both for scientific inference and for guiding opinion forming and decision making.

For the industrial substances and natural/synthetic steroids the datasets for assessing potential endocrine disrupting effects in target groups of humans and/or wildlife vary in terms of both the types of studies for which data is available and their reliability (i.e. the methods used and the quality assurance/quality control procedures adopted). This is illustrated in Table ES1 which summarises the available *in vivo* mammalian data for the industrial substances and indicates that data of definitive significance from multi-generation reproduction studies is not available for all the industrial substances considered in the review. However, some data on reproductive and developmental endpoints in mammals (which may be endocrine mediated) is available for all the substances.

For all the industrial substances, except 4-*tert* octylphenol, the information on potential adverse effects on reproductive and developmental endpoints in wildlife, which may be endocrine mediated, was extremely limited. The limited data that is available is in most instances for aquatic species (and only invertebrates and/or fish). For the natural and synthetic steroids (particularly 17β oestradiol and 17α ethinyloestradiol) there was a greater body of data for aquatic species including invertebrates and fish.

For most of the substances considered in the review there is generally no data for terrestrial or aerial organisms. However, it needs to be recognised that the importance of this data to assessing potential endocrine disrupting effects will depend on the extent to which the substances are likely to partition into these environmental compartments. Furthermore, no validated OECD methods are available which been specifically designed to assess endocrine disrupting effects in representative taxonomic groups from these compartments.

Tables ES2 and ES3 summarise the weight of evidence for endocrine disrupting effects in humans and wildlife and the uncertainties associated with the review of the 12 identified substances.

3.1 Industrial substances

3.1.1 Assessment of potential endocrine disrupting effects in humans

For a number of substances (BADGE, 4-chloro-3-methylphenol, 2,4-dichlorophenol, 4-nitrotoluene, o-phenylphenol and 4-*tert* octylphenol) the available *in vivo* data indicate that no adverse effects on reproduction and development in laboratory mammals (which may be

endocrine mediated) occur at exposure levels where general systemic toxic effects are observed. However, there is uncertainty with data for 4-chloro-3-methylphenol, 2,4-dichlorophenol, 4-nitrotoluene and resorcinol since although data on reproduction and developmental endpoints is available, a definitive multi-generational reproduction study has not been conducted. The extent to which any adverse effects observed at high exposure doses are endocrine mediated is uncertain since the available studies provide no specific consideration of effects on endocrine function (for example changes in hormone levels).

For carbon disulphide the weight of evidence indicates that this substance can cause effects on endocrine glands or hormone sensitive tissues in humans and laboratory mammals and on mammalian reproduction and development. Most effects on endocrine organs occur at concentrations at which other, clearly toxic effects predominate, particularly those on the nervous system. Other effects, more specifically functional disturbances of the female reproductive system (i.e menstrual disturbances) may occur at otherwise non-toxic concentrations, but those which are higher than environmentally relevant levels. The findings on mammalian reproduction and development are not unexpected given that carbon disulphide is classified as constituting a possible risk of impaired fertility and possible harm to the unborn child.

For resorcinol the weight of evidence indicates that the substance is a thyroid peroxidase inhibitor and hypothyroidism has been reported in humans applying dermatological preparations and creams. Over application was a problem in the early 20th century and individuals also experienced central nervous system depression and methaemoglobinaemia which are also listed as side-effects. Certain older *in vivo* laboratory animal studies have revealed reversible anti-thyroid activity. The thyroid effects in these studies resulted from continuous exposure to high doses and required a vehicle (such as peanut oil) to establish a reservoir of resorcinol and to alter the pharmacokinetics such that resorcinol was continuously bioavailable. Studies conducted as part of the National Toxicology Programme have shown no effects on the thyroid of rats or mice at doses of up to 520 mg kg body weight^{-1} day^{-1} in rats and 450 mg kg body weight^{-1} day^{-1} in mice for 13 weeks and 150 – 225 mg kg body weight^{-1} day^{-1} for 5 days per week over 2 years in rats and mice. The NTP studies have also reported adrenal weight effects (both hypertrophy and atrophy) at all doses in range finding studies in rats and mice. However, the observed responses did not show a dose-dependent relationship. There has been no thorough study of reproductive toxicity and given the role of the thyroid in development, this is a critical area of uncertainty. To address this issue the Resorcinol Task Force has already formulated a comprehensive test programme which will comprise an extensive dose range finding study and a test guideline compliant two-generation reproduction study with expanded thyroid endpoints. It will also provide additional observations on sub-chronic exposure NOEL's derived from the pre-mating dosing period.

Substances such as BADGE, 4-nitrotoluene, 4-*tert* octylphenol and resorcinol (in hair colouring dyes) are produced in closed systems and/or are used as chemical intermediates which minimises the potential for worker exposure. A number of the substances (2,4-dichlorophenol, 4-nitrotoluene and 4-*tert* octylphenol) are not used in products and the potential for consumer exposure is limited. Of the substances where an MOS approach could be applied for consumer use both BADGE (as a liner of food and drink cans), 4-nitrotoluene and resorcinol (as a hair colouring dye and in pharmaceutical products) were not found to represent a risk to consumers. The greatest risk from carbon disulphide appears to be centred on workers, rather than consumers, and particularly those in the viscose rayon industry. However, in recent years there has been more stringent control over working practices at these plants in Western Europe and compliance with existing air quality standards should minimise potential adverse effects.

3.1.2 Assessment of potential endocrine disrupting effects in wildlife

For a most of the industrial substances (BADGE, 4-chloro-3-methylphenol, 2,4-dichlorophenol, 4-nitrotoluene, o-phenylphenol and 4-*te*rt octylphenol) the available aquatic effects data shows that the threshold exposure concentrations above which reproduction in invertebrates and fish are observed is slightly lower or similar to the threshold level for general toxic effects (i.e. lethality and/or growth) in these species. However, there is generally no data in the reported studies which indicates whether the observed effects on reproduction are endocrine mediated. Indeed in invertebrates there is limited knowledge of the endocrinology of many taxonomic groups and it uncertain whether reproductive processes are modulated by oestrogens or androgens.

Using the available exposure data in an MOS approach for the aquatic compartment indicated that 4-chloro-3-methylphenol and 4-nitrotoluene do not represent a risk to aquatic organisms whereas 2,4-dichlorophenol and 4-*tert* octylphenol may represent a risk.

3.2 Natural and synthetic steroids

The natural vertebrate steroids, 17β-oestradiol and oestrone, and the synthetic steroid 17α-ethinyloestradiol all evidently cause effects on the reproduction and development of fish which are probably endocrine mediated. These effects occur at environmental relevant concentrations and, therefore, these substances can represent a risk to fish and other aquatic vertebrates. This conclusion is consistent with the results of field surveys carried out in a number of European countries (for example the COMPREHEND programme) which have identified evidence of adverse effects on the development and reproductive capability of wild fish which are exposed to natural (and synthetic) steroids discharged from sewage treatment works (for review EA 2002). The potential for effects is probably greater following exposure to natural steroids (17β-oestradiol and oestrone) than the synthetic steroid 17α-ethinyloestradiol.

3.3 Cases of particular consumer risk

One of the key objectives of the review of the 12 substances was to identify specific cases of consumer or ecosystem exposure to these substances, with particular attention to potentially vunerable consumer groups such as children. The following conclusions can be drawn from the available data:

- A number of the industrial substances (2,4-dichlorphenol, 4-nitrotoluene and 4-*tert* octylphenol) are used in the manufacture of products from which it is probable that there is no or extremely limited consumer exposure. However, information on potential consumer exposure for these substances is limited or absent and it is difficult to draw robust conclusions on the risk to vulnerable groups. Further targeted monitoring to provide this data is needed where there is a potential risk to consumers.

- For substances where there is the potential for consumer exposure (BADGE through epoxy lining of food and drink cans and 4-chloro-3-methylphenol and resorcinol through pharmaceutical products) the data indicates that there is evidently no risk to consumers including children from current exposure patterns. For 4-chloro-3-methylphenol and resorcinol in pharmaceutical products this is based on the assumption that these are used as described in the accompanying literature.

Table ES1 Summary of the available in vivo mammalian data for the industrial substances

Substance	Sub-chronic oral toxicity test (OECD 408)	One generation reproduction test (OECD 415)	Two generation reproduction test (OECD 415)	Developmental/ teratogenicity test (OECD 414)	Combined chronic toxicity/oncogenicity test (OECD 453)	Other test data
BADGE	✓[1]	✓[1]	✓[1]	✓[1]	**x**	Carcinogenicity studies
Carbon disulphide	✓[3]	x	✓[3]	✓[1]	x	Carcinogenicity studies, Human exposure studies
4-Chloro-3-methylphenol	✓[2]	x	x	✓[1]	✓[1]	Carcinogenicity studies
2,4-Dichlorophenol	x	✓[2]	x	✓[2]	x	Carcinogenicity studies
4-Nitrotoluene	✓[2]	✓[3] (3 month study)	x	x	x	Carcinogenicity studies
o-Phenylphenol	✓[1]	x	✓[1]	✓[1]	✓[1]	Carcinogenicity studies
Resorcinol	✓[1]	x	x	✓[1]	x	Carcinogenicity studies, Human exposure studies
4-tert Octylphenol	✓[2]	x	✓[1]	✓[2]	x	-
Tetra BDE	Not considered in the review					

Notes:

✓[1] – One or more studies conducted to established (OECD or US EPA) guideline and to GLP

✓[2] – One or more studies conducted to a well described procedure but not to GLP

✓[3] – Limited information on procedure by which study/studies were conducted or study/studies has limitations in terms of quality

Table ES2 Summary of the weight of evidence and uncertainties associated with the assessment of the endocrine disrupting effects in humans for the 12 identified substances of concern

Substance	Humans	
	Weight of evidence	Uncertainties
BADGE	The available data from *in vivo* studies in laboratory mammals (using oral or dermal exposure routes) indicates that BADGE does not cause adverse effects on reproductive and developmental endpoints (which may be endocrine mediated) at exposure levels where general systemic toxic effects are observed. The lowest recorded NOEL from the *in vivo* mammalian studies was 250 mg kg body weight^{-1} day^{-1} for histopathological effects in endocrine glands and hormone sensitive tissues, though the observed effects at higher doses may have resulted from direct toxic action. The available exposure data indicates that current exposure patterns to BADGE do not represent a risk to workers or consumers.	There are no major uncertainties with regard to the evaluation of potential adverse effects of BADGE on reproductive and developmental endpoints since data is available from a definitive multi-generation study as well as supporting reproduction and developmental studies. Mechanistic uncertainties exist because the available studies provide no direct measurement of changes in endocrine function (for example changes in hormone levels).
Carbon disulphide	Carbon disulphide can affect male fertility in rats through changes in sperm count and mating behaviour and the NOEL in laboratory animals for effects is at > 1000 mg/m^3. There is some evidence that this toxicity is caused by a toxic effect on the testicles or by indirect effects on the ejaculation process. However, a BUA review stated that the influence of the hypothalamus-pituitary gland-gonad axis is improbable. A number of studies have shown that carbon disulphide possesses embryotoxic effects at high doses but levels that are lower than those causing maternal toxicity. Rabbits appear to be more sensitive than rats. Teratogenic effects were described exclusively at maternally toxic doses. The NOEL for embryotoxic effects for rabbits were in the region of 900 mg/m^3 and is higher for teratogenic effects. Initial neurotoxic effects already occur at these concentrations in the 90 day tests. Studies by Tabacova and co-workers describe embryotoxic effects from carbon disulphide at 0.03 mg/m^3 and teratogenic effects at 10 mg/m^3. However, there are issues with the quality of these studies. In humans, there have been numerous studies of workers exposed occupationally that report effects on pituitary-gonadal function in men and women, as well as indications of adverse reproductive outcomes in women. In men spermatogenic, diabetogenic and adrenal effects have also been reported. These effects have generally been reported for workers in the viscose rayon industry at exposure levels considerably above current occupational exposure standards (< 30 mg/m^3)	Carbon disulphide has not been subjected to standard regulatory toxicity tests and this raises uncertainties as to the validity of certain data, particularly that for the only multi-generation reproduction study. This uncertainty is offset to a degree by the fact that there is a considerable body of relevant data for humans derived from studies on workers (principally those in the viscose rayon industry).

Table ES2 Continued

Substance	Humans	
	Weight of evidence	Uncertainties
4-chloro-3-methylphenol	The available data from *in vivo* studies in laboratory mammals (using oral or dermal exposure routes) indicates that 4-chloro-3-methylphenol does not cause adverse effects on reproductive and developmental endpoints (which may be endocrine mediated) at exposure levels where general systemic toxic effects are observed. The lowest NOEL in the *in vivo* studies was 100 mg kg body weight $^{-1}$ day^{-1} for foetotoxic effects (on intra-uterine development) At higher exposure doses where adverse effects on development (foetotoxicity) were evident no information on changes in endocrine function was available. The available data indicate that 4-chloro-3-methylphenol in hand and skin disinfectants and as a preservative in pharmaceuticals does not present a risk to consumers.	There are uncertainties with regard to the evaluation of potential adverse effects of 4-chloro-3-methylphenol on reproductive and developmental endpoints since data is not available from a definitive multi-generation study. Mechanistic uncertainties exist because the available studies provide no direct measurement of changes in endocrine function (for example changes in hormone levels). Limited exposure data have been located for workers and consumers.
2,4-Dichlorophenol	The available data from *in vivo* studies in laboratory mammals (using oral or dermal exposure routes) indicates that 2,4-dichlorophenol does not cause adverse effects on reproductive and developmental endpoints (which may be endocrine mediated) at exposure levels where general systemic toxic effects are observed. The lowest NOEL in the *in vivo* studies was 50 mg kg body weight $^{-1}$ day^{-1} for reproductive parameters. No measured exposure data has been located for 2,4-dichlorophenol but since it is produced as an intermediate in closed systems the potential exposure of workers to the substance is limited providing appropriate safety procedures are followed. No information on concentrations of 2,4-dichlorophenol to which consumers are potentially exposed during the use of products has been obtained. However, the advisory on 2,4-dichlorophenol from the United States Environmental Protection Agency (US EPA) Office of Pollution Prevention and Toxics (OPPT) and US Department of Labour Occupational Safety and Health Administration (OSHA) stated that "*The focus of concern is occupational and no risks are expected for consumers or community members.*"	There are uncertainties with regard to the evaluation of potential adverse effects of 2,4-dichlorophenol on reproductive and developmental endpoints since data is not available from a definitive multi-generation study. These issues will be addressed in a study initiated by MITI in 2002. Mechanistic uncertainties exist because the available studies provide no direct measurement of changes in endocrine function (for example changes in hormone levels).

Table ES2 Continued

Substance	Humans	
	Weight of evidence	Uncertainties
4-Nitrotoluene	The available data from *in vivo* studies in laboratory mammals (using oral or dermal exposure routes) indicates that 4-nitrotoluene does not cause adverse effects on reproductive endpoints (which may be endocrine mediated) at exposure levels where general systemic toxic effects are observed. The lowest NOEL in the *in vivo* studies was 110 mg kg body weight $^{-1}$ day^{-1} for histopathological effects on reproductive tissues, though the observed effects at higher doses may have resulted from direct cytotoxic action. The available data indicate that current exposure patterns to 4-nitrotoluene do not represent a risk to workers or consumers (including children).	There are uncertainties with regard to the evaluation of potential adverse effects of 4-nitrotoluene on reproductive and developmental endpoints since data is not available from a definitive multi-generation study as well as developmental/teratogenicity studies. A reproduction screening test has been conducted and was due to be reported in late 2002. Mechanistic uncertainties exist because the available studies provide no direct measurement of changes in endocrine function (for example changes in hormone levels).
o-Phenylphenol	The available data from *in vivo* studies in laboratory mammals (using oral or dermal exposure routes) indicates that o-phenylphenol does not cause adverse effects on reproductive and developmental endpoints (which may be endocrine mediated) at exposure levels where general systemic toxic effects are observed. The lowest NOEL in the *in vivo* studies was 250 mg kg body weight $^{-1}$ day^{-1} for foetotoxic and developmental effects. Limited exposure data for workers and consumers has been located.	There are no major uncertainties with regard to the evaluation of potential adverse effects of o-phenylphenol on reproductive and developmental endpoints since data is available from a definitive multi-generation study as well as supporting reproduction and developmental studies. Mechanistic uncertainties exist because the available studies provide no direct measurement of changes in endocrine function (for example changes in hormone levels).

Table ES2 Continued

Substance	Humans	
	Weight of evidence	Uncertainties
Resorcinol	*In vitro* studies indicate that the anti-thyroidal activity observed following resorcinol exposure is due to inhibition of thyroid peroxidase (TPO) enzymes, as evidenced by disruption of thyroid hormone synthesis and changes in the thyroid gland consistent with goitrogenesis. Certain older *in vivo* laboratory animal studies have revealed reversible anti-thyroid activity. The thyroid effects in these studies resulted from continuous exposure to high doses and required a vehicle (such as peanut oil) to establish a reservoir of resorcinol and to alter the pharmacokinetics such that resorcinol was continuously bioavailable. Studies conducted as part of the National Toxicology Programme have shown no effects on the thyroid of rats or mice at doses of up to 520 mg kg body weight^{-1} day^{-1} in rats and 450 mg kg body weight^{-1} day^{-1} in mice for 13 weeks and 150 – 225 mg kg body weight^{-1} day^{-1} for 5 days per week over 2 years in rats and mice. There is evidence of effects on adrenal weights at all doses tested in NTP rat and mouse 13-week studies. However, the observed responses did not show dose-dependent relationships. Currently available data indicates that resorcinol is not embryotoxic or teratogenic. The available exposure data indicate that resorcinol does not represent a risk to workers or consumers based on current exposure pathways.	There are uncertainties with regard to the evaluation of potential adverse effects of resorcinol on reproductive and developmental endpoints since data is not available from a definitive multi-generation study. These issues will be addressed in study being initiated by the Resorcinol Task Force, along with the uncertainties regarding the significance of effects in the adrenals observed in certain studies.
4-*tert* Octylphenol	The available data from *in vivo* studies in laboratory mammals (using oral or dermal exposure routes) indicates that 4-*tert* octylphenol does not cause adverse effects on reproductive and developmental endpoints (which may be endocrine mediated) at exposure levels where general systemic toxic effects are observed. The lowest NOEL in the *in vivo* studies was 150 mg kg body weight $^{-1}$ day^{-1} for effects on reproductive and developmental parameters. No effects on reproductive or developmental parameters in laboratory mammals are evident at low exposure doses (0.0015 – 0.002 mg kg^{-1}) The available data indicates that current exposure patterns to 4-*tert* octylphenol do not represent a risk to workers or consumers (including children).	There are no major uncertainties with regard to the evaluation of potential adverse effects of 4-*tert* octylphenol on reproductive and developmental endpoints since data is available from a definitive multi-generation study as well as supporting reproduction and developmental studies. Mechanistic uncertainties exist because most of the available studies provide no direct measurement of changes in endocrine function (for example changes in hormone levels).

Table ES2 Continued

Substance	Humans	
	Weight of evidence	Uncertainties
Tetra BDE	No review completed	
Oestrone	Not considered in the review	
17β-Oestradiol	Not considered in the review	
17α-Ethinyloestradiol	Not considered in the review	

Table ES3 Summary of the weight of evidence and uncertainties associated with the assessment of the endocrine disrupting effects in wildlife for the 12 identified substances of concerns

Substance	Wildlife	
	Weight of evidence	Uncertainties
BADGE	The available aquatic effects data shows that the threshold exposure concentration of BADGE above which reproduction of the invertebrate *Daphnia magna* is reduced (NOEC = 0.3 mg l^{-1}) is similar to the threshold level for general toxic effects (i.e. lethality). However, there is no information on the mechanism of action for the effects on reproduction observed in *Daphnia magna*.	There are uncertainties with regard to potential adverse effects of BADGE on reproduction and development in wildlife due to the absence of key data for: • A wider range of aquatic taxa, particularly fish and sediment dwelling invertebrates • Terrestrial organisms The absence of data on aerial organisms is not a major uncertainty since BADGE is not volatile and the potential for these organisms to be exposed is limited. No environmental exposure data for BADGE in the aquatic, terrestrial and aerial compartments has been located
Carbon disulphide	The available data on the effects of carbon disulphide on the development of aquatic organisms has been shown that the threshold exposure concentration above which the hatching rate of fish (*Danio rerio*) is affected is 1.0 mg l^{-1}. However, it is not clear whether these responses are endocrine mediated. This exposure level is only slightly lower than concentrations causing acute toxicity in fish (and invertebrates).	No data has been located on the potential endocrine disrupting effects of carbon disulphide on the reproduction of aquatic organisms or the reproduction/development of terrestrial or aerial organisms. The volatility of carbon disulphide means that wildlife organisms that are exposed to carbon disulphide via inhalation represent those most at risk from exposure. There is limited data on environmental concentrations of $CS_{2,}$, however it needs to recognised that environmental levels are influenced by natural releases.
4-Chloro-3-methyl phenol	The available aquatic effects data shows that the threshold exposure concentration of 4-chloro-3-methylphenol above which reproduction of the invertebrate *Daphnia magna* is reduced (NOEC = 1.25 mg l^{-1}) is slightly lower than the threshold level for general toxic effects (i.e. lethality). However there is no information on the mechanism of action for the effects on reproduction observed in *Daphnia magna*. The available exposure data indicate that 4-chloro-3-methylphenol does not represent a risk to aquatic organisms.	There are uncertainties with regard to potential adverse effects of 4-chloro-3-methylphenol on reproduction and development in wildlife due to the absence of data for a wider range of aquatic taxa, particularly fish. The absence of data for terrestrial and aerial organisms is not a major uncertainty since the physico-chemical properties of 4-chloro-3-methylphenol indicate that the substance should not partition into the terrestrial and aerial compartments. No environmental exposure data for 4-chloro-3-methylphenol in the terrestrial and aerial compartments has been located.

Table ES3 Continued

Substance	Wildlife	
	Weight of evidence	Uncertainties
2,4-Dichlorophenol	The available effects data shows that the threshold exposure concentration of 2,4-dichlorophenol above which reproduction of the aquatic invertebrate *Daphnia magna* is reduced (NOEC = 0.21 mg l^{-1}) is only slightly lower than the threshold level for general toxic effects (i.e. lethality). However there is no information on the mechanism of action for the effects observed in this species. The available exposure data indicate that 2,4-dichlorophenol may represent a risk to aquatic organisms. In contrast, the threshold exposure concentrations of 2,4-dichlorophenol above which reproduction of the terrestrial invertebrate *Folsomia candida* is reduced (NOEC = 3.8 mg kg dry weight^{-1}) is slightly higher than the threshold level for general toxic effects (i.e. lethality).	There are uncertainties with regard to potential adverse effects of 2,4-dichlorophenol on reproduction and development in wildlife due to the absence of data for a wider range of aquatic taxa, particularly fish. The absence of data on aerial organisms is not a major uncertainty since the physico-chemical properties of 2,4-dichlorophenol indicate that the substance should not partition into the aerial compartment. No environmental exposure data for 2,4-dichlorophenol in the terrestrial and aerial compartments has been located.
4-Nitrotoluene	The available aquatic effects data shows that the threshold exposure concentration of 4-nitrotoluene above which reproduction of the invertebrate *Daphnia magna* is reduced (NOEC = 0.7 mg l^{-1}) is lower than the threshold level for general toxic effects (i.e. lethality). However, there is no information on the mechanism of action for effects on the reproduction of *Daphnia magna*. The available exposure data indicate that 4-nitrotoluene does not represent a risk to aquatic organisms.	There are uncertainties with regard to potential adverse effects of 4-nitrotoluene on reproduction and development in wildlife due to the absence of key data for: • A wider range of aquatic taxa, particularly fish • Aerial organisms The absence of data on terrestrial organisms is not a major uncertainty since 4-nitrotoluene does not strongly sorb to organic carbon and the potential for these organisms to be exposed is limited. No environmental exposure data for 4-nitrotoluene in the terrestrial and aerial compartments has been located.
o-Phenylphenol	The available aquatic effects data shows that the threshold exposure concentrations of o-phenylphenol above which reproduction of the invertebrate *Daphnia magna* and fish (fathead minnow) are reduced (NOECs = 0.036 mg l^{-1} and 0.009 mg l^{-1} respectively) are lower than the theshold levels for general toxic effects (i.e. lethality). The effects observed on reproduction in fish were evidently not oestrogen mediated . However, there is no information on the mechanism of action for the effects on reproduction observed in *Daphnia magna*.	There is no data on potential adverse effects on reproduction and development in terrestrial and aerial organisms but this is not a major uncertainty since the physico-chemical properties of o-phenylphenol mean the potential for these organisms to be exposed is limited. No environmental exposure data for o-phenylphenol in the aquatic, terrestrial and aerial compartments has been located.

Table ES3 Continued

Substance	Wildlife	
	Weight of evidence	Uncertainties
Resorcinol	The available aquatic effects data from teratogenicity studies with rainbow trout and zebrafish embryos shows teratogenic effects are evident at exposure concentrations $\geq$ 100 mg l^{-1}. However, there is no available data as to whether the observed effects were endocrine mediated.	There are uncertainties with regard to the potential adverse effects of resorcinol on reproduction and development in wildlife due to the absence of key data for aquatic organisms particularly invertebrates. This is planned to be addressed by the Resorcinol Task Force. The absence of data on terrestrial and aerial organisms is not a major uncertainty since the physico-chemical properties of resorcinol mean the potential for these organisms to be exposed is limited. There are no environmental exposure data for resorcinol in the aquatic, terrestrial and aerial compartments.
4-*tert* Octylphenol	The available data shows the threshold exposure concentrations of 4-*tert* octylphenol above which reproduction and development in aquatic organisms (fish, amphibians and invertebrates) are affected (NOECs = 1-12 $\mu g\ l^{-1}$) are similar to the threshold levels for general toxic effects (i.e. growth and lethality). The effects may be oestrogen mediated. The available exposure data indicate that 4-t*ert* octylphenol may represent a risk to aquatic organisms, particularly at discharge 'hotspots'.	There are uncertainties with regard to the potential adverse effects of 4-*tert* octylphenol on reproduction and development in wildlife due to the absence of key data for terrestrial organisms. The absence of data on aerial organisms is not a major uncertainty since although 4-*tert* octylphenol is volatile it is rapidly degraded and the potential for these organisms to be exposed is limited. There are no environmental exposure data for 4-*tert* octylphenol in the terrestrial and aerial compartments, though levels in the aerial compartment are not expected to be high.
Tetra BDE	Not considered in the review	

Table ES3 Continued

Substance	Wildlife	
	Weight of evidence	Uncertainties
Oestrone	In fish it appears that effects of oestrone on reproduction and development which are considered to be endocrine mediated occur at markedly lower (and environmentally relevant) concentrations (> 1-10 ng l^{-1}) than those causing general toxicity. No effects of oestrone on the reproduction of the aquatic invertebrate copepod *Tisbe battagliai* were evident at the highest exposure concentration (100 ng l^{-1}), indicating that the processes of reproduction and development in certain invertebrate taxa (crustaceans) are evidently not affected by exposure to vertebrate steroids at typical environmental levels. However this may not be the case for other invertebrate taxa. The available aquatic exposure data (showing typical concentrations of **<0.5 - 5** ng l^{-1}) indicates that oestrone presents a risk to fish (and other aquatic vertebrates) in terms of endocrine disrupting effects. This is consistent with data from field surveys of fish populations exposed to natural (and synthetic) steroids discharged from sewage treatment works.	The data on oestrone induced and endocrine mediated effects on reproduction and development in wildlife is limited and restricted to aquatic organisms (invertebrates and fish) The results of a multi-generational study will considerably reduce the uncertainty associated with the extent of endocrine mediated responses of fish. No data are available on potential endocrine mediated effects in terrestrial and aerial organisms. Given that sorption to organic carbon is an important process resulting in the partitioning of oestrone onto soils the absence of data on potential endocrine mediated responses in terrestrial organisms is a key area of uncertainty.
17β-Oestradiol	In fish it appears that effects of 17β-Oestradiol on reproduction and development which are considered to be endocrine mediated occur at markedly lower (and environmentally relevant) concentrations (> 5 - 25 ng l^{-1}) than those causing general toxicity. The processes of reproduction and development in certain invertebrate taxa (crustaceans) are evidently not generally affected by exposure to vertebrate steroids at typical environmental levels. However this may not be the case for other invertebrate taxa. The available aquatic exposure data (showing typical concentrations of 1 – 5 ng l^{-1}) indicates that 17β-Oestradiol presents a risk to fish (and other aquatic vertebrates) in terms of endocrine disrupting effects. This is consistent with data from field surveys of fish populations exposed to natural (and synthetic) steroids discharged from sewage treatment works.	The data on 17β-Oestradiol induced and endocrine mediated responses on reproduction and development in wildlife is limited and restricted to aquatic organisms (invertebrates and fish) No data are available on potential endocrine mediated effects in terrestrial and aerial organisms. Given that sorption to organic carbon is an important process resulting in the partitioning of 17β-Oestradiol onto soils the absence of data on potential endocrine mediated responses in terrestrial organisms is a key area of uncertainty.

Table ES3 Continued

Substance	Wildlife	
	Weight of evidence	Uncertainties
17α-Ethinyloestradiol	In fish it appears that threshold effects of 17α-ethinyloestradiol on reproduction and development which are considered to be endocrine mediated occur at markedly lower (and environmentally relevant) concentrations (> 0.3 - 1 ng l^{-1}) than those causing general toxicity. The processes of reproduction and development in certain aquatic invertebrate taxa (crustaceans) are evidently not affected by exposure to vertebrate steroids at typical environmental concentrations. However this may not be the case for other invertebrate taxa. The available exposure data indicates that 17α-ethinyloestradiol can in certain circumstances present a risk to fish (and other aquatic vertebrates) in terms of endocrine disrupting effects. However, detectable aquatic concentrations in surface waters are in most cases below the threshold levels capable of resulting in endocrine disrupting effects. The assessment of risk is confounded by the current analytical limitations in the sensitivity of detection of 17α-ethinyloestradiol.	The data on 17α-ethinyloestradiol induced and endocrine mediated effects on reproduction and development in wildlife is limited and restricted to aquatic organisms (invertebrates and fish). No data are available on potential endocrine mediated effects in terrestrial and aerial organisms. Given that sorption to organic carbon is an important process and sewage sludge may be applied to land the absence of data on potential endocrine mediated responses in terrestrial organisms is an area of uncertainty.

4. General issues relating to the assessment of endocrine disrupting effects

In addition to the conclusions on the individual substances reviewed in the report it is evident that there are a number of generic issues associated with the assessment of endocrine disrupting effects which apply to all potential substances of interest and not just those considered in the review.

The assessment of endocrine disrupting effects in humans and wildlife is an evolving area and a considerable body of activity is on-going at both national and international levels. It was evident from the recent Report of a European Workshop on Endocrine Disrupters held in Aronsborg (Sweden) that there are a number of key areas of uncertainty which need to be addressed to enhance the evaluation of the extent of endocrine disrupting effects of substances of concern and the risks they present to humans and/or wildlife. Key areas requiring further activity are:

- The development of validated methods which provide robust information on endocrine disrupting effects in particular target groups, specifically invertebrates where there is a lack of knowledge on the endocrinology of many taxonomic groups;

- The conduct and interpretation of mammalian and non-mammalian tests in relation to potential low-dose effects;

- Collation (and if required generation) of information on the normal background variability in reproduction and developmental responses of mammalian and wildlife species;

- Assessment of the risks presented by the potential endocrine disrupting effects of synthetic substances in relation to background exposure to natural compounds (for example vertebrate steroids and phyto-oestrogens).

5. Implementation of a framework for reviewing other substances of concern

Following the conduct of the reviews in this project a framework for conducting reviews of other substances identified as potential endocrine disrupters in a prioritisation exercise has been proposed (see Figure ES1). The framework (Boxes 2 and 3 in Figure ES1) follows the approach described in Section 2 of the report and incorporates a step-wise evaluation of data on the substances against the three issues given in Section 2 of the report, namely whether:

- the weight of evidence for a substance indicates endocrine disrupting effects occur in target groups of humans and/or wildlife;

- endocrine disrupting effects occur at lower concentrations of the substance than those causing general non-endocrine mediated (eco)toxicological effects in the target groups;

- the target groups of humans and/or wildlife are likely to be exposed to the substance in the environment at doses/concentrations capable of causing endocrine disrupting effects.

In the framework it is also important to consider:

1. Is there sufficient data of definitive significance available to draw robust and meaningful conclusions on the extent to which a substance causes or has the potential to cause

endocrine disruption effects in target groups of humans and/or wildlife at levels below those causing general non-endocrine (eco)toxicological responses?;

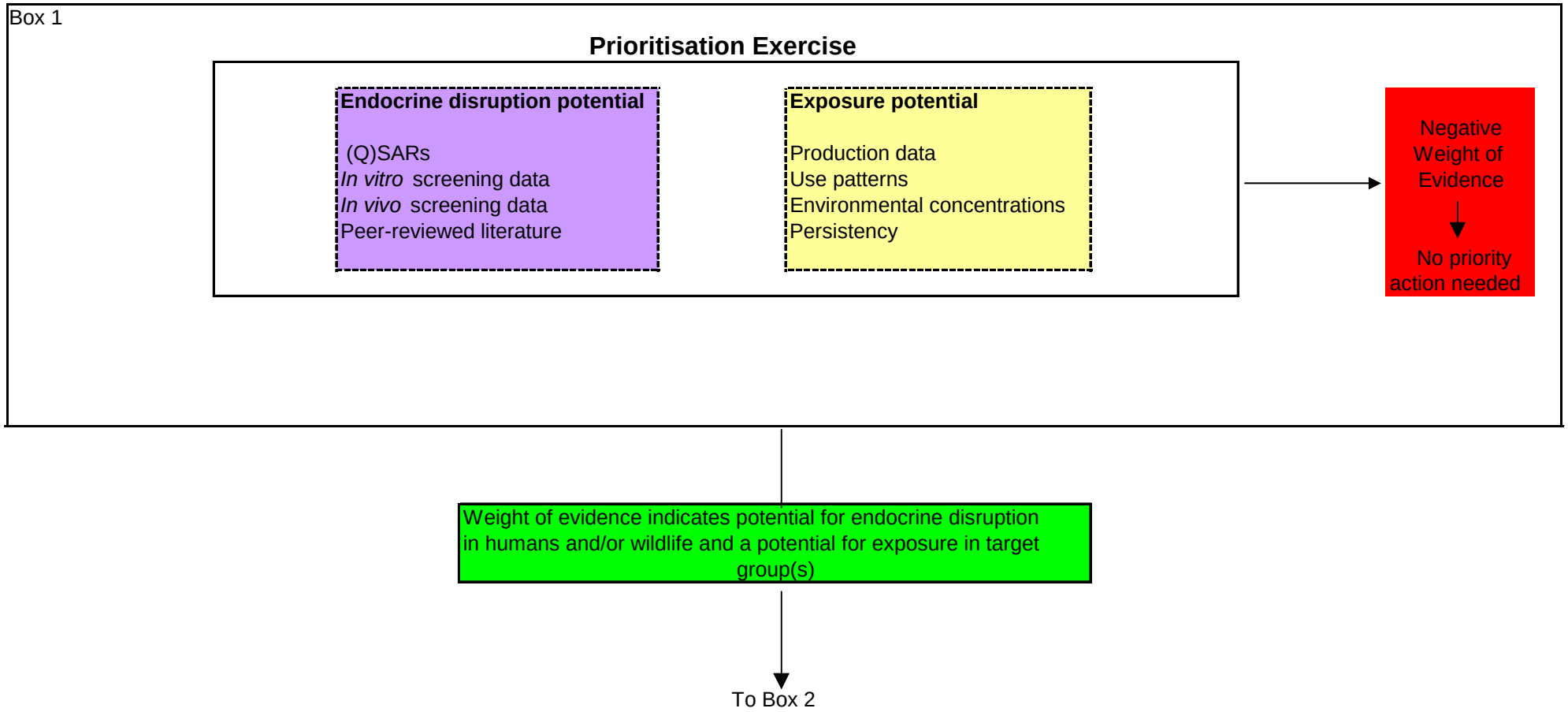
2. What additional information is needed to allow robust and meaningful conclusions to be drawn if there is currently insufficient data for a substance?.

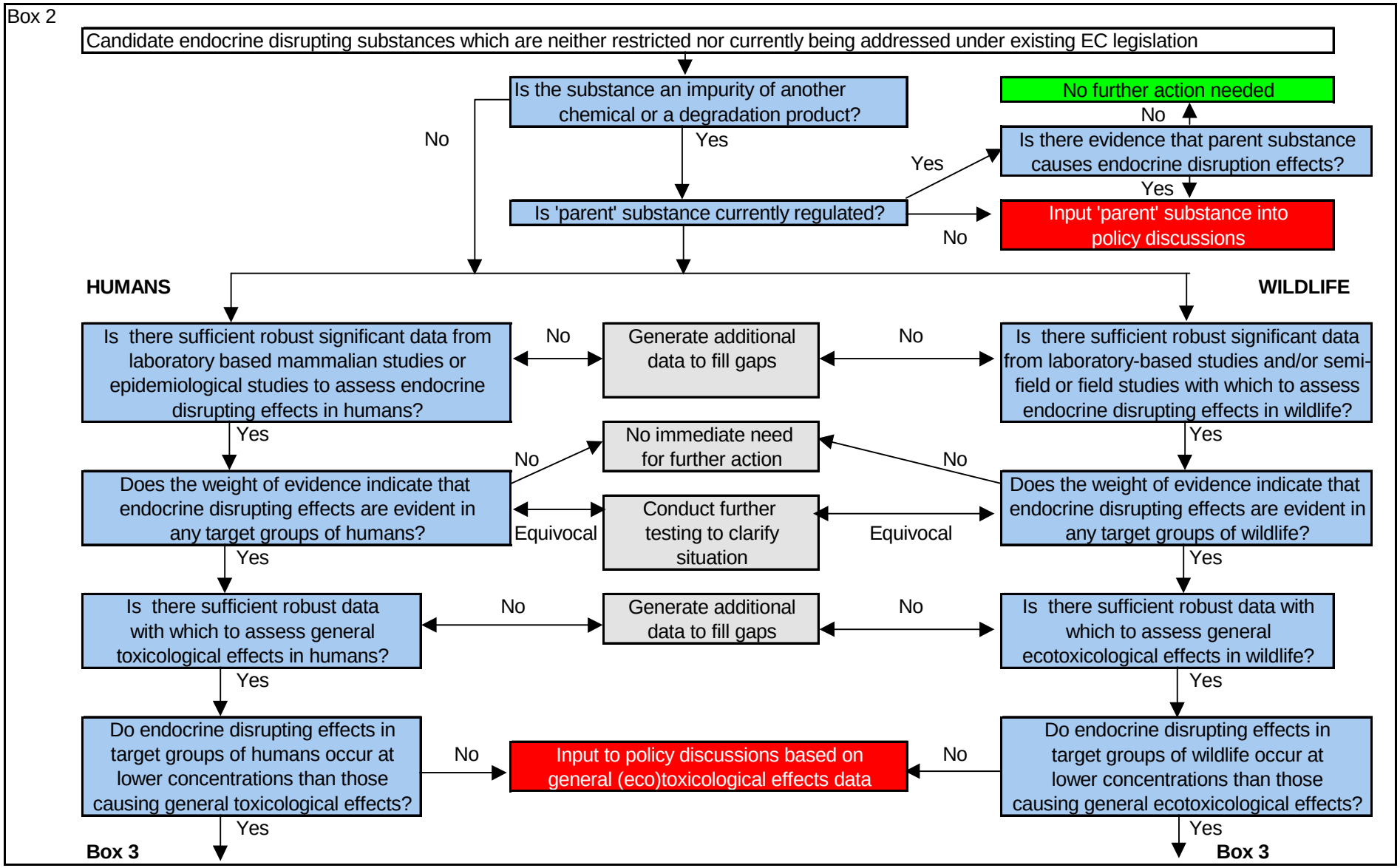
The framework is designed to build on a prioritisation exercise using the procedure being developed by BKH (Box 1 in Figure ES1) and it is envisaged that this screening approach will be used to prioritise the substances for which a review is conducted.

At the prioritisation stage substances for which there is evidence of endocrine disruption in humans and/or wildlife and a potential for exposure of the target group(s) should be considered a priority for more detailed review using the procedure described in Section 2. Evidence of endocrine disruption in a target group but an absence of data on the potential for exposure should lead to the acquisition of relevant basic exposure data so that a decision can be made on whether a more detailed review of the substance is required. If there is evidence of exposure potential but no information on potential endocrine disrupting effects then no detailed review should be conducted until some robust data on endocrine disrupting effects has been generated.

Where there is sufficient data, an absence of evidence or negative weight of evidence for potential endocrine disrupting effects in a target group and no potential for exposure can be used to indicate that further detailed consideration of the substance is not a priority action.

For the assessment of endocrine disrupting effects of a substance at the prioritisation exercise stage the emphasis should be placed on *in vivo* data where this is available. The review of the 9 industrial substances has shown that effects observed *in vitro* assays are not always translated into effects in whole organisms, especially at doses/concentrations which may reflect the lowest observed toxicity in a target group.


Box 1
Prioritisation Exercise
Endocrine disruption potential
(Q)SARs
In vitro screening data
In vivo screening data
Peer-reviewed literature
Exposure potential
Production data
Use patterns
Environmental concentrations
Persistency
Negative Weight of Evidence
No priority action needed
Weight of evidence indicates potential for endocrine disruption in humans and/or wildlife and a potential for exposure in target group(s)
To Box 2


Box 2
Candidate endocrine disrupting substances which are neither restricted nor currently being addressed under existing EC legislation
Is the substance an impurity of another chemical or a degradation product?
No
Yes
No further action needed
No
Is there evidence that parent substance causes endocrine disruption effects?
Yes
Yes
Is 'parent' substance currently regulated?
Yes
Input 'parent' substance into policy discussions
No
HUMANS
WILDLIFE
Is there sufficient robust significant data from laboratory based mammalian studies or epidemiological studies to assess endocrine disrupting effects in humans?
No
Generate additional data to fill gaps
No
Is there sufficient robust significant data from laboratory-based studies and/or semi-field or field studies with which to assess endocrine disrupting effects in wildlife?
Yes
Yes
No immediate need for further action
No
No
Does the weight of evidence indicate that endocrine disrupting effects are evident in any target groups of humans?
Equivocal
Conduct further testing to clarify situation
Equivocal
Does the weight of evidence indicate that endocrine disrupting effects are evident in any target groups of wildlife?
Yes
Yes
Is there sufficient robust data with which to assess general toxicological effects in humans?
No
Generate additional data to fill gaps
No
Is there sufficient robust data with which to assess general ecotoxicological effects in wildlife?
Yes
Yes
Do endocrine disrupting effects in target groups of humans occur at lower concentrations than those causing general toxicological effects?
No
Input to policy discussions based on general (eco)toxicological effects data
No
Do endocrine disrupting effects in target groups of wildlife occur at lower concentrations than those causing general ecotoxicological effects?
Yes
Yes
Box 3
Box 3

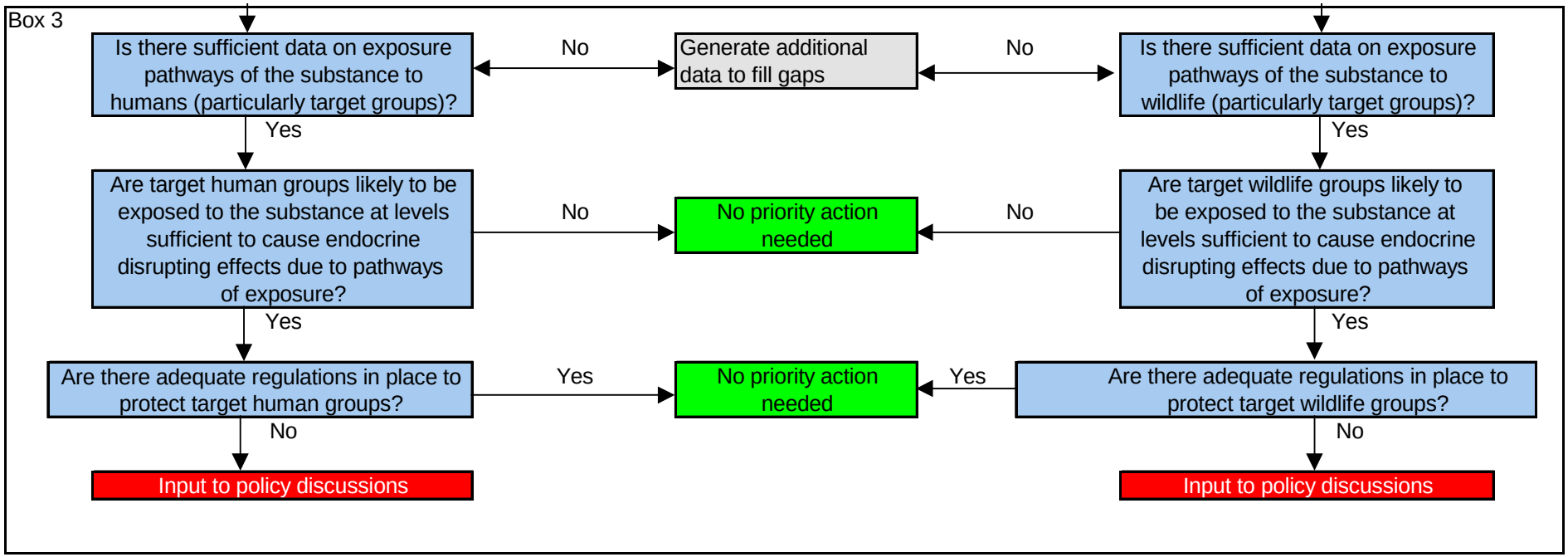


Figure ES1 Framework for the prioritisation and review of potential endocrine disrupting substances

EUROPEAN COMMISSION

STUDY ON THE SCIENTIFIC EVALUATION OF 12 SUBSTANCES IN THE CONTEXT OF ENDOCRINE DISRUPTER PRIORITY LIST OF ACTIONS

WRc-NSF Ref: UC 6052
December 2002

STUDY ON THE SCIENTIFIC EVALUATION OF 12 SUBSTANCES IN THE CONTEXT OF ENDOCRINE DISRUPTER PRIORITY LIST OF ACTIONS

Report No.: UC 6052

November 2002

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Contract No.: 12834-0

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FOREWORD

In December 1999, the European Commission published a Community Strategy for Endocrine Disrupters (COM(1999)706) in which it announced its intention to establish a priority list of substances for further evaluation of their role in endocrine disruption.

In June 2000, BKH Consulting Engineers (NL), under contract to the Commission, prepared a report entitled "Towards the establishment of a priority list of substances for further evaluation of their role in endocrine disruption – preparation of a candidate list of substances as a basis for priority-setting". The report identified a candidate list of 553 substances, from which evidence of endocrine disruption or potential endocrine disruption was found for 118 substances. An analysis of the legal status of these 118 substances revealed that 109 were already subject to bans or restrictions or were being addressed under existing Community legislation, although for reasons not necessarily related to endocrine disruption. (see Figure F1: Establishment of a priority list of substances for further evaluation of their role in Endocrine Disruption).

Following a wide consultation on the BKH report, it was decided to give priority in the short-term to an in-depth evaluation of the 9 candidate substances with evidence of endocrine disruption or potential endocrine disruption which were neither restricted nor being addressed under existing Community legislation, together with an additional 3 synthetic/natural hormones present in the environment. This decision reflected a broad agreement among stakeholders that a more in-depth study of specific candidate substances than that contained in the 2000 BKH Report would be necessary before any proposals for restrictions could be envisaged. It also reflected the Commission intention not to duplicate work on substances for which risk assessments were underway under existing Community legislation. It should however be noted that the 109 substances already regulated or being addressed under existing Community legislation do not disappear from the candidate list and may become future candidates for definitive testing once agreed test methods for endocrine disruption are available.

In addition to the in-depth evaluation of the 9+3 substances, it was decided to give equal priority to gathering data/information on persistence, production volumes and legal status on another 435 candidate substances for which there was insufficient data in the 2000 BKH Report to decide on ED or potential for ED (due not to lack of data but to lack of resources to gather the data).

Thus in 2001, two studies were launched in parallel. The first, on 12 (9+3) substances, was carried out by WRc-NSF (UK) and is the subject of this report. The second, on 435 substances, was carried out by BKH Consulting Engineers (NL).

The reader is referred to Commission document COM(2001)262 of 14 June 2001 on the implementation of the Community Strategy for Endocrine Disrupters for a more detailed account of the context of both of these studies. The reader is also reminded that the word "priority" in the context of this work does not indicate the relative importance of a substance in terms of endocrine disruption but refers rather to making the best use of available resources in the process of further evaluation of all candidate substances.

Finally, the results of these studies will be used by the Commission, in consultation with the Member States and other stakeholders, to provide input to policy discussions at European level.

Kathryn Tierney,
Environment DG,
European Commission.

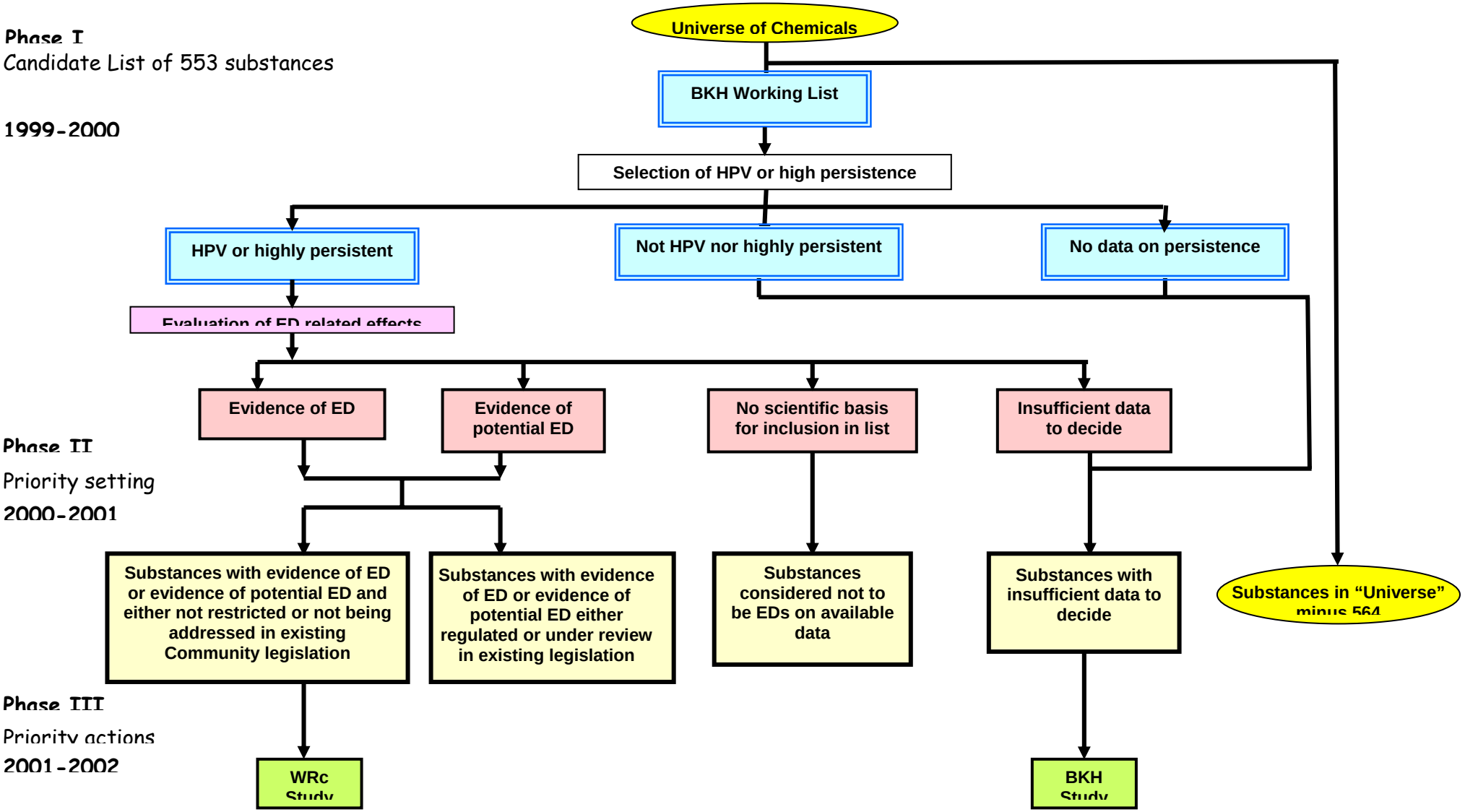


Figure F1 Establishment of a priority list of substances for further evaluation of their role in Endocrine Disruption

EXECUTIVE SUMMARY

1. Background

In June 2001, the Commission adopted a follow up Communication to the Council and European Parliament on the implementation of the Community Strategy for Endocrine Disrupters [COM(2001)262]. In this Communication (EC 2001) the Commission proposed a priority list of actions to further evaluate the role of specific "candidate" substances in endocrine disruption. One of these priority actions was the initiation of an in-depth evaluation of a group of 12 candidate substances consisting of 9 industrial substances (2,2'-bis(4-(2,3-epoxypropyl)phenyl)propane, Carbon disulphide, 4-Chloro-3-methylphenol, 2,4-Dichlorophenol, 4-Nitrotoluene, o-Phenylphenol, Resorcinol, 4-*tert* Octylphenol and 2,2',4,4'-Tetrabrominated diphenyl ether or tetra BDE) and three natural/synthetic hormones (Oestrone, Oestradiol and Ethinyloestradiol).

This report comprises a review of the 12 substances with the following objectives:

1. Conduct an in-depth evaluation of nine (9) candidate substances for which scientific evidence of endocrine disruption or potential endocrine disruption was identified in the BKH report and which are neither restricted nor currently being addressed under existing Community legislation;

2. Conduct an in-depth evaluation of three (3) synthetic/natural hormones, oestrone, oestradiol and ethinyloestradiol, with a particular focus on up to date evidence of environmental exposure and related effects.

3. Identify specific cases of consumer or ecosystem exposure to these substances, with particular attention to potentially vulnerable consumer groups such as children.

At a stakeholder meeting on 21-22 Februray 2002 which discussed the strategy to be adopted for the review a representative from Sweden stated that one of the industrial substances tetra-brominated diphenylether (tetra BDE) was a component of Penta BDE which was in the process of being banned. Therefore, in the interests of efficiency, resources were not assigned to a review of tetra BDE

2. Approach adopted

Within the European Commission Strategy for Endocrine Disrupters this report, and the evaluation framework that has been developed, is designed to represent a stage between the identification of potential substances of concern (in a prioritisation) and any potential action following input of these substances into policy discussions. The evaluation framework has been developed to review the nature and extent of endocrine disrupting effects of identified chemicals (and potentially others in the future) and is based on robust datasets. The derived framework is criteria-based (where possible) so that decisions are made in an objective rather than a subjective manner. However, expert judgement is required at each stage and it is important to record the basis of decisions to aid transparency. It also needs to be recognised that the framework does **not** involve carrying out a full Risk Assessment of a substance under the Existing Substances Regulation 793/93, but it is appropriate to use established procedures from the Technical Guidance Document (EC 1994) where these are relevant. The

adoption of this approach is designed to maximise consistency in terms of terminology used and the procedures adopted (for example in the areas of data relevance and study validity).

The developed evaluation framework considers a number of issues most importantly:

1. Does the available data indicate there is evidence that a chemical causes endocrine disrupting effects in target groups of humans and/or wildlife?

2. Do endocrine disrupting effects of the chemical in target groups of humans and/or wildlife occur at lower concentrations than those causing effects on general systemic toxicological endpoints?

3. Are particular target groups of workers, consumers or wildlife organisms in the environment likely to be exposed to concentrations of chemicals which exceed effects thresholds due to current emission patterns.

In the review the International Programme for Chemical Safety (IPCS) definition of an endocrine disrupter has been adopted, namely that it is "*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*". As a result the review considers other mechanisms of action than oestrogenicity and anti-oestrogenicity including androgenicity and anti-androgenicity, effects on thyroid function and effects on hormone secretion and synthesis and steroidogenesis, where relevant data are available.

In the context of the review it is recognised that there are various laboratory-based *in vivo* and *in vitro* methods utilising a range of (eco)toxicological endpoints that are claimed by different sources to be relevant to the assessment of endocrine disruption in humans and/or wildlife. However, since this field is still in an early stage of development there is uncertainty regarding the significance of many of the current findings.

From the numerous recent reviews of potential test methods (such as the Detailed Review Paper prepared by OECD in 1997) there is a clear consensus in terms of the hierarchy of the relevance of test methods. In this hierarchy longer-term *in vivo* studies considering effects on reproduction and/or development (and including mechanistic information) are of greater relevance than short-term *in vivo* screening tests which are of greater relevance than *in vitro* assays. The greater relevance of chronic *in vivo* tests or those assessing effects during critical windows of sensitivity is also evidenced by the fact that these are the key (eco)toxicological methods being developed in the OECD Endocrine Disruption Testing and Assessment (EDTA) Programme. This hierarchy approach to data relevance has been adopted in the review along with a weight of evidence consideration of the available data.

For consideration of the risk of a substance to target groups of humans and/or wildlife a Margin of Safety (MOS) approach has been adopted. The MOS (for consumer use) is calculated by dividing the lowest No Observable Adverse Effect Level (NOAEL) of a compound by its Systemic Exposure Dose (SED) during normal use. If the MOS exceeds 100, the substance is regarded as safe for use. The value of 100 can be modified to account for perceived sensitive target groups (for example children). This approach is based on that given in the Scientific Committee for Consumer Products and Non-Food Products intended for Consumers (SCCNFP) "Notes of Guidance for Testing of Cosmetic Ingredients for their Safety Evaluation" (SCCNFP/0321/00 Final, 2001). A similar approach is applied to workers and also to wildlife groups using appropriate exposure and effects data. It should be recognised that this approach does not provide a as robust a measure of potential risk as a PEC/PNEC

comparison but that it is designed to highlight situations where further effort may be needed to clarify the nature and extent of exposure to a target group.

3. Outcome of the reviews

In considering the data on the potential endocrine disrupting effects of individual natural and synthetic substances on target groups of humans and/or wildlife it needs to be recognised that a vast majority of the available data is from studies conducted using current internationally recognised test guidelines or studies carried out using alternative (possibly non-regulatory) procedures. As such the studies may provide information on potential adverse effects on reproduction and development in the species studied but, because they are not specifically designed to address endocrine disruption, do not usually provide data on changes in endocrine function (for example changes in hormone levels). In the assessment of endocrine disruption in humans and wildlife the question of 'proof' of adverse effects and of underlying causative factors is crucial both for scientific inference and for guiding opinion forming and decision making.

For the industrial substances and natural/synthetic steroids the datasets for assessing potential endocrine disrupting effects in target groups of humans and/or wildlife vary in terms of both the types of studies for which data is available and their reliability (i.e. the methods used and the quality assurance/quality control procedures adopted). This is illustrated in Table ES1 which summarises the available *in vivo* mammalian data for the industrial substances and indicates that data of definitive significance from multi-generation reproduction studies is not available for all the industrial substances considered in the review. However, some data on reproductive and developmental endpoints in mammals (which may be endocrine mediated) is available for all the substances.

For all the industrial substances, except 4-*tert* octylphenol, the information on potential adverse effects on reproductive and developmental endpoints in wildlife, which may be endocrine mediated, was extremely limited. The limited data that is available is in most instances for aquatic species (and only invertebrates and/or fish). For the natural and synthetic steroids (particularly 17β oestradiol and 17α ethinyloestradiol) there was a greater body of data for aquatic species including invertebrates and fish.

For most of the substances considered in the review there is generally no data for terrestrial or aerial organisms. However, it needs to be recognised that the importance of this data to assessing potential endocrine disrupting effects will depend on the extent to which the substances are likely to partition into these environmental compartments. Furthermore, no validated OECD methods are available which been specifically designed to assess endocrine disrupting effects in representative taxonomic groups from these compartments.

Tables ES2 and ES3 summarise the weight of evidence for endocrine disrupting effects in humans and wildlife and the uncertainties associated with the review of the 12 identified substances.

3.1 Industrial substances

3.1.1 Assessment of potential endocrine disrupting effects in humans

For a number of substances (BADGE, 4-chloro-3-methylphenol, 2,4-dichlorophenol, 4-nitrotoluene, o-phenylphenol and 4-*tert* octylphenol) the available *in vivo* data indicate that no adverse effects on reproduction and development in laboratory mammals (which may be

endocrine mediated) occur at exposure levels where general systemic toxic effects are observed. However, there is uncertainty with data for 4-chloro-3-methylphenol, 2,4-dichlorophenol, 4-nitrotoluene and resorcinol since although data on reproduction and developmental endpoints is available, a definitive multi-generational reproduction study has not been conducted. The extent to which any adverse effects observed at high exposure doses are endocrine mediated is uncertain since the available studies provide no specific consideration of effects on endocrine function (for example changes in hormone levels).

For carbon disulphide the weight of evidence indicates that this substance can cause effects on endocrine glands or hormone sensitive tissues in humans and laboratory mammals and on mammalian reproduction and development. Most effects on endocrine organs occur at concentrations at which other, clearly toxic effects predominate, particularly those on the nervous system. Other effects, more specifically functional disturbances of the female reproductive system (i.e menstrual disturbances) may occur at otherwise non-toxic concentrations, but those which are higher than environmentally relevant levels. The findings on mammalian reproduction and development are not unexpected given that carbon disulphide is classified as constituting a possible risk of impaired fertility and possible harm to the unborn child.

For resorcinol the weight of evidence indicates that the substance is a thyroid peroxidase inhibitor and hypothyroidism has been reported in humans applying dermatological preparations and creams. Over application was a problem in the early 20th century and individuals also experienced central nervous system depression and methaemoglobinaemia which are also listed as side-effects. Certain older *in vivo* laboratory animal studies have revealed reversible anti-thyroid activity. The thyroid effects in these studies resulted from continuous exposure to high doses and required a vehicle (such as peanut oil) to establish a reservoir of resorcinol and to alter the pharmacokinetics such that resorcinol was continuously bioavailable. Studies conducted as part of the National Toxicology Programme have shown no effects on the thyroid of rats or mice at doses of up to 520 mg kg body weight^{-1} day^{-1} in rats and 450 mg kg body weight^{-1} day^{-1} in mice for 13 weeks and 150 – 225 mg kg body weight^{-1} day^{-1} for 5 days per week over 2 years in rats and mice. The NTP studies have also reported adrenal weight effects (both hypertrophy and atrophy) at all doses in range finding studies in rats and mice. However, the observed responses did not show a dose-dependent relationship. There has been no thorough study of reproductive toxicity and given the role of the thyroid in development, this is a critical area of uncertainty. To address this issue the Resorcinol Task Force has already formulated a comprehensive test programme which will comprise an extensive dose range finding study and a test guideline compliant two-generation reproduction study with expanded thyroid endpoints. It will also provide additional observations on sub-chronic exposure NOEL's derived from the pre-mating dosing period.

Substances such as BADGE, 4-nitrotoluene, 4-*tert* octylphenol and resorcinol (in hair colouring dyes) are produced in closed systems and/or are used as chemical intermediates which minimises the potential for worker exposure. A number of the substances (2,4-dichlorophenol, 4-nitrotoluene and 4-*tert* octylphenol) are not used in products and the potential for consumer exposure is limited. Of the substances where an MOS approach could be applied for consumer use both BADGE (as a liner of food and drink cans), 4-nitrotoluene and resorcinol (as a hair colouring dye and in pharmaceutical products) were not found to represent a risk to consumers. The greatest risk from carbon disulphide appears to be centred on workers, rather than consumers, and particularly those in the viscose rayon industry. However, in recent years there has been more stringent control over working practices at these plants in Western Europe and compliance with existing air quality standards should minimise potential adverse effects.

3.1.2 Assessment of potential endocrine disrupting effects in wildlife

For a most of the industrial substances (BADGE, 4-chloro-3-methylphenol, 2,4-dichlorophenol, 4-nitrotoluene, o-phenylphenol and 4-*te*rt octylphenol) the available aquatic effects data shows that the threshold exposure concentrations above which reproduction in invertebrates and fish are observed is slightly lower or similar to the threshold level for general toxic effects (i.e. lethality and/or growth) in these species. However, there is generally no data in the reported studies which indicates whether the observed effects on reproduction are endocrine mediated. Indeed in invertebrates there is limited knowledge of the endocrinology of many taxonomic groups and it uncertain whether reproductive processes are modulated by oestrogens or androgens.

Using the available exposure data in an MOS approach for the aquatic compartment indicated that 4-chloro-3-methylphenol and 4-nitrotoluene do not represent a risk to aquatic organisms whereas 2,4-dichlorophenol and 4-*tert* octylphenol may represent a risk.

3.2 Natural and synthetic steroids

The natural vertebrate steroids, 17β-oestradiol and oestrone, and the synthetic steroid 17α-ethinyloestradiol all evidently cause effects on the reproduction and development of fish which are probably endocrine mediated. These effects occur at environmental relevant concentrations and, therefore, these substances can represent a risk to fish and other aquatic vertebrates. This conclusion is consistent with the results of field surveys carried out in a number of European countries (for example the COMPREHEND programme) which have identified evidence of adverse effects on the development and reproductive capability of wild fish which are exposed to natural (and synthetic) steroids discharged from sewage treatment works (for review EA 2002). The potential for effects is probably greater following exposure to natural steroids (17β-oestradiol and oestrone) than the synthetic steroid 17α-ethinyloestradiol.

3.3 Cases of particular consumer risk

One of the key objectives of the review of the 12 substances was to identify specific cases of consumer or ecosystem exposure to these substances, with particular attention to potentially vunerable consumer groups such as children. The following conclusions can be drawn from the available data:

- A number of the industrial substances (2,4-dichlorphenol, 4-nitrotoluene and 4-*tert* octylphenol) are used in the manufacture of products from which it is probable that there is no or extremely limited consumer exposure. However, information on potential consumer exposure for these substances is limited or absent and it is difficult to draw robust conclusions on the risk to vulnerable groups. Further targeted monitoring to provide this data is needed where there is a potential risk to consumers.

- For substances where there is the potential for consumer exposure (BADGE through epoxy lining of food and drink cans and 4-chloro-3-methylphenol and resorcinol through pharmaceutical products) the data indicates that there is evidently no risk to consumers including children from current exposure patterns. For 4-chloro-3-methylphenol and resorcinol in pharmaceutical products this is based on the assumption that these are used as described in the accompanying literature.

Table ES1 Summary of the available in vivo mammalian data for the industrial substances

Substance	Sub-chronic oral toxicity test (OECD 408)	One generation reproduction test (OECD 415)	Two generation reproduction test (OECD 415)	Developmental/ teratogenicity test (OECD 414)	Combined chronic toxicity/oncogenicity test (OECD 453)	Other test data
BADGE	✓[1]	✓[1]	✓[1]	✓[1]	**x**	Carcinogenicity studies
Carbon disulphide	✓[3]	x	✓[3]	✓[1]	x	Carcinogenicity studies, Human exposure studies
4-Chloro-3-methylphenol	✓[2]	x	x	✓[1]	✓[1]	Carcinogenicity studies
2,4-Dichlorophenol	x	✓[2]	x	✓[2]	x	Carcinogenicity studies
4-Nitrotoluene	✓[2]	✓[3] (3 month study)	x	x	x	Carcinogenicity studies
o-Phenylphenol	✓[1]	x	✓[1]	✓[1]	✓[1]	Carcinogenicity studies
Resorcinol	✓[1]	x	x	✓[1]	x	Carcinogenicity studies, Human exposure studies
4-tert Octylphenol	✓[2]	x	✓[1]	✓[2]	x	-
Tetra BDE	Not considered in the review					

Notes:

✓[1] – One or more studies conducted to established (OECD or US EPA) guideline and to GLP

✓[2] – One or more studies conducted to a well described procedure but not to GLP

✓[3] – Limited information on procedure by which study/studies were conducted or study/studies has limitations in terms of quality

Table ES2 Summary of the weight of evidence and uncertainties associated with the assessment of the endocrine disrupting effects in humans for the 12 identified substances of concern

Substance	Humans	
	Weight of evidence	Uncertainties
BADGE	The available data from *in vivo* studies in laboratory mammals (using oral or dermal exposure routes) indicates that BADGE does not cause adverse effects on reproductive and developmental endpoints (which may be endocrine mediated) at exposure levels where general systemic toxic effects are observed. The lowest recorded NOEL from the *in vivo* mammalian studies was 250 mg kg body weight^{-1} day^{-1} for histopathological effects in endocrine glands and hormone sensitive tissues, though the observed effects at higher doses may have resulted from direct toxic action. The available exposure data indicates that current exposure patterns to BADGE do not represent a risk to workers or consumers.	There are no major uncertainties with regard to the evaluation of potential adverse effects of BADGE on reproductive and developmental endpoints since data is available from a definitive multi-generation study as well as supporting reproduction and developmental studies. Mechanistic uncertainties exist because the available studies provide no direct measurement of changes in endocrine function (for example changes in hormone levels).
Carbon disulphide	Carbon disulphide can affect male fertility in rats through changes in sperm count and mating behaviour and the NOEL in laboratory animals for effects is at > 1000 mg/m^3. There is some evidence that this toxicity is caused by a toxic effect on the testicles or by indirect effects on the ejaculation process. However, a BUA review stated that the influence of the hypothalamus-pituitary gland-gonad axis is improbable. A number of studies have shown that carbon disulphide possesses embryotoxic effects at high doses but levels that are lower than those causing maternal toxicity. Rabbits appear to be more sensitive than rats. Teratogenic effects were described exclusively at maternally toxic doses. The NOEL for embryotoxic effects for rabbits were in the region of 900 mg/m^3 and is higher for teratogenic effects. Initial neurotoxic effects already occur at these concentrations in the 90 day tests. Studies by Tabacova and co-workers describe embryotoxic effects from carbon disulphide at 0.03 mg/m^3 and teratogenic effects at 10 mg/m^3. However, there are issues with the quality of these studies. In humans, there have been numerous studies of workers exposed occupationally that report effects on pituitary-gonadal function in men and women, as well as indications of adverse reproductive outcomes in women. In men spermatogenic, diabetogenic and adrenal effects have also been reported. These effects have generally been reported for workers in the viscose rayon industry at exposure levels considerably above current occupational exposure standards (< 30 mg/m^3)	Carbon disulphide has not been subjected to standard regulatory toxicity tests and this raises uncertainties as to the validity of certain data, particularly that for the only multi-generation reproduction study. This uncertainty is offset to a degree by the fact that there is a considerable body of relevant data for humans derived from studies on workers (principally those in the viscose rayon industry).

Table ES2 Continued

Substance	Humans	
	Weight of evidence	Uncertainties
4-chloro-3-methylphenol	The available data from *in vivo* studies in laboratory mammals (using oral or dermal exposure routes) indicates that 4-chloro-3-methylphenol does not cause adverse effects on reproductive and developmental endpoints (which may be endocrine mediated) at exposure levels where general systemic toxic effects are observed. The lowest NOEL in the *in vivo* studies was 100 mg kg body weight $^{-1}$ day^{-1} for foetotoxic effects (on intra-uterine development) At higher exposure doses where adverse effects on development (foetotoxicity) were evident no information on changes in endocrine function was available. The available data indicate that 4-chloro-3-methylphenol in hand and skin disinfectants and as a preservative in pharmaceuticals does not present a risk to consumers.	There are uncertainties with regard to the evaluation of potential adverse effects of 4-chloro-3-methylphenol on reproductive and developmental endpoints since data is not available from a definitive multi-generation study. Mechanistic uncertainties exist because the available studies provide no direct measurement of changes in endocrine function (for example changes in hormone levels). Limited exposure data have been located for workers and consumers.
2,4-Dichlorophenol	The available data from *in vivo* studies in laboratory mammals (using oral or dermal exposure routes) indicates that 2,4-dichlorophenol does not cause adverse effects on reproductive and developmental endpoints (which may be endocrine mediated) at exposure levels where general systemic toxic effects are observed. The lowest NOEL in the *in vivo* studies was 50 mg kg body weight $^{-1}$ day^{-1} for reproductive parameters. No measured exposure data has been located for 2,4-dichlorophenol but since it is produced as an intermediate in closed systems the potential exposure of workers to the substance is limited providing appropriate safety procedures are followed. No information on concentrations of 2,4-dichlorophenol to which consumers are potentially exposed during the use of products has been obtained. However, the advisory on 2,4-dichlorophenol from the United States Environmental Protection Agency (US EPA) Office of Pollution Prevention and Toxics (OPPT) and US Department of Labour Occupational Safety and Health Administration (OSHA) stated that "*The focus of concern is occupational and no risks are expected for consumers or community members.*"	There are uncertainties with regard to the evaluation of potential adverse effects of 2,4-dichlorophenol on reproductive and developmental endpoints since data is not available from a definitive multi-generation study. These issues will be addressed in a study initiated by MITI in 2002. Mechanistic uncertainties exist because the available studies provide no direct measurement of changes in endocrine function (for example changes in hormone levels).

Table ES2 Continued

Substance	Humans	
	Weight of evidence	Uncertainties
4-Nitrotoluene	The available data from *in vivo* studies in laboratory mammals (using oral or dermal exposure routes) indicates that 4-nitrotoluene does not cause adverse effects on reproductive endpoints (which may be endocrine mediated) at exposure levels where general systemic toxic effects are observed. The lowest NOEL in the *in vivo* studies was 110 mg kg body weight $^{-1}$ day^{-1} for histopathological effects on reproductive tissues, though the observed effects at higher doses may have resulted from direct cytotoxic action. The available data indicate that current exposure patterns to 4-nitrotoluene do not represent a risk to workers or consumers (including children).	There are uncertainties with regard to the evaluation of potential adverse effects of 4-nitrotoluene on reproductive and developmental endpoints since data is not available from a definitive multi-generation study as well as developmental/teratogenicity studies. A reproduction screening test has been conducted and was due to be reported in late 2002. Mechanistic uncertainties exist because the available studies provide no direct measurement of changes in endocrine function (for example changes in hormone levels).
o-Phenylphenol	The available data from *in vivo* studies in laboratory mammals (using oral or dermal exposure routes) indicates that o-phenylphenol does not cause adverse effects on reproductive and developmental endpoints (which may be endocrine mediated) at exposure levels where general systemic toxic effects are observed. The lowest NOEL in the *in vivo* studies was 250 mg kg body weight $^{-1}$ day^{-1} for foetotoxic and developmental effects. Limited exposure data for workers and consumers has been located.	There are no major uncertainties with regard to the evaluation of potential adverse effects of o-phenylphenol on reproductive and developmental endpoints since data is available from a definitive multi-generation study as well as supporting reproduction and developmental studies. Mechanistic uncertainties exist because the available studies provide no direct measurement of changes in endocrine function (for example changes in hormone levels).

Table ES2 Continued

Substance	Humans	
	Weight of evidence	Uncertainties
Resorcinol	*In vitro* studies indicate that the anti-thyroidal activity observed following resorcinol exposure is due to inhibition of thyroid peroxidase (TPO) enzymes, as evidenced by disruption of thyroid hormone synthesis and changes in the thyroid gland consistent with goitrogenesis. Certain older *in vivo* laboratory animal studies have revealed reversible anti-thyroid activity. The thyroid effects in these studies resulted from continuous exposure to high doses and required a vehicle (such as peanut oil) to establish a reservoir of resorcinol and to alter the pharmacokinetics such that resorcinol was continuously bioavailable. Studies conducted as part of the National Toxicology Programme have shown no effects on the thyroid of rats or mice at doses of up to 520 mg kg body weight^{-1} day^{-1} in rats and 450 mg kg body weight^{-1} day^{-1} in mice for 13 weeks and 150 – 225 mg kg body weight^{-1} day^{-1} for 5 days per week over 2 years in rats and mice. There is evidence of effects on adrenal weights at all doses tested in NTP rat and mouse 13-week studies. However, the observed responses did not show dose-dependent relationships. Currently available data indicates that resorcinol is not embryotoxic or teratogenic. The available exposure data indicate that resorcinol does not represent a risk to workers or consumers based on current exposure pathways.	There are uncertainties with regard to the evaluation of potential adverse effects of resorcinol on reproductive and developmental endpoints since data is not available from a definitive multi-generation study. These issues will be addressed in study being initiated by the Resorcinol Task Force, along with the uncertainties regarding the significance of effects in the adrenals observed in certain studies.
4-*tert* Octylphenol	The available data from *in vivo* studies in laboratory mammals (using oral or dermal exposure routes) indicates that 4-*tert* octylphenol does not cause adverse effects on reproductive and developmental endpoints (which may be endocrine mediated) at exposure levels where general systemic toxic effects are observed. The lowest NOEL in the *in vivo* studies was 150 mg kg body weight $^{-1}$ day^{-1} for effects on reproductive and developmental parameters. No effects on reproductive or developmental parameters in laboratory mammals are evident at low exposure doses (0.0015 – 0.002 mg kg^{-1}) The available data indicates that current exposure patterns to 4-*tert* octylphenol do not represent a risk to workers or consumers (including children).	There are no major uncertainties with regard to the evaluation of potential adverse effects of 4-*tert* octylphenol on reproductive and developmental endpoints since data is available from a definitive multi-generation study as well as supporting reproduction and developmental studies. Mechanistic uncertainties exist because most of the available studies provide no direct measurement of changes in endocrine function (for example changes in hormone levels).

Table ES2 Continued

Substance	Humans	
	Weight of evidence	Uncertainties
Tetra BDE	No review completed	
Oestrone	Not considered in the review	
17β-Oestradiol	Not considered in the review	
17α-Ethinyloestradiol	Not considered in the review	

Table ES3 Summary of the weight of evidence and uncertainties associated with the assessment of the endocrine disrupting effects in wildlife for the 12 identified substances of concerns

Substance	Wildlife	
	Weight of evidence	Uncertainties
BADGE	The available aquatic effects data shows that the threshold exposure concentration of BADGE above which reproduction of the invertebrate *Daphnia magna* is reduced (NOEC = 0.3 mg l^{-1}) is similar to the threshold level for general toxic effects (i.e. lethality). However, there is no information on the mechanism of action for the effects on reproduction observed in *Daphnia magna*.	There are uncertainties with regard to potential adverse effects of BADGE on reproduction and development in wildlife due to the absence of key data for: • A wider range of aquatic taxa, particularly fish and sediment dwelling invertebrates • Terrestrial organisms The absence of data on aerial organisms is not a major uncertainty since BADGE is not volatile and the potential for these organisms to be exposed is limited. No environmental exposure data for BADGE in the aquatic, terrestrial and aerial compartments has been located
Carbon disulphide	The available data on the effects of carbon disulphide on the development of aquatic organisms has been shown that the threshold exposure concentration above which the hatching rate of fish (*Danio rerio*) is affected is 1.0 mg l^{-1}. However, it is not clear whether these responses are endocrine mediated. This exposure level is only slightly lower than concentrations causing acute toxicity in fish (and invertebrates).	No data has been located on the potential endocrine disrupting effects of carbon disulphide on the reproduction of aquatic organisms or the reproduction/development of terrestrial or aerial organisms. The volatility of carbon disulphide means that wildlife organisms that are exposed to carbon disulphide via inhalation represent those most at risk from exposure. There is limited data on environmental concentrations of $CS_{2,}$, however it needs to recognised that environmental levels are influenced by natural releases.
4-Chloro-3-methyl phenol	The available aquatic effects data shows that the threshold exposure concentration of 4-chloro-3-methylphenol above which reproduction of the invertebrate *Daphnia magna* is reduced (NOEC = 1.25 mg l^{-1}) is slightly lower than the threshold level for general toxic effects (i.e. lethality). However there is no information on the mechanism of action for the effects on reproduction observed in *Daphnia magna*. The available exposure data indicate that 4-chloro-3-methylphenol does not represent a risk to aquatic organisms.	There are uncertainties with regard to potential adverse effects of 4-chloro-3-methylphenol on reproduction and development in wildlife due to the absence of data for a wider range of aquatic taxa, particularly fish. The absence of data for terrestrial and aerial organisms is not a major uncertainty since the physico-chemical properties of 4-chloro-3-methylphenol indicate that the substance should not partition into the terrestrial and aerial compartments. No environmental exposure data for 4-chloro-3-methylphenol in the terrestrial and aerial compartments has been located.

Table ES3 Continued

Substance	Wildlife	
	Weight of evidence	Uncertainties
2,4-Dichlorophenol	The available effects data shows that the threshold exposure concentration of 2,4-dichlorophenol above which reproduction of the aquatic invertebrate *Daphnia magna* is reduced (NOEC = 0.21 mg l^{-1}) is only slightly lower than the threshold level for general toxic effects (i.e. lethality). However there is no information on the mechanism of action for the effects observed in this species. The available exposure data indicate that 2,4-dichlorophenol may represent a risk to aquatic organisms. In contrast, the threshold exposure concentrations of 2,4-dichlorophenol above which reproduction of the terrestrial invertebrate *Folsomia candida* is reduced (NOEC = 3.8 mg kg dry weight^{-1}) is slightly higher than the threshold level for general toxic effects (i.e. lethality).	There are uncertainties with regard to potential adverse effects of 2,4-dichlorophenol on reproduction and development in wildlife due to the absence of data for a wider range of aquatic taxa, particularly fish. The absence of data on aerial organisms is not a major uncertainty since the physico-chemical properties of 2,4-dichlorophenol indicate that the substance should not partition into the aerial compartment. No environmental exposure data for 2,4-dichlorophenol in the terrestrial and aerial compartments has been located.
4-Nitrotoluene	The available aquatic effects data shows that the threshold exposure concentration of 4-nitrotoluene above which reproduction of the invertebrate *Daphnia magna* is reduced (NOEC = 0.7 mg l^{-1}) is lower than the threshold level for general toxic effects (i.e. lethality). However, there is no information on the mechanism of action for effects on the reproduction of *Daphnia magna*. The available exposure data indicate that 4-nitrotoluene does not represent a risk to aquatic organisms.	There are uncertainties with regard to potential adverse effects of 4-nitrotoluene on reproduction and development in wildlife due to the absence of key data for: • A wider range of aquatic taxa, particularly fish • Aerial organisms The absence of data on terrestrial organisms is not a major uncertainty since 4-nitrotoluene does not strongly sorb to organic carbon and the potential for these organisms to be exposed is limited. No environmental exposure data for 4-nitrotoluene in the terrestrial and aerial compartments has been located.
o-Phenylphenol	The available aquatic effects data shows that the threshold exposure concentrations of o-phenylphenol above which reproduction of the invertebrate *Daphnia magna* and fish (fathead minnow) are reduced (NOECs = 0.036 mg l^{-1} and 0.009 mg l^{-1} respectively) are lower than the theshold levels for general toxic effects (i.e. lethality). The effects observed on reproduction in fish were evidently not oestrogen mediated . However, there is no information on the mechanism of action for the effects on reproduction observed in *Daphnia magna*.	There is no data on potential adverse effects on reproduction and development in terrestrial and aerial organisms but this is not a major uncertainty since the physico-chemical properties of o-phenylphenol mean the potential for these organisms to be exposed is limited. No environmental exposure data for o-phenylphenol in the aquatic, terrestrial and aerial compartments has been located.

Table ES3 Continued

Substance	Wildlife	
	Weight of evidence	Uncertainties
Resorcinol	The available aquatic effects data from teratogenicity studies with rainbow trout and zebrafish embryos shows teratogenic effects are evident at exposure concentrations $\geq$ 100 mg l^{-1}. However, there is no available data as to whether the observed effects were endocrine mediated.	There are uncertainties with regard to the potential adverse effects of resorcinol on reproduction and development in wildlife due to the absence of key data for aquatic organisms particularly invertebrates. This is planned to be addressed by the Resorcinol Task Force. The absence of data on terrestrial and aerial organisms is not a major uncertainty since the physico-chemical properties of resorcinol mean the potential for these organisms to be exposed is limited. There are no environmental exposure data for resorcinol in the aquatic, terrestrial and aerial compartments.
4-*tert* Octylphenol	The available data shows the threshold exposure concentrations of 4-*tert* octylphenol above which reproduction and development in aquatic organisms (fish, amphibians and invertebrates) are affected (NOECs = 1-12 $\mu g\ l^{-1}$) are similar to the threshold levels for general toxic effects (i.e. growth and lethality). The effects may be oestrogen mediated. The available exposure data indicate that 4-t*ert* octylphenol may represent a risk to aquatic organisms, particularly at discharge 'hotspots'.	There are uncertainties with regard to the potential adverse effects of 4-*tert* octylphenol on reproduction and development in wildlife due to the absence of key data for terrestrial organisms. The absence of data on aerial organisms is not a major uncertainty since although 4-*tert* octylphenol is volatile it is rapidly degraded and the potential for these organisms to be exposed is limited. There are no environmental exposure data for 4-*tert* octylphenol in the terrestrial and aerial compartments, though levels in the aerial compartment are not expected to be high.
Tetra BDE	Not considered in the review	

Table ES3 Continued

Substance	Wildlife	
	Weight of evidence	Uncertainties
Oestrone	In fish it appears that effects of oestrone on reproduction and development which are considered to be endocrine mediated occur at markedly lower (and environmentally relevant) concentrations (> 1-10 ng l^{-1}) than those causing general toxicity. No effects of oestrone on the reproduction of the aquatic invertebrate copepod *Tisbe battagliai* were evident at the highest exposure concentration (100 ng l^{-1}), indicating that the processes of reproduction and development in certain invertebrate taxa (crustaceans) are evidently not affected by exposure to vertebrate steroids at typical environmental levels. However this may not be the case for other invertebrate taxa. The available aquatic exposure data (showing typical concentrations of **<0.5 - 5** ng l^{-1}) indicates that oestrone presents a risk to fish (and other aquatic vertebrates) in terms of endocrine disrupting effects. This is consistent with data from field surveys of fish populations exposed to natural (and synthetic) steroids discharged from sewage treatment works.	The data on oestrone induced and endocrine mediated effects on reproduction and development in wildlife is limited and restricted to aquatic organisms (invertebrates and fish) The results of a multi-generational study will considerably reduce the uncertainty associated with the extent of endocrine mediated responses of fish. No data are available on potential endocrine mediated effects in terrestrial and aerial organisms. Given that sorption to organic carbon is an important process resulting in the partitioning of oestrone onto soils the absence of data on potential endocrine mediated responses in terrestrial organisms is a key area of uncertainty.
17β-Oestradiol	In fish it appears that effects of 17β-Oestradiol on reproduction and development which are considered to be endocrine mediated occur at markedly lower (and environmentally relevant) concentrations (> 5 - 25 ng l^{-1}) than those causing general toxicity. The processes of reproduction and development in certain invertebrate taxa (crustaceans) are evidently not generally affected by exposure to vertebrate steroids at typical environmental levels. However this may not be the case for other invertebrate taxa. The available aquatic exposure data (showing typical concentrations of 1 – 5 ng l^{-1}) indicates that 17β-Oestradiol presents a risk to fish (and other aquatic vertebrates) in terms of endocrine disrupting effects. This is consistent with data from field surveys of fish populations exposed to natural (and synthetic) steroids discharged from sewage treatment works.	The data on 17β-Oestradiol induced and endocrine mediated responses on reproduction and development in wildlife is limited and restricted to aquatic organisms (invertebrates and fish) No data are available on potential endocrine mediated effects in terrestrial and aerial organisms. Given that sorption to organic carbon is an important process resulting in the partitioning of 17β-Oestradiol onto soils the absence of data on potential endocrine mediated responses in terrestrial organisms is a key area of uncertainty.

Table ES3 Continued

Substance	Wildlife	
	Weight of evidence	Uncertainties
17α-Ethinyloestradiol	In fish it appears that threshold effects of 17α-ethinyloestradiol on reproduction and development which are considered to be endocrine mediated occur at markedly lower (and environmentally relevant) concentrations (> 0.3 - 1 ng l^{-1}) than those causing general toxicity. The processes of reproduction and development in certain aquatic invertebrate taxa (crustaceans) are evidently not affected by exposure to vertebrate steroids at typical environmental concentrations. However this may not be the case for other invertebrate taxa. The available exposure data indicates that 17α-ethinyloestradiol can in certain circumstances present a risk to fish (and other aquatic vertebrates) in terms of endocrine disrupting effects. However, detectable aquatic concentrations in surface waters are in most cases below the threshold levels capable of resulting in endocrine disrupting effects. The assessment of risk is confounded by the current analytical limitations in the sensitivity of detection of 17α-ethinyloestradiol.	The data on 17α-ethinyloestradiol induced and endocrine mediated effects on reproduction and development in wildlife is limited and restricted to aquatic organisms (invertebrates and fish). No data are available on potential endocrine mediated effects in terrestrial and aerial organisms. Given that sorption to organic carbon is an important process and sewage sludge may be applied to land the absence of data on potential endocrine mediated responses in terrestrial organisms is an area of uncertainty.

4. General issues relating to the assessment of endocrine disrupting effects

In addition to the conclusions on the individual substances reviewed in the report it is evident that there are a number of generic issues associated with the assessment of endocrine disrupting effects which apply to all potential substances of interest and not just those considered in the review.

The assessment of endocrine disrupting effects in humans and wildlife is an evolving area and a considerable body of activity is on-going at both national and international levels. It was evident from the recent Report of a European Workshop on Endocrine Disrupters held in Aronsborg (Sweden) that there are a number of key areas of uncertainty which need to be addressed to enhance the evaluation of the extent of endocrine disrupting effects of substances of concern and the risks they present to humans and/or wildlife. Key areas requiring further activity are:

- The development of validated methods which provide robust information on endocrine disrupting effects in particular target groups, specifically invertebrates where there is a lack of knowledge on the endocrinology of many taxonomic groups;

- The conduct and interpretation of mammalian and non-mammalian tests in relation to potential low-dose effects;

- Collation (and if required generation) of information on the normal background variability in reproduction and developmental responses of mammalian and wildlife species;

- Assessment of the risks presented by the potential endocrine disrupting effects of synthetic substances in relation to background exposure to natural compounds (for example vertebrate steroids and phyto-oestrogens).

5. Implementation of a framework for reviewing other substances of concern

Following the conduct of the reviews in this project a framework for conducting reviews of other substances identified as potential endocrine disrupters in a prioritisation exercise has been proposed (see Figure ES1). The framework (Boxes 2 and 3 in Figure ES1) follows the approach described in Section 2 of the report and incorporates a step-wise evaluation of data on the substances against the three issues given in Section 2 of the report, namely whether:

- the weight of evidence for a substance indicates endocrine disrupting effects occur in target groups of humans and/or wildlife;

- endocrine disrupting effects occur at lower concentrations of the substance than those causing general non-endocrine mediated (eco)toxicological effects in the target groups;

- the target groups of humans and/or wildlife are likely to be exposed to the substance in the environment at doses/concentrations capable of causing endocrine disrupting effects.

In the framework it is also important to consider:

1. Is there sufficient data of definitive significance available to draw robust and meaningful conclusions on the extent to which a substance causes or has the potential to cause

endocrine disruption effects in target groups of humans and/or wildlife at levels below those causing general non-endocrine (eco)toxicological responses?;

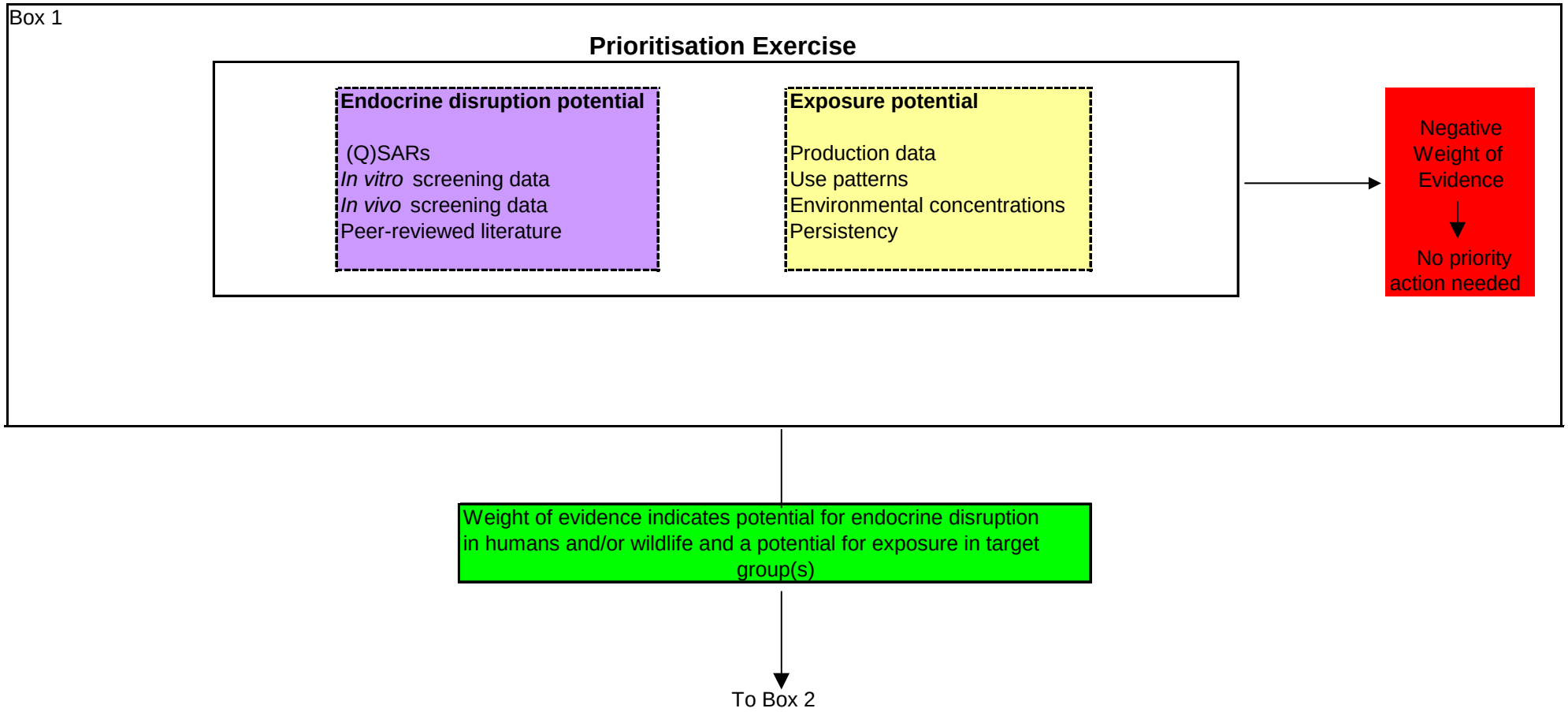
2. What additional information is needed to allow robust and meaningful conclusions to be drawn if there is currently insufficient data for a substance?.

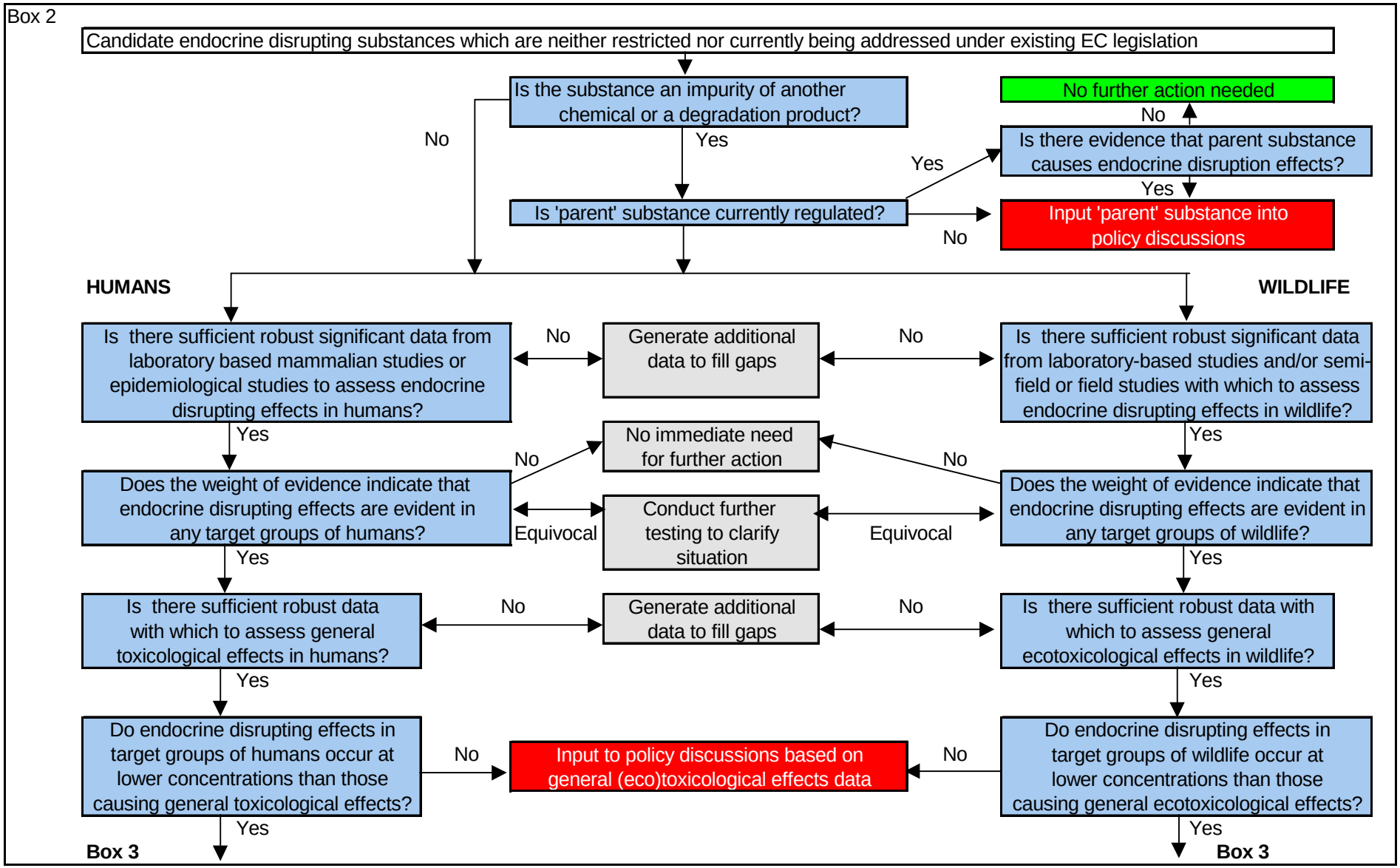
The framework is designed to build on a prioritisation exercise using the procedure being developed by BKH (Box 1 in Figure ES1) and it is envisaged that this screening approach will be used to prioritise the substances for which a review is conducted.

At the prioritisation stage substances for which there is evidence of endocrine disruption in humans and/or wildlife and a potential for exposure of the target group(s) should be considered a priority for more detailed review using the procedure described in Section 2. Evidence of endocrine disruption in a target group but an absence of data on the potential for exposure should lead to the acquisition of relevant basic exposure data so that a decision can be made on whether a more detailed review of the substance is required. If there is evidence of exposure potential but no information on potential endocrine disrupting effects then no detailed review should be conducted until some robust data on endocrine disrupting effects has been generated.

Where there is sufficient data, an absence of evidence or negative weight of evidence for potential endocrine disrupting effects in a target group and no potential for exposure can be used to indicate that further detailed consideration of the substance is not a priority action.

For the assessment of endocrine disrupting effects of a substance at the prioritisation exercise stage the emphasis should be placed on *in vivo* data where this is available. The review of the 9 industrial substances has shown that effects observed *in vitro* assays are not always translated into effects in whole organisms, especially at doses/concentrations which may reflect the lowest observed toxicity in a target group.


Box 1
Prioritisation Exercise
Endocrine disruption potential
(Q)SARs
In vitro screening data
In vivo screening data
Peer-reviewed literature
Exposure potential
Production data
Use patterns
Environmental concentrations
Persistency
Negative Weight of Evidence
No priority action needed
Weight of evidence indicates potential for endocrine disruption in humans and/or wildlife and a potential for exposure in target group(s)
To Box 2


Box 2
Candidate endocrine disrupting substances which are neither restricted nor currently being addressed under existing EC legislation
Is the substance an impurity of another chemical or a degradation product?
No
Yes
No further action needed
No
Is there evidence that parent substance causes endocrine disruption effects?
Yes
Yes
Is 'parent' substance currently regulated?
Yes
Input 'parent' substance into policy discussions
No
HUMANS
WILDLIFE
Is there sufficient robust significant data from laboratory based mammalian studies or epidemiological studies to assess endocrine disrupting effects in humans?
No
Generate additional data to fill gaps
No
Is there sufficient robust significant data from laboratory-based studies and/or semi-field or field studies with which to assess endocrine disrupting effects in wildlife?
Yes
Yes
No immediate need for further action
No
No
Does the weight of evidence indicate that endocrine disrupting effects are evident in any target groups of humans?
Equivocal
Conduct further testing to clarify situation
Equivocal
Does the weight of evidence indicate that endocrine disrupting effects are evident in any target groups of wildlife?
Yes
Yes
Is there sufficient robust data with which to assess general toxicological effects in humans?
No
Generate additional data to fill gaps
No
Is there sufficient robust data with which to assess general ecotoxicological effects in wildlife?
Yes
Yes
Do endocrine disrupting effects in target groups of humans occur at lower concentrations than those causing general toxicological effects?
No
Input to policy discussions based on general (eco)toxicological effects data
No
Do endocrine disrupting effects in target groups of wildlife occur at lower concentrations than those causing general ecotoxicological effects?
Yes
Yes
Box 3
Box 3

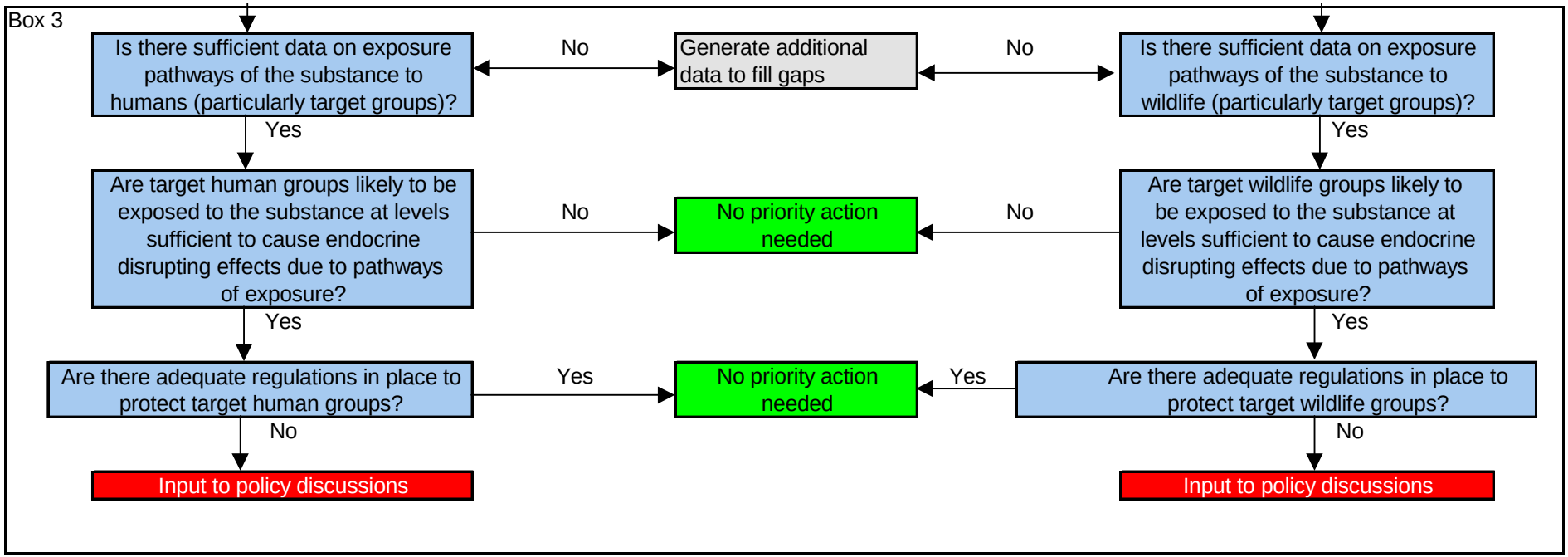


Figure ES1 Framework for the proiritisation and review of potential endocrine disrupting substances

1. INTRODUCTION

1.1 Background

In vertebrates the endocrine system, which is composed of glands that secrete chemical messengers, regulates and co-ordinates physiological responses and functions of the body. Hormones are biologically highly active substances, which in vertebrates are produced in the endocrine glands in one part of the body and travel through the bloodstream to specific target tissues where they interact to initiate essential biological responses. A key feature of vertebrate hormones is that dramatic changes in cellular activity are caused by extremely low doses and interactions of such chemical messengers regulate, for example the processes of growth, metabolism, physiology, sexual development and maturation as well as reproduction (Eubanks 1997).

In recent years it has been recognised that the normal operation of the endocrine (hormonal) system in a range of organisms can be disrupted by a number of man-made and naturally-occurring chemicals, thereby affecting those physiological processes which are under hormonal control. Endocrine disrupters may either mimic natural hormones or inhibit the actions of hormones, thus principally affecting the regulation of the endocrine system and potentially inter-dependent systems such as the immune and nervous systems.

There has been increasing concern regarding the presence of natural[1] and synthetic endocrine disrupting substances in food, water or other environmental media, and the potential risk they pose to the reproductive systems of wildlife and man (including declining sperm quality and increased incidences of genital tract abnormalities, breast and testicular cancer). This hypothesised link continues to receive much scientific, public and media attention and there is pressure for regulators to take action to reduce and/or prevent the use of a number of synthetic chemicals which exhibit endocrine disrupting properties. Research to date has mainly focused on the potential for compounds to inadvertently mimic the biological activities of the natural female sex-hormone oestrogen, and which cause a feminising or 'oestrogenic' effect (for example intersex in fish). However, there has also been interest in the ability of substances to block male sex hormones (androgens) and to interfere with thyroid hormones.

In its opinion of 4 March 1999, the CTSEE recognised the growing concern on possible harmful consequences of exposure to xenobiotic compounds that are capable of modulating the endocrine system and thus have the potential to adversely affect human and wildlife reproductive health. At the time, this concern had not been substantiated with respect to human health in that no causative role for endocrine disrupting chemicals in diseases and abnormalities possibly related to an endocrine disturbance have been verified. However, the

[1] It needs to be recognised that plant hormones (compounds synthesised by plants exhibiting hormone-like properties in vertebrate systems) are present in many vegetables such as beans, cabbage, grains, hops, peas, soya bean, spinach and spouts. Furthermore, the release of natural vertebrate steroids such as oestradiol and oestrone in aquatic systems has been shown to have pronounced effects on male fish species in some European countries.

CSTEE re-commended to further evaluate the human health effects that have been associated with endocrine disrupters, and to identify the underlying causes. On the other hand, impaired reproduction and development of several wildlife species have been causally linked to exposure to endocrine disrupting chemicals and have resulted in local or regional population changes. Thus, a European Commission Community Strategy for Endocrine Disrupters was seen by the CSTEE as a timely initiative to address the public concern.

In December 1999 the Commission published the Community Strategy on Endocrine Disrupters [COM(1999)706]. The objectives of this Communication (EC 1999) were to identify the problem of endocrine disruption, its causes and consequence and to identify appropriate policy action on the basis of the precautionary principle in order to respond rapidly and effectively to the problem.

Recommendations were made for short-, medium- and long-term actions with the ***short-term actions*** including the establishment of a priority list of substances for further evaluation of their role in endocrine disruption. Another short-term action was the identification of specific cases of consumer use or ecosystem exposure which might warrant special attention.

The initial stage in the preparation of a candidate list of substances as a basis for priority setting was carried out in a study by BKH Consulting Engineers (The Netherlands) under contract to the European Commission (Environment DG). The study focussed on man-made chemicals used primarily in industry, agriculture and consumer goods and involved four major prioritisation steps (see Section 2). The final report from the study in June 2000 ‘Towards the establishment of a priority list of substances for further evaluation of their role in endocrine disruption” identified a total of 553 ***candidate*** substances using a criteria-based approach (BKH 2000).

After the submission of the report, stakeholders including EU Member and Associated States, Commission Scientific Committees, industry associations and non-governmental organisations were consulted on a priority-setting exercise using the BKH report as the basis. In September 2000, the Commission Scientific Committee for Toxicity, Ecotoxicity and the Environment (CSTEE) in association with the Scientific Committee for Plants adopted an opinion on the scientific relevance of the BKH report (CSTEE 2000).

In the opinion the CSTEE considered that it is important to realise that:

“endocrine disruption is not a toxicological endpoint *per se* as is cancer or allergy, but that it is a descriptor for a functional change that may lead to adverse health effects”.

Furthermore, CTSEE strongly warned against the development of endocrine disruption as a classification category, used for example in labelling. Rather, endocrine disruption should be seen in the context of well-established endpoints, primarily reproductive toxicity and impaired development. Notwithstanding the definition of an endocrine disrupter, the CSTEE cautioned against confusing a secondary effect on endocrine disruption caused by a primary damage of hormone producing organ with a primary alteration of the function of an endocrine system leading to adverse health effects.

Stakeholders provided comments on the BKH Report and in general the majority of *Member States and non-governmental organisations* took the view that the BKH approach was a pragmatic and reasonable one for a “first cut” of the data. At the same time the need for additional work to improve and develop the list was emphasised, in particular the need to update the list in a transparent manner with clear criteria for inclusion and exclusion of

substances, and the need for a more detailed assessment of substances before any new regulatory action could be envisaged.

The *chemical industry* expressed its concern that "the BKH process might be perceived as a valid risk assessment because it appeared to combine hazard and exposure in a single assessment in a simplistic way".

In June 2001, the Commission adopted a follow up Communication to the Council and European Parliament on the implementation of the Community Strategy for Endocrine Disrupters (COM(2001)262). In this Communication (EC 2001) the Commission proposed a priority list of actions to further evaluate the role of specific "candidate" substances in endocrine disruption. One of these priority actions was the initiation of an in-depth evaluation of a group of 12 candidate substances for which there is scientific evidence of endocrine disruption or potential endocrine disruption, but which are neither restricted nor currently being addressed under existing community legislation. The list consisted of 9 industrial substances and three natural/synthetic hormones (see Table 1.1).

Table 1.1 Twelve substances for further evaluation of their role in endocrine disruption

CAS Number	Substance
1675-54-3	2,2'-bis(4-(2,3-epoxypropyl)phenyl)propane = 2,2'-[(1-methylethylidene) bis(4,1-phenyleneoxymethylene]bisoxirane
75-15-0	Carbon disulphide
59-50-7	4-chloro-3-methylphenol
120-83-3	2,4-dichlorophenol
99-99-0	4-nitrotoluene
90-43-7	o-phenylphenol
108-46-3	Resorcinol
-	2,2',4,4'-tetrabrominated diphenyl ether (2,2',4,4'-tetraBDE)
140-66-9	4-tert-octylphenol = (1,1,3,3-Tetramethyl-4-butylphenol)
53-16-7	Oestrone
50-28-2	Oestradiol
57-63-6	Ethinyloestradiol

To achieve this objective the Directorate General for Environment (DG ENV) of the European Commission has commissioned WRc-NSF to evaluate 12 candidate substances with respect to their role in endocrine disruption and possible effects on human health and the environment.

1.2 Objectives of the study

The objectives stated in the tender specification are to:

1. Conduct an in-depth evaluation of nine (9) candidate substances for which scientific evidence of endocrine disruption or potential endocrine disruption was identified in the BKH report and which are neither restricted nor currently being addressed under existing Community legislation;

2. Conduct an in-depth evaluation of three (3) synthetic/natural hormones, oestrone, oestradiol and ethinyloestradiol, with a particular focus on up to date evidence of environmental exposure and related effects.

3. Identify specific cases of consumer or ecosystem exposure to these substances, with particular attention to potentially vulnerable consumer groups such as children.

1.3 References

BKH (2000) Towards the establishment of a priority list of substances for further evaluation of their role in endocrine disruption – preparation of a candidate list of substances as a basis for priority setting, Study Report by BKH Consulting Engineers, NL, June 2000.

CSTEE (2000) Opinion of the Scientific Committee for Toxicity, Ecotoxicity and Environment on the BKH report, dated 5 September 2000.

EC (1999) Communication on the Community Strategy on Endocrine Disrupters [COM(1999)706].

EC (2001) Report of a European Workshop on Endocrine Disrupters on 18-20 June 2001 in Aronsborg, Sweden, European Commission, Brussels.

Eubanks, M.W. (1997) Hormones and health. *Environmental Health Perspectives*, **105**, 5.

2. FRAMEWORK FOR THE REVIEW OF CHEMICALS

2.1 Introduction

In this section a framework is described which has been used to review the nature and extent of endocrine disrupting effects of identified chemicals (and potentially others in the future) based on robust datasets. The framework is designed to represent a stage between the identification of potential substances of concern and any potential action following input of these substances into policy discussions. It needs to be recognised that the framework does **not** involve carrying out a full Risk Assessment of a substance under the Existing Substances Regulation 793/93, but it is appropriate to use established procedures from the Technical Guidance Document (EC 1994) where these are relevant. The adoption of this approach is designed to maximise consistency in terms of terminology used and the procedures adopted (for example in the areas of data relevance and study validity).

The approach for the targeted review of substances in relation to endocrine related activity consists of two basic tasks (see Figure 2.1):

1. Identifying a robust dataset by:

a) Collecting and collating all relevant effects and exposure data as required (that is **Data Collection and Collation**);

b) Evaluating the nature and validity of the available data in terms of:

- the relevance of the effects and exposure data, particularly for i) effects endpoints that have been measured and the relevance of that endpoint to the effects of potential endocrine disruption mechanisms and ii) the fate and behaviour of substances in the environment (that is **Data Relevance**)

- the quality, reliability and reproducibility of a particular study and its protocol, together with the extent of peer review (that is **Study Validity**)

c) Evaluating the significance of the data based on assessments of the Data Relevance and Study Validity (that is **Data Weighting**);

2. Assessing the implications of the dataset in terms of whether there is sufficient robust information to draw conclusions on the nature and extent of endocrine disruption in humans and/or wildlife (based on a weight of evidence approach), and (if not) what further evidence is required to be able to draw conclusions (that is **Data Implications**).

The approach proposed is consistent with that advocated by Assmuth and Louekari (2001) in relation to the BKH report which stated that “in order to devise efficient procedures for the identification of endocrine disrupters, analyses are needed of how those already on the lists have been identified and what factors are to be taken into consideration in developing efficient risk identification systems. It is also consistent with the conclusions of the CSTEE on the overall list derived in the BKH report from lists of other groups in that “ *the compilation of such lists has been perfomed with varying levels of scientific input and competence, so that the use of these lists for priority setting and further risk assessment must be done with considerable caution*”.

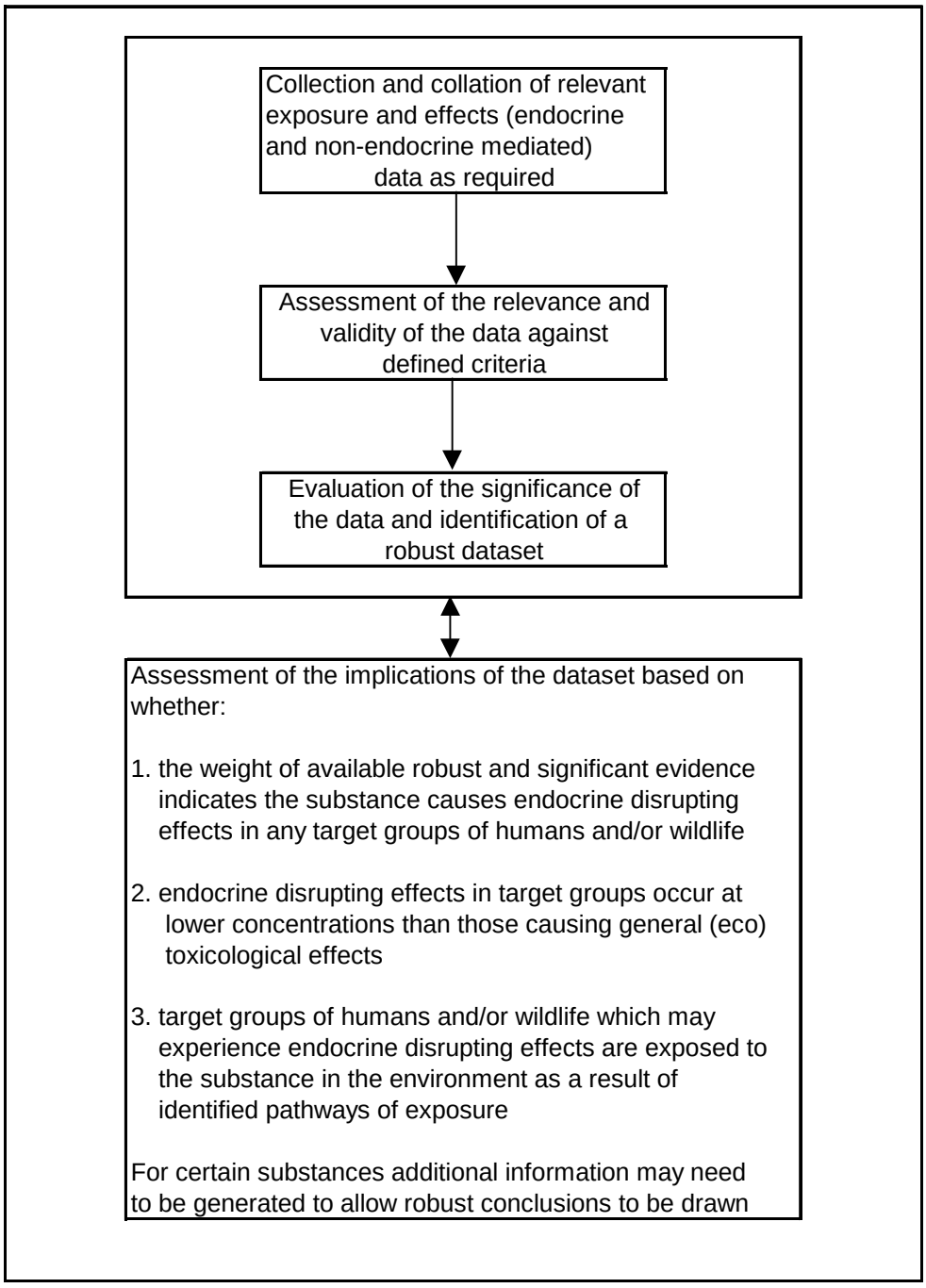


Figure 2.1 Summary of the framework adopted to review identified substances

The derived framework is criteria-based (where possible) so that decisions are made in an objective rather than a subjective manner. However, expert judgement is required at each stage and it is important to record the basis of decisions to aid transparency.

In the assessment of endocrine disruption in humans and wildlife the question of 'proof' of adverse effects and of underlying causative factors is crucial both for scientific inference and for guiding opinion forming and decision making. In the development of the framework a weight of evidence approach is advocated which builds on the document prepared by CEFIC-EMSG "Towards the establishment of a weight of evidence approach to prioritising action in relation to endocrine disruption". The strategy is also consistent with:

- Assmuth and Louekeri (2001) which states that "the proof of adverse effects usually requires a weight of evidence approach combining information on exposures, mechanisms, surrogate animal responses and epidemiological effects and confounding factors, for all the potentially causative substances or other agents";

- the Report on "Hormonally Active Agents in the Environment" prepared by the Committee on Hormonally Active Agents in the Environment of the United States National Research Council (NRC 1999).

- The recent IPCS Global Assessment of the State-of-the-Science of Endocrine Disruptors (IPCS 2002) which states that "a collective weight of evidence is essential in determining under what conditions observed effects resulting from exposure to EDCs occur via endocrine mediated responses".

2.2 Identification of a robust dataset

2.2.1 Data collection and collation

In the review of a substance it is important to obtain all relevant effects and exposure data which is available from the published literature and also the 'grey' literature (particularly where published data is limited). Table 2.1 summarises the commercially available databases which were searched and also the search strings which were used which included (the substances name, endocrine disruption, oestrogen, androgen, thyroid, toxicity, ecotoxicity, fate, environmental concentrations).

Table 2.1 Sources searched for information on the 12 substances

On line searches	British Library on line Toxline OVID including Agricola, BIOSIS, CAB Abstracts, CSA – Aquatic Science and Fisheries, Environmental Science and Pollution Management and Life Sciences Environmental Toxicology and Chemistry online
Specific Web sites	European Commission European Environment Agency Organisation for Economic Cooperation and Development RIVM United States Environmental Protection Agency
CD Roms	IUCLID (2000) Current Contents – Agriculture, Biology and Environmental Sciences and Life Sciences

Relevant grey literature was obtained by consultation with the relevant sector groups of CEFIC responsible for the different chemicals in Table 1.1 for exposure and effects data and by contacting the European Environment Agency for environmental monitoring data.

A large body of the available data on endocrine disrupting effects has focused on the adverse effects of synthetic chemicals which may result from oestrogenic or anti-oestrogenic mechanisms. However, there are a number of other mechanisms by which substances may exert an effect on the endocrine system of a target group of organisms and all these potential mechanisms of action need to be considered. Therefore, the review for each substance has also sought information on adverse effects which may result from androgenic, anti-androgenic, thyroid and anti-thyroid and adrenal responses.

Endocrine disrupting effects on humans are assessed based on data from:

- studies of workers exposed to the substance during production or use and/or consumers exposed to the substance via products.

- laboratory studies using whole male and female rats, mice, guinea pig and monkeys as human models (whilst recognising the issues associated with the extrapolation between species[2]) or isolated sub-cellular, cellular or tissue preparations;

In the evaluation of the human health effects of a substance four major types of human data may be available:

1. *analytical epidemiological studies* on exposed populations which examine relationships (associations or causal links) between human exposure and effects (such as biological effect markers, early signs of chronic effects, disease occurrence or mortality). Study designs include:

- case-control (case-referent) studies where a group of individuals with (cases) and without (controls/referents) a particular effect are identified and compared to determine differences in exposure;

- cohort studies where groups of “non-exposed” and “exposed” individuals are identified and differences in effect occurrence are studied;

- cross-sectional studies, where a population (for example a workforce) is studied, so that morbidity at any given point in time can be assessed in relation to concurrent exposure.

2. *descriptive or correlation epidemiological studies* which examine differences in disease rates among human populations in relation to age, gender, race and differences in temporal or environmental conditions;

[2] The difficulty of extrapolating between species is compounded because different processes behave differently with respect to inter-species differences. There is a high degree of genetic conversation between taxa and genes and gene products governing pattern formation are structurally similar and appear to operate similarly even in evolutionary distant species (Tomarev *et al* 1997). In contrast, differences between different strains of a species, which may result from differences of only genetic locus, can have marked effects on responses to toxicants.

3. *controlled studies in human volunteers* which assess exposure levels associated with acute effects, for example human patch tests for dermal irritation studies;

4. *case reports* which describe a particular effect in an individual or group of individuals who have been exposed to a substance.

Endocrine disrupting effects on wildlife are assessed based on data from:

- studies of populations and/or communities exposed to the substances under semi-field or field conditions;

- laboratory studies using whole mammals, birds, reptiles, amphibians, fish or invertebrates or isolated sub-cellular, cellular or tissue preparations.

Generally the data which is available for the 12 substances is largely derived from whole organism studies on groups of mammals, and fish and to a lesser degree for birds, amphibians and reptiles. Information on the largest taxonomic group, the invertebrates, is limited due to the diversity of endocrine systems between taxa and the absence of available validated test methods which are relevant to the assessment of potential endocrine disrupting effects (EC 2001). However, to obtain a robust assessment of the potential endocrine disrupting effects of a substance it is necessary to have reliable data from a range of taxonomic groups within a particular environmental compartment (for example invertebrates, fish and amphibians within the aquatic ecosystem). This is important in the assessment of the potential risk of endocrine disrupting effects in an environmental compartment as data will probably not be available on the potential population or community level effects resulting from endocrine disruption.

It has to be recognised that the data available from the laboratory studies will generally be for species which have been selected on the basis of their practicality for toxicity testing. This will mean that the species often show short life cycles (thereby reducing the timescales and costs of multi-generation studies) and data on endocrine disrupting effects will typically not be available for long lived species. It also needs to be recognised that the review of each of the substances in Table 1.1 will by definition have to be carried out using existing data. This may mean that individual studies examining effects on reproduction and/or development (even if carried out to OECD procedures) may not always include endpoints which provide definitive data on endocrine disruption. As a result further specific information may be needed to reduce the uncertainty of whether a substance causes endocrine disruption in target groups of humans and/or wildlife. In addition the assessment of adverse effects on wildlife is complicated by the limited information on what constitutes normal endocrine function for many species, particularly invertebrates.

2.2.2 Assessment of the relevance of the data

Endocrine disrupters can act directly at a specific receptor or indirectly and their effects on a range of physiological systems (including metabolism, immunity and behaviour) can vary depending on the target tissue, the timing of exposure and interactions with other endocrine disrupters (particularly endogenous substances). However, because endocrine disrupters can mimic or modulate the activity of endogenous hormones it can be difficult to distinguish altered responses from the range of normal background hormone regulated responses (under non-exposed conditions).

When developing criteria to assess the relevance of data on endocrine disruption generated from human and wildlife studies it also has to be recognised that chemicals can cause sub-lethal effects (such as reproduction, development and growth) and lethality in target groups through a variety of mechanisms of toxic action with endocrine disruption being one type. In this respect the CSTEE has considered it important to realise that "*endocrine disruption is not a toxicological endpoint per se as is cancer or allergy but that it is a descriptor for a functional change that may lead to adverse health effects*". The US EPA also does not view endocrine disruption to be an endpoint *per se* but rather a mode of action that potentially would lead to other outcomes for example carcinogenic, reproductive, developmental, neurotoxic or immunological properties which are routinely evaluated toxicologically and considered in reaching regulatory decisions. Therefore, in the review of substances the International Programme of Chemical Safety definition has been used (see Section 1.2) such that ""*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*"[3].

From the IPCS definition it is evident that endocrine disrupting activity can only be adequately defined in terms of effects in intact animals of the parental or subsequent generations. It is also necessary to demonstrate that a response is truely adverse (in terms of physical, developmental and reproductive capability in humans and wildlife) and is not a transient fluctuation within the normal background range for the response which has no biological significance. Finally it is necessary to distinguish effects that arise as a consequence of primary disruption of the endocrine system from those occurring secondary to overt toxicity in other organs or systems.

Assessing the relevance of human studies

Table 2.2 summarises the strengths and limitations of different types of human data and their usefulness for hazard/risk assessment. For the purposes of the strategy it can be seen that the most valuable type of data is from analytical epidemiological studies and to a lesser degree controlled studies in human volunteers and case reports.

[3] The US EPA definition is that an endocrine disrupter is an exogenous chemical substance or mixture that alters the function(s) of the endocrine system and thereby causes adverse effects to an organism, its progeny, or (sub)populations

Table 2.2 Summary of the strengths and limitations and usefulness of different types of human data (after EC 1994)

Type of data	Strengths and limitations	Usefulness for hazard/risk evaluation
Analytical epidemiological studies	The strength of these epidemiological studies for specific health effects depends on range of factors including the type of analyses and on the magnitude and specificity of the response. Confidence in the findings is increased when comparable results are obtained in several independent studies on populations exposed to the same agent under different conditions and using different study designs	High
Descriptive epidemiological studies	These studies can only identify patterns or trends in disease occurrence over time or in different geographical locations but cannot ascertain the causal agent or degree of human exposure	Low
Controlled human exposure studies	Few human experimental toxicity studies are available due to the practical and ethical considerations involved in deliberate exposure of individuals. These studies are often limited by a relatively small number of subjects, short duration of exposure and low dose levels resulting in poor sensitivity in detecting effects	Moderate
Case reports	These studies are particularly relevant when they demonstrate effects which cannot be observed in laboratory mammalian studies	Moderate

Assessing the relevance of *in vivo* and *in vitro* studies

There are various laboratory-based *in vivo* and *in vitro* methods utilising a range of (eco)toxicological endpoints that are claimed by different sources to be relevant to the assessment of endocrine disruption in humans and wildlife. However, since this field is still in an early stage of development there is uncertainty regarding the significance of many of the current findings.

From the numerous recent reviews of potential test methods (such as the Detailed Review Paper prepared by OECD in 1997) there is a clear consensus in terms of the hierarchy of the relevance of test methods. In this hierarchy longer-term *in vivo* studies considering effects on reproduction and/or development (and including mechanistic information) are of greater relevance than short-term *in vivo* screening tests which are of greater relevance than *in vitro* assays. The greater relevance of chronic *in vivo* tests or those assessing effects during critical windows of sensitivity is also evidenced by the fact that these are the key (eco) toxicological methods being developed in the OECD Endocrine Disruption Testing and Assessment (EDTA) Programme. The mechanistic limitations of *in vitro* methods were discussed in detail in the OECD Detailed Review Paper (OECD 1997) and other reports (CSTEE 1999, NRC 1999) and relate principally to the following factors:

- *in vitro* methods are dependent on specific receptors or response elements which may or may not act similarly as those *in vivo*. Futhermore, in whole organisms factors such as absorption, metabolism and bioaccumulation, influence the outcome of studies and these factors are not relevant in *in vitro* assays.

- *in vitro* systems are based on known specific receptors and cannot address other receptors and mechanisms.

In vivo tests

At present the OECD has an on going process to develop and implement a suite of validated *in vivo* methods to screen for or monitor exposure to substances in mammals and non-mammals (birds, reptiles, amphibians and fish) that could cause adverse effects on endocrine activity (EC 2001). However, most of the data that is available on the substances of interest comes from currently available tests and a major question that arises is whether the endpoints examined in routine toxicological studies are suitable to detect adverse effects mediated by endocrine disrupters. It is recognised that current OECD tests used to provide data for regulatory purposes are not specifically designed to detect endocrine disrupting chemicals. However, when a battery of these tests probing a variety of toxicological endpoints in mammals are conducted (including effects on fertility, pregnancy, general reproductive performance and sexual behaviour as well as peri- and post-natal development and sexual maturation) these data, when properly interpreted are more than adequate to identify endocrine effects caused by chemicals possessing hormonal activity and/or interference (Cockburn and Leist 1999). Furthermore, Stevens *et al* (1997a,b) have postulated that until there is clear evidence to the contrary it is scientifically sound to assess and evaluate effects caused via an endocrine disruptive mechanism in the same way as adverse effects caused by different modes of action.

For the hazard characterisation of endocrine disrupting chemicals in humans and wildlife a survey of 16 OECD Member Countries considered that the most relevant endpoints were:

- morphological and functional effects in the reproductive system of mammalian target species;
- effects on sex-linked hormone levels in mammalian target species;
- morphological effects in the reproductive system of aquatic target species;
- morphological effects in the reproductive system of terrestrial target species.

It was recognised that, where necessary, existing guidelines and protocols will be modified and new guidelines would be introduced if they were considered to be necessary

As a result the current OECD mammalian toxicology protocols that relate particularly to endocrine disrupters are the developmental (teratogenicity) toxicity (OECD TG 414) test and one and two-generation reproduction tests (OECD TG 415 and TG 416). The developmental test provides information on the potential hazard to the unborn that may arise from exposure of the mother during pregnancy. However, the ability to detect effects on sexual differentiation is rather limited unless really profound endocrine disruption has occurred. There is only a gross external and internal morphological examination of the offspring that cannot detect subtleties in their intra-uterine development that is sensitive to an altered hormonal environment.

The one and two generation reproduction toxicity tests provide information concerning the effects of a test substance on male and female reproductive performance. The one generation reproductive test assesses effects on gonadal function, oestrous cycling, mating behaviour, conception, parturition, lactation and weaning while the two generation reproduction test assess the effects on all the above parameters as well as the growth and development of the offspring. The tests may also provide preliminary information about developmental toxic effects of the test substance such as neonatal morbidity, mortality, behaviour and teratogenesis. These tests are not designed to determine specific cause and effects in all cases. Response endpoints monitored in the one and two generation mammalian

reproduction studies include: fertility, litter size, and litter weight and survival and growth of the offspring. All of these responses can be altered by exposure to endocrine disrupters. Other end points, such as vaginal cyclicity, reproductive-organ weight, gonadal morphology, accessory sex-organ weight, sperm count, and anogenital distance could be affected by endocrine disrupters, but the changes are not necessarily specific to any particular hormone or portion of the endocrine system.

In birds and fish the avian reproduction test (OECD TG 206) and early life stage test (OECD TG 210) are the most appropriate standardised methods currently available for assessing endocrine disrupting effects (see Table 2.3). Other current methodologies used to assess effects on reproduction and/or development of invertebrates (*Daphnia magna* reproduction test - OECD TG 211) may not be appropriate for assessing potential endocrine mediated effects. Indeed in invertebrates there is limited knowledge of the endocrinology of many taxonomic groups and it uncertain whether reproductive processes are modulated by oestrogens or androgens.

Other types of *in vivo* mammalian studies, including shorter term screening assays such as the Uterotrophic assay (measuring the weight of the uterus in ovariectomised or immature rodents) and the Hersberger assay (measuring musculus levator ani muscle weight in castrated rodents) also provide relevant information, and data from such studies should be included in the any weight of evidence review. The Reproduction/Developmental Toxicity Screening Test (OECD TG 421) does not provide complete information on all aspects of reproduction and development. In particular it offers only limited means of detecting post-natal effects of pre-natal exposure, or effects that may be induced during post-natal exposure. Small sample sizes and early post-natal termination of the test (day 4 of postnatal life) are the limiting problem. However, it should be remembered that the positive results in screening assays are not conclusive evidence of adverse health effects and are of lower relevance than longer-term *in vivo* studies in making a judgement about endocrine disruption. Nevertheless shorter term *in vivo* screening assays (whether for mammals or non-mammals) do provide useful data by indicating whether or not there is potential for adverse effects.

Within the *in vivo* tests changes in levels of hormone and/or biomarkers may also be measured. Changes in these parameters should be irreversible during the exposure period to be of relevance. For the biomarkers it is also important to distinguish (if possible) between indicators of exposure and indicators of effect. Indicators of effects are taken to be those parameters which can be linked to higher level anatomical or pathological changes.

In the ECETOC Monograph on Guidance on Evaluation of Reproductive Toxicity Data (ECETOC 2002) the issue of endocrine disruption was considered as an emerging issue. It was stated that "*Endocrine disruption can be detected in a number of suitably adapted 'routine' reproductive toxicity assays. This is achieved by the incorporation of endpoints that are under hormonal control, and thus sensitive to disturbance by endocrine disrupters, and some amendments to the overall protocol where appropriate. Such endpoints include the age of sexual maturation of offspring (for example preputial separation in males and vaginal opening in females), disturbance of sexual differentiation (for example anogenital distance), sperm parameters (for example number, morphology, motility), circulating hormone levels and regularity and duration of the oestrous cyclicity, as well as more conventional endpoints such as histopathology and weights of organs of the reproductive tract. However, it must be borne in mind that many of these endpoints are not specifically indicative of an endocrine mediated mechanism of toxicity per se, but may also be influenced by overall growth and health status of the animal, or by toxicants that influence homeostasis through other mechanisms. For example, the oestrous cycle is perturbed in cases of bodyweight reduction, and reduced*

sperm counts may be indicative of a male germ cell cytotoxicant. Consequently, these endpoints must be interpreted with reference to other endocrine-sensitive endpoints and to additional observations on growth and histopathology. They are most useful as a measure of effect rather than a mechanism of toxicity".

In vitro tests

In vitro tests can be reliable for detecting potential endocrine modulating activity *per se* and therefore are a useful tool in the overall context of endocrine toxicity testing, particularly for discerning mechanisms of action. A number of *in vitro* screening systems are available which involve the interaction of chemicals with vertebrate steroid receptors. Although the number of *in vitro* assays for taxa other than mammals is limited (mainly using fish cells and tissues), receptors, such as for oestrogen, androgen, and thyroid, and their essential roles may for certain responses be conserved across vertebrates. In contrast, the endocrine systems of invertebrates are poorly understood.

The data collection and collation exercise aims to incorporate all available *in vitro* data, and for the purposes of assessing the relevance of *in vitro* endpoints, attention has focused on both a hierarchy of information and the quality of the particular measurement system, namely

- whether the assay is designed to indicate simple receptor binding potential or the more indicative receptor binding coupled with transcriptional activation;
- whether the assay is a cellular or sub-cellular assay, which would be indicative of whether or not the endocrine receptor was likely to be exposed to metabolites of the parent compound;
- whether the assay examines relevant endocrine parameters such as steroid metabolism.

Summary

Table 2.4 provides an indication of the relevance to endocrine disruption in humans and wildlife of different types of *in vivo* and *in vitro* test data. It should be noted that the hierarchy is solely for the relevance of the endpoint, and is not indicative of the final weighting applied to the result which is described in Section 2.2.4.

2.2.3 Assessment of the validity of studies

In order that robust data is used in the assessment of the endocrine and non-endocrine mediated eco(toxicological) effects in humans and wildlife it is necessary to evaluate the validity of each study in terms of the extent to which the study and the results can be reproduced in another laboratory. Traditionally, (eco)toxicological studies carried out to provide data for regulatory purposes (for example for Classification, Labelling and Packaging under Directive 67/548/EEC) have been performed to internationally recognised and validated standard protocols (for example OECD Test Guidelines) and under a quality assurance/control scheme (such as Good Laboratory Practice). Such studies can then typically be repeated with a high level of confidence. Evaluation of protocols for the determination of endocrine disruption is difficult, since a suite of standard protocols are not currently available to evaluate for this specific mechanism but are under development.

Table 2.3 Summary of potential for current OECD test methods for assessing endocrine disrupting effects

Description of test	OECD TG Number (US EPA Guideline)	Endocrine related endpoints and parameters	Status of test endpoints
Mammalian studies			
Sub-chronic oral toxicity study	408/409 (82-1)	Morphology and weight of gonads and accessory organs	Not definitive
Developmental (teratogenicity) toxicity study	414 (83-3)	Effects on organogenesis, malformations, sexual differentiation and sex ratios of foetuses	Not definitive[1]
One generation reproduction toxicity study	415 (-)	Duration of gestation in P generation adults, number and sex of pups, stillbirths, live births and the presence of gross abnormalities of F_1 generation. growth (as weight) of F_1 offspring, gross necropsy (with special reference to the reproductive organs) and, if necessary, histopathology of P generation adults and dead or moribund pups	Not definitive
Two generation reproduction toxicity study	416 (83-4)	Duration of gestation in P and F_1 generation adults, number and sex of pups, stillbirths, live births and the presence of gross abnormalities of F_1 generation. growth (as weight) of F_1 and F_2 offspring, gross necropsy (with special reference to the reproductive organs) and, if necessary, histopathology of P and F_1 generation adults and dead or moribund pups, effects on sperm count, sperm motility and sperm morphology of P and F_1 generation males	Definitive
Reproduction/development toxicity screening test	421 (-)	Macroscopic abnormalities of reproductive organs, gross abnormalities in pups, weight of testis and epididymids	Not definitive

1 - Changes to the protocol adopted by the OECD have made the test more likely to detect the potential of a chemical to perturb endocrine homeostatis in dams and foetuses.

Table 2.3 Continued

Description of test	OECD TG Number	Endocrine related endpoints and parameters	Relevance of test endpoints
Non-mammalian studies			
Avian reproduction test	206	Egg production, eggs set, viability, hatchability (including normal hatchlings), survival of young, egg shell thickness, body weights of young (at 14 days of age), food consumption of the young (first and second week after hatching) and gross pathological examination of all adult birds	Definitive
Fish, Early life stage toxicity test	210	Hatching of fertilised eggs, abnormal appearance and behaviour of hatchlings, survival of hatchlings, growth of hatchlings to free feeding stage (based on measurements of weight and length)	Not definitive

Table 2.4 Relevance of mammalian and non-mammalian *in vivo* and *in vitro* assays for the assessment of endocrine disrupting effects

Status	Type of mammalian or non-mammalian assays	
	In vivo	*In vitro*
High relevance	• Endpoints[1] in mammalian or non-mammalian multi-generation tests that are specifically controlled by the endocrine system • Endpoints[1] in targeted repeat dose mammalian (28-90 day) or non-mammalian toxicology tests that are specifically controlled by the endocrine system • Endpoints[1] in mammalian or non-mammalian tests covering exposure during critical windows of sensitivity in the life cycle that are specifically controlled by the endocrine system	
Medium relevance	• Endpoints[2] in mammalian or non-mammalian multi-generation tests, which may be influenced by the endocrine system, but are also potentially affected by non-endocrine factors (for example other mechanisms of action) • Endpoints[2] in targeted repeat dose mammalian (28-90 day) or non-mammalian toxicology tests which may be influenced by the endocrine system but are also potentially affected by non-endocrine factors (for example other mechanisms of action) • Endpoints[2] in mammalian or non-mammalian tests covering exposure during critical windows of sensitivity in the life cycle which may be influenced by the endocrine system but are also potentially affected by non-endocrine factors (for example other mechanisms of action) • Endpoints[2] from short-term or screening assays specifically controlled by the endocrine system	• Endpoint is based upon receptor binding potential, coupled with transcriptional activation, in a whole cell or sub-cellular assay • Receptor binding potential in a whole cell assay • Assessment of steroid metabolism in a whole cell assay
Low relevance	• Endpoints in tests not controlled by the endocrine system (However, positive results of this type are important in the overall consideration of adverse effects) • Irreversible changes in biomarkers that have not been linked to higher level anatomical and/or physiological responses	• Endpoint is based on receptor binding activity in a sub-cellular assay • Endpoint is based on cell growth or other endpoint not a direct measurement of receptor mediated activity • Endpoint of steroid metabolism in a sub-cellular assay

1 – Including irreversible changes in hormone levels or biomarkers which are accompanied by presence of consequent toxicological effects

2 – Including irreversible changes in hormone levels or biomarkers (that have been linked to higher level anatomical and/or physiological responses) in the absence of any toxicological effects

Other, perhaps more novel protocols may produce endocrine-specific information, but their reliability needs careful evaluation. Proposed criteria for reported data are listed in Tables 2.5 (human epidemiological studies), 2.6 (*in vivo* studies) and 2.7 (*in vitro* studies), and have been selected as criteria which are indicative of work which has been undertaken to a good standard of scientific practice.

Table 2.5 General requirements for the validity of human studies

Type of study	Criteria
Epidemiological studies	• Proper selection and characterisation of the control and exposed groups • Adequate characterisation of exposure • Sufficient length of follow-up for disease occurrence • Valid ascertainment of effect(s) • Proper consideration of bias and confounding factors • Reasonable statistical power to detect effects
Controlled human exposure studies	• Use of a double-blind study design • Inclusion of a matched control group and an adequate number of subjects to detect an effect

Note: If the above conditions are satisfied a study can usually be considered valid. However, these criteria represent an outline framework for evaluation of data validity and each study needs to be evaluated on case-by-case basis

Table 2.6 General requirements for the validity of *in vivo* laboratory studies

Element of study	Criteria
Basic experimental design	• Top dose should be a maximum tolerated dose level for mammalian tests • There should be a minimum of two (usually three) test concentrations for mammalian studies, and typically 3 to 5 concentrations in non-mammalian studies, ideally with one at a concentration expected to cause no effects • Suitable controls should be included as well as the test concentrations, including a carrier control if a carrier solvent is used in the tests o All controls and treatments should preferably be replicated for screening assays (necessity of this requirement may be assessed based upon complexity of the experiment, and may be considered extraneous, based upon expert judgement) • Toxicity to the intact organism (animal) and any organ being used as an endpoint should be assessed o Data relevant to test validity criteria should be measured and reported (for example in aquatic studies, the type of water, pH, dissolved oxygen, temperature and preferably hardness) • Analysis of diet(s) for potentially relevant contaminants in oral studies should be conducted
Exposure regime	• Purity and source of test material should be specified • Confidence limits for (chemical) analytical techniques should be verifiably assessed and taken into account during analysis of results o Exposure concentrations should be analysed o Test concentrations should be maintained at reasonably constant levels. o Flow-through aquatic studies are usually better at maintaining test concentrations than static studies due to the regular replenishment of test substance(s)
Test organisms	• The stocking density, or animal numbers, should be appropriate o The test should incorporate an appropriate feeding regime (where necessary) o Extraneous sources of stress should be minimised (such as noise, lighting and vibrations) • The test organism should be defined, and of suitable age, sex and health o Use of incompatible materials in the test apparatus should be avoided. (If concentrations are analysed and control mortalities reported, this becomes less important).
Analysis of results	• Results should be analysed in the context of both concurrent and historical control data. o Ideally the results should show a dose or concentration dependent effect and the results should be analysed for confidence limits or statistical significance.

Note: Tests meeting all the above criteria will be considered valid. Tests meeting the criteria with bolded bullet points only will normally be assigned a use with care status. All other tests merit non-valid status.

Table 2.7 General requirement for the validity of *in vitro* laboratory studies

Element of study	Criteria
Basic experimental design	• There should be a minimum of three (usually five) test concentrations, ideally with one at a concentration expected to cause no response o Intervals between test concentrations should be less than one order of magnitude • Suitable controls should be included as well as the test concentrations, including a carrier control if a carrier solvent if used in the tests • All controls and treatments should be replicated • Top dose should show slight cytotoxicity
Measured concentrations	-
Other aspects of test procedure	• Source and/or purity of test material should be specified • Confidence limits for (chemical) analytical techniques should be verifiably assessed and taken into account during analysis of results.
Analysis of results	o For a positive response, the results should normally show a concentration dependent response • Results should be analysed for confidence limits or statistical significance, and data presented to allow verification

Note: Tests meeting all the above criteria will be considered valid. Tests meeting the criteria with bolded bullet points only will normally be assigned a use with care status. All other tests merit non-valid status.

It is proposed that tests carried out in accordance with these criteria form a suitable basis for assessing the validity of studies. On this basis, it is proposed that the hierarchy for study validity should be ranked as follows in Table 2.8.

An assessment of the validity of a study takes into account:

- The extent to which protocols have been validated and the limits within which conclusions can be drawn
- The extent to which the (eco)toxicological endpoints are understood
- Basic experimental design including the adequacy of controls and suitability of dose or concentration ranges
- Exposure data including the purity of the test material and the extent of the verification of exposure doses or concentrations
- Test species including their suitability, general health and environmental conditions
- Analysis of results including an assessment of the statistical validity of observed effects
- Transparency of the study report

Table 2.8 Hierarchy of validity of study data

Status	Type of data
Valid	**All** criteria for the experimental design and conditions, and for reporting transparency are met - *Full details of experimental method are available and these indicate that studies have been carried out to an acceptable standard*
Use with care	The **main** criteria for the experimental design and conditions, and for reporting transparency are met - *Key details of the experimental method are available which indicate that studies have been carried out to an acceptable standard*
Not valid	Insufficient information is available for the experimental design and conditions to reproduce the studies with any degree of certainty. Alternatively, the reported methodology gives rise to serious uncertainty in the interpretation of results

2.2.4 Assessment of the significance of the data

Establishing the 'weight' that should be ascribed to any set of data takes into account both the 'Relevance' and 'Validity' of the data as evaluated in Sections 2.2.2 and 2.2.3. In effect, the 'weight' is measured as the level of significance that can be ascribed to a data set in reaching conclusions about endocrine disruption (see Table 2.9). In the framework four levels of significance have been defined:

- Definitive
- Indicative
- Low
- Unusable

Within the evaluation framework it is only possible to assess a substance as an endocrine disrupter if there is data on laboratory-based long-term *in vivo* assays (such as multi-generational reproduction and development tests and targeted repeat-dose studies) or studies which address exposure during critical widows of sensitivity and the approach has been designed to reflect this with such data being of **"Definitive Significance**".

For non-standard protocol endpoints, the assessment of endpoint relevance has been a subjective decision which was based on sound expert judgement. If such a judgement proved impossible then the data are treated as being of 'low significance' until such time that additional research is able to clarify the relevance of the data.

While robust *in vivo* screening studies and certain *in vitro* data are useful in making judgements about the presumption of hazard they are not currently linked directly to, or are predictive of adverse/toxicological effects associated with endocrine disruption. Consequently such results can only be considered as being of "**Indicative Significance**".

In the evaluation framework only data of definitive and indicative significance are used, data which are of low significance and those deemed unusable are not considered. In all instances, the relevance rating given to data has been clearly documented.

Table 2.9 Assessing the significance of data on endocrine effects

Significance of data	Requirements
Definitive	• Data from mammalian or non-mammalian *in vivo* tests of high relevance which are valid • Data from (eco)epidemiological studies of high relevance which are valid
Indicative	• Data from mammalian or non-mammalian *in vivo* tests of high relevance which need to be used with care • Data from mammalian or non-mammalian *in vivo* tests of medium relevance which are valid • Data from (eco)epidemiological studies of high relevance which need to be used with care • Data from (eco)epidemiological studies of medium relevance which are valid • Data from *in vitro* tests of medium relevance which are valid
Low	• Data from mammalian or non-mammalian *in vivo* tests of medium relevance which need to be used with care • Data from mammalian or non-mammalian *in vivo* tests of low relevance which are valid • Data from (eco)epidemiological studies of medium relevance which need to be used with care • Data from *in vitro* tests of medium relevance which need to be used with care • Data from *in vitro* tests of low relevance which are valid
Unusable	• Data from mammalian or non-mammalian *in vivo* tests and *in vitro* tests of low relevance which need to be used with care

2.3 Assessment of the implications of the dataset

2.3.1 Approach

Having identified a robust dataset for a substance (as described in Section 2.2) the next key element of the framework is to assess the consequences of the available information. In the evaluation framework the dataset for each substance is used to assess whether:

- the weight of evidence for a substance indicates endocrine disrupting effects occur in target groups of humans and/or wildlife;

- endocrine disrupting effects occur at lower concentrations of the substance than those causing general non-endocrine mediated (eco)toxicological effects in the target groups;

- the target groups of humans and/or wildlife are likely to be exposed to the substance in the environment at doses/concentrations capable of causing endocrine disrupting effects.

In the strategy it is also important to consider:

3. Is there sufficient data of definitive significance available to draw robust and meaningful conclusions on the extent to which a substance causes or has the potential to cause endocrine disruption effects in target groups of humans and/or wildlife at levels below general non-endocrine (eco)toxicological responses?;

4. What additional information is needed to allow robust and meaningful conclusions to be drawn if there is currently insufficient data for a substance?.

2.3.2 Evidence of endocrine disrupting effects in target groups of humans and wildlife

Approach to consideration of evidence

The available data for the endocrine disrupting effects of each substance is separated into that for two human target groups (workers and consumers) and three wildlife target groups (aquatic, terrestrial and aerial) and further sub-divided based on the level of significance (that is definitive or indicative).

In each laboratory-based *in vivo* study an assessment is made (where data are available) of:

1. nature of the dose or concentration response curves and whether this indicates a transition from no effect to effects for a particular endpoint over a small dose or concentration range.

2. the magnitude of changes in endpoints which are statistically significant in terms of the magnitude of changes in the endpoint which can be expected in control animals (that is normal background variability). This is important so that the biological significance of the changes in the endpoint can be considered.

3. the nature of the potency of the substance in terms of the extent of the effects observed relative to those for a mechanistic standard appropriate to the mode of action of the substance.

For the evaluations of the potential of a substance to cause endocrine disrupting effects in a target group there needs to be data from a minimum of three studies of definitive significance to allow the weight of evidence approach to be used. In the assessment it is necessary to balance positive and negative results to obtain a weight of evidence view. Clearly the decision making process is more straight-forward if the data strongly point to evidence or absence of endocrine disrupting effects. However, in practice, many substances may show both positive and negative results and even the data of definitive significance may provide equivocal results. If the balance is neutral or close to neutral then it may be necessary to undertake additional high quality studies of definitive significance to draw definitive robust conclusions.

In the assessment indicative data alone can be used to identify a potential for endocrine disruption but more importantly to guide further testing when appropriate methods become available.

For evaluations of endocrine disrupting effects in humans where human data and mammalian studies are available well reported relevant human data for any given endpoint is normally given preference (EC 1994). However, the potential differences in sensitivity of human studies and laboratory studies in mammalian models should be taken into account on a case-by-case basis. Analytical epidemiological studies with negative results cannot prove the absence of intrinsic endocrine disrupting properties of a substance but well documented “negative” studies of good quality are extremely valuable.

The data for each target group of humans and wildlife is used (if possible) to:

1. Determine whether the weight of evidence indicate that the substance causes endocrine disrupting effects;

2. Determine the mode of action(s) by which the substance exerts its endocrine disrupting effects;

3. Identify the dose or concentration ranges over which endocrine disrupting effects are found and also the threshold level at which effects are evident. This involves defining ranges of No Observed Effect Levels (NOELs)/No Observed Adverse Effect Levels (NOAELs) or No Observed Effect Concentrations (NOECs) and the lowest relevant NOEL/NOAEL or NOEC for each target group. However, it is important to assess how the lowest value compares with the other data for a target group to ascertain whether it could represent an extremely low value which is atypical of the other values.

4. Determine the relative potency of the substance relative to an appropriate mechanistic standard(s) based on the mode of action(s) of the substance.

Consideration of low-dose effects

In recent years there has been considerable debate in the scientific community with regard to the evidence on low-dose effects and non-monotonic dose-response relationships for endocrine disrupting chemicals in mammalian species (see NRC 1999, EC 2001). At the request of the United States Environmental Protection Agency (US EPA) the National Toxicology Program organised an independent and open peer review to evaluate these issues, where "low-dose effects" referred to *biologic changes that occur in the range of human exposures or at doses lower than those typically used in the standard testing paradigm of the US EPA for evaluating reproductive and developmental toxicity* (Melnick *et al* 2002). The demonstration that an effect is adverse was not required because in many cases the long-term health consequences of altered endocrine function during development have not been fully characterised.

In the peer review process individual animal data was submitted by principal investigators of primary research groups active in this field and there was independent statistical re-analysis of selected parameters prior to the meeting by a sub-panel of statisticians. The expert peer review panel also considered mechanistic data that might influence the plausibility of low-dose effects and identified study designs or other biologic factors that might account for differences in reported outcomes among studies. The selected studies included treatments with bisphenol A, diethylstilbestrol, 17β-oestradiol, ethinyloestradiol, nonylphenol, octylphenol, genistein, methoxychlor and vinclozolin.

The overall conclusions of the peer review panel were that low dose effects, as defined above, were demonstrated in laboratory animals exposed to certain endocrine active agents. The effects are dependent on the compound studied and the endpoint(s) measured. In some cases where low dose effects have been reported, the findings have not been replicated. In addition the toxicological significance of many of these effects has not been determined. It was concluded that the shape of the dose-response curves for these effects varies with the end point and dosing regimen, and may be low-dose linear, threshold-appearing or non-monotonic.

The panel also recognised that the traditional mammalian multi-generation reproduction study protocol has not revealed major reproductive or developmental effects in laboratory animals exposed to endocrine-active agents at doses approaching their NOAELs set by the standard

testing paradigm. However, few multi-generation studies have been conducted over expanded dose ranges, and end points such as cancer of reproductive organs or neurobehavioural effects are generally not evaluated in multi-generation studies.

The panel recommended additional research to

- replicate previously reported key lose-dose findings
- characterise target tissue dosimetry during critical period of development
- identify sensitive molecular markers that would be useful in understanding mechanistic events associated with low-dose effects
- determine the long-term health consequences of low-dose effects of endocrine active agents

The findings of the panel indicated that the current testing paradigm used for the assessment of reproductive and developmental toxicity should be revisited to identify whether changes are needed regarding dose selection, animal model selection, age when animals are evaluated and the endpoints being measured following exposure to endocrine active agents. As a result the available data for the 12 substances may not be sufficiently robust to be able to define whether low dose effects are an issue.

2.3.3 Comparison of endocrine disrupting effects and general (eco)toxicological effects in target groups of humans and wildlife

In certain instances a substance is recognised to cause what are apparently, endocrine derived adverse effects at high doses or concentrations, while other toxic effects are detectable at lower doses/concentrations. It is these non-endocrine related effects that determine a substances toxicity, and consequently, are used in any decision making process. Indeed, the apparent endocrine effects may be merely a secondary effect of other toxic assaults (for example liver damage).

For comparison with data on endocrine disrupting effects of a substance, available data on general (eco) toxicological effects in target groups of humans (workers and consumers), laboratory mammals and wildlife (aquatic, terrestrial and aerial species) of the type required for the risk assessment of an existing substance has been collated. This data is quality assessed using the procedures defined in the Technical Guidance Document (EC 1994) and used to identify ranges of acute and chronic effects as well as the lowest “No Observed (Adverse) Effect Level” in a target group. For industrial substances this information has been obtained from existing data collations such as IUCLID files, CICADs and SIARs.

In the comparison for each target group the ranges of data (where data are available) are examined as well as the lowest values. For both the endocrine disrupting effects and general (eco)toxicological effects it is important to assess how the lowest identified value compares with the other data to ascertain whether it could represent an extremely low value which is atypical of the other values.

The comparison also needs to take into account potential issues of interspecies variability where, for example, the lowest value for endocrine disrupting effects in aquatic organisms

occurs in fish (with no data for invertebrates) whereas general ecotoxicological effects are most pronounced in invertebrates with fish being of reduced sensitivity.

In drawing conclusions on how the nature of endocrine disrupting effects for a target group compare against general (eco)toxicological effects comparison of lowest values for each data type are used.

Interpretation of the ratio for a substance is carried out on a case by case basis.

2.3.4 Assessment of whether any target group of humans and wildlife which exhibit endocrine disrupting effects at lower threshold concentrations than general (eco)toxicological effects are likely to be exposed to these threshold concentrations

The most relevant information on concentrations of identified substances to which humans (workers and consumers) or wildlife (aquatic, terrestrial or aerial species) are exposed is that obtained directly by the collection and chemical analysis of environmental samples (water, sediment soil and air) or tissue samples from humans (adipose tissue, blood, breast milk and urine) and tissues of aquatic, terrestrial and aerial invertebrates, fish, birds and mammals. However, for such data to be relevant it is vital that;

1. environmental samples are taken in the appropriate compartment (aquatic, terrestrial and aerial) based on the physico-chemical properties of the substance (for example water solubility, octanol water partition coefficient, organic carbon water partition coefficient and vapour pressure) and its environmental partitioning;

2. the time at which samples are collected coincides with any specific periods at which target groups of wildlife which are most susceptible to any endocrine disrupting effects of a substance. This usually requires a time series of data to be collected.

For substances where industrial, consumer or environmental monitoring data is not available it is may be necessary to estimate concentrations to which humans and wildlife may be exposed using appropriate modelling techniques.

Table 2.10 summarises the type of exposure data required for this element of the framework and this clearly requires an input from industrial producers and users of substances as well as regulatory agencies.

Table 2.10 Summary of the type of data required to assess exposure of target groups of humans and/or wildlife to a substance

Data requirements for exposure assessment	
Humans	Wildlife
Industrial and consumer uses of the substance	Routes of release of substance to the environment
Possible routes of exposure in humans (such as inhalation, oral uptake and dermal uptake)	Possible routes of exposure in wildlife
Occupational and consumer exposure scenarios	Emission scenarios
Exposure frequencies and duration	Exposure frequencies and duration
Concentrations in the workplace	Concentrations in environmental compartments
Concentrations of the substance in the tissues of humans	Concentrations of the substance in the tissues of aquatic, terrestrial and aerial species

Exposure assessment in workers and consumers

Exposure assessment for workers can be carried out using the Estimation and Assessment Substance Exposure (EASE) model linked with expert judgement. It uses information on the physical properties of the substance during processing to describe the tendency to become airborne, and incorporates into the assessment the use patterns of the substance from full containment to direct handling. This leads to a series of exposure fields for which ranges of exposure have been described based on information collected in the United Kingdom. For dermal exposure assessment and additional parameter, level of contact, is included in the system.

Assessment of exposure for consumer products, other than those for which more specific procedures apply, is based on use (ECETOC 1994, Health and Safety Executive 1994). Both normal use and reasonably forseeable misuse need to be considered. The aim is to use 'worst case' only as a preliminary calculation and 'reasonable worst case' as the normal calculation. 'Reasonable worst case' covers normal use patterns, including cases where consumers may use several products containing the same substance, as well as the majority of forseeable extreme use and misuse. There are separate algorithms for each route of exposure (Table 2.11) and these depend on assumptions contained in product use scenarios. Typical product use scenarios include those for cosmetics and personal care products, household cleaning products, aerosols, paints and plasticisers. Default values for exposure levels are used when measured information is not available. The measured information will depend on the scenario and a combination of algorithm and product use scenario in the model.

Table 2.11 Algorithms used for estimating consumer exposure (after Health and Safety Commission 1994)

Type of exposure	Equations (Units)	Parameters	
		Symbol	Description
Inhalation	$C_{air} = q \times w_f \times R \times V_r^{-1}$ (mg m^3)	C_{air}	Average concentration in air (mg m^{-3})
		q	Amount of product used (mg)
		wf	Weight fraction of substance in product
	$I_{inhalationA} = C_{air} \times V_{inhalation} \times t$ (mg $event^{-1}$)	R	Respirable or inhalable fraction of product (default = 1)
		Vr	Room volume (m^3)
	$I_{inhalationB} = I_{inhalationB} \times BW^{-1}$ (mg kg BW^{-1} $event^{-1}$)	$V_{inhalation}$	Ventilation rate in adults (default = 0.8 m^3 h^{-1} or 20 m^3 day^{-1})
		t	Duration of exposure (hours)
	$I_{inhalationC} = I_{inhalationB} \times n$ (mg kg BW^{-1} $event^{-1}$)	$I_{inhalation}$	Amount of substance inhaled/respired
		BW	Body weight (default adult values: female = 60 kg, male = 70 kg)
		n	Number of events (per day)
Dermal	$C_{dermal} = d \times w_f$ (mg cm^{-1})	C_{dermal}	Average concentration in product (mg)
		d	Density of product (mg m^{-3})
		E_{dermal}	Amount of substance on skin
	$E_{dermalB} = C_{dermal} \times T_{dermal} \times S_{dermal}$ (mg $event^{-1}$)	T_{dermal}	Thickness of layer of product (cm)
		S_{dermal}	Surface area of exposed skin (cm^2)
	$E_{dermalB} = E_{dermalA} \times n \times BW$ (mg kg BW^{-1} $event^{-1}$)		
Oral	$I_{oral} = q \times w_f \times f_{oral}$	I_{oral}	Amount of substance injested (mg)

	(mg event^{-1})	f_{oral}	Fraction of product swallowed

For consumers it is important to evaluate the exposure to the substances of groups which may be particularly vulnerable to endocrine disrupters, including pregnant women and foetuses and breast fed children.

For the assessment of potential endocrine disrupting effects in consumers it needs to be recognised that besides the main exposure route via ingestion of food, uptake of substances can also occur by the consumption of drinking water. However, currently neither the exact concentrations of potential endocrine disrupting substances in drinking water and thus the consumed amounts nor their potential in vivo adverse effects are verified or even known for the area of the European Union. As a consequence of both, the precautionary principle which is to be applied in European Regulations and Directives and the lack of knowledge in respect of concentrations of potential endocrine disrupting substances in drinking water (and potential adverse effects) the European Commission (DG ENV) has initiated a joint project with the Fraunhofer Institute for Molecular Biology IME (Schmallenberg, Germany) and the ESWE–Institute for Water Research and Water Technology (Wiesbaden, Germany) for a study on Endocrine Disrupters and Drinking Water.

The measured or estimated exposure concentrations can then be compared with the NOELs/NOAELs to obtain an effects-exposure ratio for target groups of humans where endocrine disrupting effects occur at lower concentrations than those eliciting general, non-endocrine mediated (eco) toxicological effects.

The approach adopted for consumer use is based on that given in the Scientific Committee for Consumer Products and Non-Food Products intended for Consumers (SCCNFP) “Notes of Guidance for Testing of Cosmetic Ingredients for their Safety Evaluation” (SCCNFP/0321/00 Final, 2001). On a general basis, the Margin of Safety (MOS) is calculated by dividing the lowest No Observable Adverse Effect Level (NOAEL) of a compound by its Sytemic Exposure Dose (SED) during normal forseeable use. If the MOS exceeds 100, the substance is regarded as safe for use. The value of 100 can be modified to account for perceived sensitive target groups (for example children).

Exposure assessment in wildlife

For each industrial substance a Mackay Level 1 fugacity model (Version 2.1) will be run to ascertain the partitioning of a known amount of the substance (1000 tonnes) within different environmental compartments. Table 2.12 summarises the volumes of the different compartments used within the model. The assumed organic carbon levels (%) in suspended sediment, bottom sediment and soil were = 0.2%, 0.04% and 0.02% respectively. The model was run using physico-chemical data on molecular weight, water solubility, log K_{ow} and Henry’s Law Constant.

Table 2.12 Volumes of the different compartments used within the Mackay Level 1 fugacity model

Compartment	Volumes of different compartments using Mackay Fugacity Level 1 model
Water	2×10^{11}
Suspended sediment	10^{6}
Bottom sediment	10^{8}
Fish	2×10^{5}
Air	10^{14}
Aerosol	2000
Soil	9×10^{9}

Using the information on the environmental compartment(s) to which the substance is expected to partition data can then be sought on measured environmental concentrations in that compartment(s).

If measured environmental concentration data is not available environmental exposures of synthetic substances can be estimated using the EUSES model which is a simple deterministic multimedia model which incorporates a basic level (Mackay type) fugacity model for the estimation of partitioning between the different environmental compartments and more detailed descriptions of sub-processes along various exposure routes at different spatial levels (local, regional and continental). However, the model requires a certain level of input data if it is to provide meaningful information on environmental concentrations.

A margin of safety (MOS) approach has also been adopted for consideration of effects on wildlife species where the lowest NOEC for endocrine disrupting effects in species from a given compartment (aquatic, terrestrial or aerial) is considered in the light of mean measured or predicted exposure concentrations for that compartment.

Summary

Overall it should be recognised that the exposure assessment for any identified substance will need to be considered on a case by case basis depending on the physico-chemical properties of the substances, the emission scenarios and the susceptibility to endocrine disrupting effects of target groups of humans and wildlife.

2.4 Issues outside the scope of the current reviews

In the review of each of the 12 substances it has only been possible to draw conclusions based on the available data and to identify data gaps which can be filled using currently available methodologies or those nearing completion. In the future new techniques will be developed to assess endocrine disrupting properties of chemicals and these may provide data on the 12 substances which means the current conclusions have to be revisited. The approach taken at that moment will be determined by the Commission in consultation with stakeholders and the Scientific Committees of the Commission.

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3. INFORMATION ON THE 12 SUBSTANCES PROVIDED IN THE BKH REPORT

3.1 Summary

In this section a description of the current state of knowledge on the 12 substances from work done to date by BKH has been given.

Table 3.1 summarises the types of data on the nine industrial chemicals generated in the BKH study for which there is "evidence of endocrine disruption or potential endocrine disruption but which are neither restricted nor currently being addressed under community legislation. For many of the substances (with the exception of 4-nitrotoluene, resorcinol and 4-tert octylphenol) limited data was reported in the BKH report on parameters important in assessing the potential exposure of humans and wildlife to these substances (for example physico-chemical properties, environmental fate and behaviour data and environmental concentration data). Reported effects data (in particular for endocrine disrupting effects) was also limited for many of the chemicals.

Table 3.2 summarises the classification of nine industrial chemicals against the BKH criteria for production volume, persistence, endocrine disruption category and exposure category. All of the substances except 2,2',4,4'-tetrabominated diphenyl ether were characterised as High Production Volume (HPV) chemicals (that is produced in Europe in volumes of greater than 1000 tonnes). However, of the nine substances only 2,2',4,4'-tetrabominated diphenyl ether was classified as persistent on the basis of the criterion used in the BKH report (that is substances that take more than months to biodegrade, combined with a biodegradation probability of <0.1).

The summary indicates that limited data on endocrine effects of the different substances to humans and wildlife was available (with the exception of 4-tert octylphenol) and no literature data was identified for 4 of the 9 industrial substances. Although there was limited data on endocrine disruption effects (see Table 3.3) the nature of the criteria used in the report meant that all of the nine industrial chemicals were classified as being either:

- Category 1 - substances with at least one study providing evidence of endocrine disruption in an intact organism
- Category 2 - substances with the potential for endocrine disruption based on:

  - *in vitro* data indicating the potential for endocrine disruption in intact organisms;

  - *in vivo* effects that may, or may not, be endocrine disruption mediated;

  - structural analyses and metabolic consideration.

Table 3.1 Summary of the types of data collated in the BKH Report for the nine industrial chemicals

Parameter	Substance								
	2,2'bis(4-(2,3-epoxypropyl) phenyl) propane	Carbon disulphide	4-chloro-3-methylphenol	2,4-dichloro-phenol	4-nitro-toluene	o-phenyl-phenol	Resorcinol	2,2',4,4'-tetra BDE	4-tert octyl-phenol
Production volume	No data	✓	✓	✓	✓	✓	✓	✓	✓
Use pattern	✓	✓	✓	✓	✓	✓	✓	✓	✓
Physico-chemical data									
Water solubility	No data	No data	No data	No data	✓	No data	✓	No data	✓
Octanol-water partition coefficient (Log Kow)	✓	No data	No data	No data	✓	No data	✓	No data	✓
Henry's Law Constant	No data	No data	No data	No data	No data	No data	No data	No data	✓
Environmental fate and behaviour									
Photodegradation	No data	No data	No data	No data	✓	No data	✓	No data	✓
Stability in water	✓	No data	No data	No data	✓	No data	✓	No data	No data
Biodegradation	✓	No data	✓	No data	✓	✓	✓	No data	✓
Bioaccumulation	No data	No data	✓	No data	✓	✓	No data	No data	✓
Data on environmental concentrations	No data	No data	No data	No data	No data	No data	✓	No data	✓
Endocrine disruption data									
Mammalian - *In vivo*	✓	✓	✓	No data	✓	✓	✓	✓	✓
Mammalian - *In vitro*	✓	No data	✓	✓	✓	✓	✓	No data	✓
Wildlife - *In vivo*	No data	No data	✓	No data	✓	✓	✓	No data	✓
Wildlife - *In vitro*	No data	No data	No data	✓	✓	✓	No data	No data	✓
Mammalian toxicology data									
Acute	✓	No data	✓	No data	✓	✓	✓	No data	✓
Chronic	✓	No data	✓	No data	✓	✓	✓	No data	✓
Ecotoxicology data									
Acute	No data	No data	✓	No data	No data	✓	✓	No data	✓
Chronic	No data	No data	✓	No data	✓	✓	✓	No data	No data

Table 3.2 Summary of the classification of the nine industrial chemicals against the BKH report criteria

CAS No,	Substance (BKH Number)	List(s) from which taken[1]	HPV Chemical[2]	Persistent[2]	Endocrine disrupting effects[3]			ED Category[4,5]	Exposure Concern[4]
					Negative	Positive	Total		
1675-54-3	2,2'-bis(4-(2,3-epoxy propoxy)phenyl) propane (318)	Norway	Yes	No	0	2	2	**2** (2M, 3W)	-
75-15-0	Carbon disulphide (543)	WWF (USA)	Yes	No	0	1	1	**2** (2M, 3W)	-
59-50-7	4-Chloro-3-methylphenol (196)	Greenpeace	Yes	No	0	1	1	**2** (2M, 3W)	-
120-83-2	2,4-Dichlorophenol (194)	WWF (Canada)	Yes	No	1	2	3	**2** (2M, 3W)	-
99-99-0	4-Nitrotoluene (538)	Japan, WWF (Canada)	Yes	No	0	1	1	**1** (1M, 3W)	Low
90-43-7	o-Phenylphenol (371)	WWF (More than 1 list)	Yes	No	0	2	2	**2** (2M, 2W)	-
108-46-3	Resorcinol (560)	WWF (USA)	Yes	No	0	2	2	**1** (1M, 3W)	High
046	2,2',4,4'-Tetrabrominated diphenyl ether (435)	Sweden	No	Yes	0	2	2	**2** (2M, 3W)	-
140-66-9	4-tert-Octylphenol (216)	Germany, UK, Norway, OSPAR, Greenpeace, Dutch Health Council	Yes	No	2	13	15	**1** (1M, 1W)	Medium

Key: [1] – Information given in Annex 9 of the BKH report

[2] – Information given in Annex 6 of the BKH report

[3] – Information given in Annexes 6, 7, 8 and 12 of the BKH report

[4] – Information given in Annexes 10 and 12 of the BKH report

[5] – Incorporated scientific evidence used at the Expert Meeting which may not have been included in Annexes 7 and 8 of the BKH report

Table 3.3 Summary of the endocrine disruption data for identified substances given in Annexes 7 and 8 of the BKH Report

Substance	Test type	Class	Species/ receptor	Exposure route	Criterion	Dose-concentration	Unit	Effect	Potency	Reference
HUMANS										
Carbon disulphide	Epidemio-logical	+th	Human	-	-	-	-	Decreased T4 levels	-	Cavalieri (1975)
2,4,Dichloro-phenol	*In-vitro*	+th	-	-	LOEL	-	-	Binding with high affinity to trans- thyreton (T3)	-	Van den Berg *et al* (1991)
"	*In-vitro*	-e	Human	-	NOEL	0.01	mM	Cell proliferation	-	Jobling *et al* (1995)
Resorcinol	Epidemio-logical	+th	Human	-	-	-	-	Decreased PBI, ulceration of goitre	-	Lindsay and Gaitan(1989) Quentin and Hobson (1951)
"	*In-vivo*	+th	Rat	-	-	-	-	Decreased PBI, ulceration of goitre	-	Doniach and Logothetopoulov (1953), Doniach and Fraser (1950) Arnott and Doniat (1952)
2,2', 4,4'-tetra BDE	*In-vivo*	+re	Rat	Oral	LOEL	2.5	mg/kg body weight	Decreased hepatic and pulmonary retinoid levels		Hakanson cited in SEPA (1998)
"	*In-vivo*	+th	Rat	Oral	LOEL	-	-	Decreased levels of total plasma T4		Darnerud and Sinjari (1996)
4-tert octyl - phenol	*In-vivo*	+e	Adult female rat	Subcutaneous	-	20 - 40	mg/animal	Persistent oestrus	-	Blake *et al* (1997)
"	*In-vivo*	+e	Female rat (LE)	Oral	LOEL	100	g/kg body weight daily	Accelerated vaginal opening	-	Gray and Ostby (1998)
"	*In-vivo*	+e	Female rat (LE)	Subcutaneous	LOEL	200	mg/kg body weight	Lordosis	-	Gray and Ostby (1998)

Table 3.3 Continued

Substance	Test type	Class	Species/ receptor	Exposure route	Criterion	Dose-concentration	Unit	Effect	Potency	Reference
4-*tert* octylphenol	*In-vivo*	+e	Neonatal female rat	Subcutaneous	-	1.0	mg animal	Vaginal opening and oestrous cycling	-	Blake *et al* (1997)
"	*In-vivo*	+e	Ovariectonized female rat (LE)	Subcutaneous	LOEL	200	mg/kg body weight day	Increased uterine weight	-	Gray and Ostby (1998)
"	*In-vivo*	+e	Ovariectonized female rat (LE)	Oral	LOEL	200	mg/kg body weight day	Increased uterine weight	-	Gray and Ostby (1998)
"	*In-vivo*	+e	Rat	Subcutaneous	LOEL	600	mg/kg	Increased uterine weight	-	Bicknell *et al* (1995)
"	*In-vivo*	-e	Wistar Rat (WU) Hsd/Cpb spf	Oral	-	100	mg/kg body weight day	Increased number of corpora lutea	-	Piersma *et al* (1998)
"	*In-vivo*	+e	Rat	Oral	LOEL	1000	µg/l	Decreased testis weight and sperm production	-	Sharpe *et al* (1995)
"	*In-vitro*	+e	Human MCF-7 cells	-	-	-	-	Stimulation of proliferation	-	White *et al* (1994)
"	*In-vitro*	+e	Human YES assay	-	-	-	-	Binding affinity	-	Arnold *et al* (1996)
"	*In-vitro*	+e	Human ZR-75 cells	-	-	-	-	Stimulation of proliferation	-	White *et al* (1994)
"	*In-vitro*	+z	Mouse, spleneocytes	Medium	LOEC	0.5	µM	Oestrogen-sensitive decreased viability		Blake *et al* (1997)
"	*In-vitro*	+e	Human embryonal kidney 293 cells	Water		1000	nM	Luciferase reporter gene induction	0.61	Kuiper *et al* (1998)
"	*In-vitro*	+e	Human MCF – 7 cells	-	EC_{100}	100	nM	Stimulation of cell proliferation	0.0003	Soto *et al* (1995)

Table 3.3 Continued

Substance	Test type	Class	Species/ receptor	Exposure route	Criterion	Dose-concentration	Unit	Effect	Potency	Reference

4-*tert* octyl - phenol	*In-vitro*	+e	Human MCF – 7 cells	-	EC_{50}	0.1 – 1.0	μM	Increased transcription	0.00001 – 0.0001	Jobling and Sumpter (1993)
"	*In-vitro*	+e	Insect cell line Sf 9	Water	IC_{50}	-	-	Solid-phase RBA – assay for human ERα	< 0.0002	Kuiper *et al* (1998)
"	*In-vitro*	+e	Insect cell line Sf 9	Water	IC_{50}	-	-	Solid-phase RBA – assay for human ERβ	< 0.0007	Kuiper *et al* (1998)
"	*In-vitro*	+e	Yeast transfected	-	EC^{50}	0.2	μM	Stimulation of oestrogen-dependent transcription	0.001	Arnold *et al* (1996)
"	*In-vitro*	+e	Yeast transfected	-	EC_{50}	1.0	μM	Stimulation of oestrogen-dependent transcription	0.0004	Routledge and Sumpter (1996)
"	*In-vitro*	+e	Yeast transfected	-	EC_{50}	0.2	μM	Stimulation of oestrogen-dependent transcription	0.001	Arnold *et al* (1996)
WILDLIFE										
2,4 Dichloro-phenol	*In vitro*	+e	*Salmo* sp.	-	LOEC	> 10	mM	Inhibition of binding of marked 17β-oestradiol on oestrogen rector	-	Jobling *et al* (1995)
4-*tert* octyl - phenol	*In vivo*	-e	Guppy	-	NOEC	50 - 300	g/l	Altered sperm count, body coloration gonadosomatic index (GSI)	-	Baatrup (1999)
"	*In vitro*	+e	Trout FHVSA cells	-	-	-		Stimulation of proliferation	-	Jobling and Sumpter (1993)
"	*In vitro*	+e	Trout FHVSA cells	-	-	-		Stimulation of proliferation	-	White *et al* (1994)

Key: e – suspected endochrine effects, th- effects on thyroid, z- other effects

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4. REVIEW OF DATA FOR 2,2-BIS(4-(2,3-EPOXYPROPOXY) PHENYL) PROPANE (BADGE)

Table of Contents

Notes:

This section contains information collected and collated from a range of sources including published papers, reports of studies conducted by industrial companies or sector groups and data compilations such as the Draft OECD SIDS Dossier on the HPV Phase 6 Chemical Bisphenol A Diglycidyl Ether (OECD 1999). The data from the Draft OECD SIDS dossier has been taken as accurate and individual source documents have not been checked unless they are considered to be key studies which have a major influence on the outcome of the review. All information taken from the Draft OECD SIDS dossier has been referenced as being from that source and individual references have not been given in the references.

This review has been carried out in accordance with the evaluation framework described in Section 2. In the review the International Programme for Chemical Safety (IPCS) definition of an endocrine disrupter has been adopted, namely that it is "*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*".

In the context of the review it is recognised that there are various laboratory-based *in vivo* and *in vitro* methods utilising a range of (eco)toxicological endpoints that are claimed by different sources to be relevant to the assessment of endocrine disruption in humans and wildlife. However, since this field is still in an early stage of development there is uncertainty regarding the significance of many of the current findings.

From the numerous recent reviews of potential test methods (such as the Detailed Review Paper prepared by OECD in 1997) there is a clear consensus in terms of the hierarchy of the relevance of test methods. In this hierarchy longer-term *in vivo* studies considering effects on reproduction and/or development (and including mechanistic information) are of greater relevance than short-term *in vivo* screening tests which are of greater relevance than *in vitro* assays. The greater relevance of chronic *in vivo* tests or those assessing effects during critical windows of sensitivity is also evidenced by the fact that these are the key (eco) toxicological methods being developed in the OECD Endocrine Disruption Testing and Assessment (EDTA) Programme. This hierarchy approach to data relevance has been adopted in the review along with a weight of evidence consideration of the available data.

The review has been carried out to address three key questions:

1. Does the available data indicate there is evidence that a chemical causes endocrine disrupting effects in target groups of humans and/or wildlife?

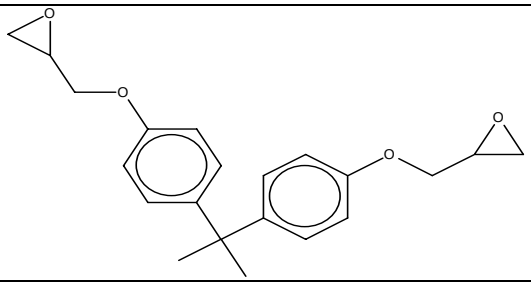
2. Do endocrine disrupting effects of the chemical in target groups of humans and/or wildlife occur at lower concentrations than those causing effects on general systemic toxicological endpoints?

3. Are particular target groups of workers, consumers or organisms in the environment likely to be exposed to concentrations of chemicals which exceed effects thresholds due to current emission patterns.

It should be recognised that this review is not designed to be a full Risk Assessment of a substance under the Existing Substances Regulation 793/93.

4.1 Physico-chemical data for BADGE

4.1.1 Summary details on the substance

CAS Number	1675-54-3
EINECS Number	216-823-5
IUPAC Name	2,2-bis(4-(2,3-epoxypropoxy)phenyl) propane
Other names	Bis(p-2,3-epoxy propoxy) phenyl) propane, Bisphenol A diglycidyl ether, BADGE, DGEBPA, Diglycidyl ether of Bisphenol A, Diomethane giglycidyl ether, Diphenylol propane diglycidyl ether, 2,2'-(1-methylethylidene) bis(4,1-phenyleneoxymethylene) bis-oxirane, 2,2-Bis(4-hydroxyphenyl)propane diglycidyl ether, 2,2-Bis(p-glycidoxy)phenyl]propane, 2,2-bis(p-glycidyl-oxy-phenyl)-propane, 2.2'-bis(p-2,3-epoxy propoxy)phenyl)propane
Molecular weight	340 - 700 The basic epoxy resin involves the reaction of epichlorohydrin with bisphenol A (2 moles ECH + 1 moleBPA). These compounds include the ethers of bisphenol A [2,2-bis(4-hydroxyphenyl)propane] and, for resins of higher molecular weight, condensation products of their further reaction or advancement with bisphenol A. The resins are usually mixtures and may contain homologues of higher weight, isomers, branched-chain homologues, and occasionally, monoglycidyl ethers. The essentially pure BADGE resin of molecular weight 340 is a crystalline solid, other lower-molecular weight resins are liquids, and as the molecular weight increases the resins become increasingly viscous and finally solids.
Chemical formula	$C_{21}H_{24}O_4$
Chemical structure	

4.1.2 Physico-chemical properties and environmental fate information (from the Draft OECD SIDS dossier, OECD 1999)

The data on the physico-chemical properties of BADGE and its environmental fate (see Table 4.1) indicate that the substance is inherently biodegradable and will tend to sorb to sludge in waste water treatment plants with very small amounts being discharges to receiving waters. In the aquatic environment BADGE is hydrolysed to form the water soluble product bis-diol.

Volatilisation is unlikely to represent a major removal process from the aquatic environment based on the Henry's Law Constant of 6.85 x 10^{-4} Pa-m^3 mol^{-1} (< 6.94 x 10^{-9} atm-m^3 mol^{-1}) being lower than a value range of 1 - 100 Pa-m^3 mol^{-1} which is considered to indicate volatility.

Table 4.1 Physico-chemical properties and environmental fate data (from the Draft OECD SIDS dossier, OECD 1999)

Physico-chemical property	Value (and comments)
Physical state at ambient temperature	Liquid
Water solubility	<0.5 mg l^{-1} at 25 ^oC
Octanol-water partition coefficient (log Kow)	3.7 – 3.9
Organic carbon water partition coefficient (log Koc)	No data
Henry's Law Constant	6.85 x 10^{-4} Pa-m^3 mol^{-1} (< 6.94 x 10^{-9} atm-m^3 mol^{-1}) at 25 ^oC
Type of degradation	Information
Aquatic - abiotic	BADGE hydrolyses in water to form the water soluble product bisphenol A bis (2,3-dihydroxypropyl ether) known as bis-diol with a half-life of 86 hours at pH 7
Aquatic - biotic	BADGE was reported as inherently biodegradable (12% loss after 28 days) in an aerobic Zahn-Wellens test (OECD 302B)
Terrestrial	No data
Atmospheric	No data

A Mackay Level 1 fugacity model has shown that for a discharge of 1000 tonnes of BADGE 65.5% of the substance will partition into the soil (Table 4.2), with 11.7% and 19.9% partitioning into the water and air. Amounts present in other compartments are minimal.

Table 4.2 Summary of the results of a Mackay Level 1 fugacity model

Compartment	Volumes of different compartments	% of substance present in different compartments
Water	2 x 10^{11}	11.7
Suspended sediment	10^6	0.046
Bottom sediment	10^8	1.46
Fish	2 x 10^5	0.037
Air	10^{14}	19.9
Aerosol	2000	1.36
Soil	9 x 10^9	65.5

4.2 Production and Uses

4.2.1 Production Patterns

BADGE (Bisphenol A Di Glycidyl Ether) is the major component in commercial liquid epoxy resins which themselves are manufactured by co-reacting bisphenol A with epichlorohydrin. Liquid epoxy resins are either used as a binder in cured epoxy systems or further advanced with bisphenol A to higher molecular weight solid epoxy resins. The manufacture of glycidyloxy compounds usually occurs in closed systems designed with engineering controls to minimise or prevent human exposure.

4.2.2 Use Patterns

Bisphenol-A based epoxy resins are typically formulated with curing agents to yield high-performance crosslinked systems. Epoxy resins with differing molecular weights are used in various applications. For example, high molecular weight epoxy resins (MW> 3000) are used as a polymeric binder in heat cured coil coatings or interior coatings for food and drink containers. Liquid and low molecular weight epoxy resins (MW < 2000) are typically used as binders in industrial protective coatings, powder coatings, industrial floorings, printed circuit boards, composites, electronic encapsulation, fillers and adhesives. The majority of epoxy applications are industrial with negligible consumer exposure. The total market volume of epoxy resins used in Europe is estimated at 286000 tonnes per year with about 35000 tonnes (12%) being used in epoxy based interior coatings for food and drink cans.

4.3 Toxicokinetics, metabolism and bioaccumulation

4.3.1 Toxicokinetics and metabolism

The toxicokinetics and metabolism of BADGE in intact animal systems and in tissue fractions has been studied in detail. Bentley *et al* (1989) investigated the hydrolysis of the epoxide functionalities of DGEBPA by the microsomal and cytosolic fractions of mouse liver and skin. It was reported that DGEBPA was rapidly hydrolised by the epoxide hydrolase of both tissues, with skin microsomal activity being about 10 times greater than that found in the cytosol of skin. In other experiments using *in vitro* systems of liver fractions obtained from mouse, rat and rabbit the two epoxide groups of BADGE were very rapidly hydrolysed to form the corresponding bis-diol of BADGE (bisphenol A bis(2,3-dihydroxypropyl ether). Further experiments showed no changes in the metabolism under conditions, which inhibited the epoxide hydrolase activity or promoted other breakdown mechanisms (for example oxidative metabolism). Formation of Bisphenol A from BADGE was not observed in any of these *in-vitro* experiments. The metabolism of five different glycidyl ethers, including BADGE has been examined in human, rat and mouse liver, lung, and skin preparations (Boogaard *et al* 2000 a,b). The results of the metabolic studies found no evidence for any significant formation of Bisphenol A in incubations of BADGE with human or rodent liver or lung microsomal or cytosolic fractions, nor in incubations with intact viable human or rodent skin.

In intact animal systems, the fate of BADGE was studied in mice following oral or dermal administration (Climie *et al* 1981 a,b). Upon oral administration, BADGE was rapidly and extensively excreted with the faecal route being the major route of elimination while a small fraction of the dose was recovered in the urine. The pattern of the metabolites identified in the urine and the faeces was consistent with the results of the *in vitro* studies and confirmed that the primary product of the metabolism of BADGE was the formation of the corresponding diol, which was further conjugated and/or converted to the corresponding carboxylic acids. Bisphenol A could not be detected in the excreta. Additional evidence indicating the inability of the mammalian biotransformation systems to convert BADGE to Bisphenol A was obtained in a similar experiment with BADGE diol, where Bisphenol A was not found in the urine or the

faeces. Coveney (1983) reported that metabolic pathways in the rabbit appeared similar to those in the mice. Subsequently metabolic studies with Bisphenol A in the rat after oral or parenteral administration showed that Bisphenol A was eliminated primarily as unchanged material or as the mono-glucuronide conjugate of unchanged Bisphenol A (Pottenger *et al* 2000).

Nolan *et al* (1981) reported route-dependent differences in plasma ^{14}C concentration-time profiles, tissue/plasma ^{14}C ratios, and urinary excretion following intra-venous or oral administration of [^{14}C]DGEBPA to rats. The [^{14}C]DGEBPA was labelled at the isopropylidene methylene carbon. The plasma radioactivity that resulted from the oral administration of [^{14}C]DGEBPA was eliminated more rapidly than the radioactivity resulting from intra-venous administration.

The available data demonstrate that mammalian metabolic systems are unable to transform BADGE into Bisphenol A and it can be concluded that human consumption of food containing low levels of BADGE will not lead to systemic exposure of Bisphenol A.

4.3.2 Bioaccumulation

In the presence of water, BADGE undergoes chemical hydrolysis of the epoxide functionality to produce the water soluble product bis-diol indicating BADGE is not likely to be persistent in the body. The low octanol-water partition coefficient (log Kow) of 3.7 to 3.9 for BADGE indicates that the substance is not expected to bioaccumulate in biota.

4.4 Studies relevant to the assessment of potential endocrine disrupting effects

4.4.1 Studies relevant to the assessment of potential endocrine disrupting effects in humans

4.4.1.1 In vitro studies

A. Receptor competitive binding assays

In a competitive binding assay BADGE did not show any affinity for the oestrogen receptor at the highest concentration tested and was unable to displace 17β-oestradiol from a rat uterine oestrogen receptor in a concentration series from 0.1 to 100 μM (that is concentrations 10^6 times higher than those at which 17β-oestradiol caused effects (Olea *et al* 1996, Perez *et al* 1998).

Satoh *et al* (2001), in a poster presented at the Japan Society of Endocrine Disruption Research in 2001, reported that BADGE had a low binding affinity for the oestrogen receptor, (no experimental details provided). The authors did, however, report some binding to the androgen receptor although no details were provided.

B. Recombinant yeast assays

Vinggaard (1998) evaluated the effects of BADGE and its hydrolysis product [bisphenol A bis(2,3-dihydoxypropyl ether] in a recombinant yeast screen and found that no responses were recorded at concentrations ranging from 0.5 - 500 μM (1700-35000 μg l^{-1}). Control substances confirmed the sensitivity and activity of the test system.

C. Mammalian cell growth assays

Olea *et al* (1996) and Perez *et al* (1988) investigated the effects of BADGE on the E-Screen (a modified human MCF-7 cell proliferation assay). The substance caused a minimal proliferative effect (<2 fold) at a concentration of 10 μM (3400 - 7000 μg l^{-1}) which represents the limit of the aqueous solubility of BADGE. At lower concentrations no proliferative effects were observed. It was stated that BADGE itself was not responsible for the cell proliferation observed at 10 μM, but rather that the weak response was due to the conversion of BADGE to Bisphenol A. However, biotransformation studies have demonstrated that BADGE cannot be biotransformed into Bisphenol A (Climie *et al* 1981a,b; Hutson 1998). As a result the minimal cell proliferation in the E-Screen[1] cells reported by Olea *et al* (1996) was probably due to a non-oestrogenic mechanism. In the MCF-7 cells BADGE also showed no significant effects on oestrogen sensitive markers such as progesterone receptor induction or pS2 accumulation at concentrations of 0.1 to 10 μM.

Satoh *et al* (2001), in a poster presented at the Japan Society of Endocrine Disruption Research in 2001, reported that BADGE did not cause proliferation of MCF-7 cells in the E-screen assay.

Summary of *in vitro* data

Table 4.3 summarises the available *in vitro* data for BADGE which only relates to *in vitro* assays assessing oestrogenic and androgenic mechanisms of action in mammalian cells and tissues. The data indicates an absence of induction of oestrogen-sensitive gene products and no or weak binding of BADGE to the human oestrogen receptor. A single study (with no experimental details given) reports binding of BADGE to the androgen receptor. No data has been identified on the effects of BADGE on thyroid function and effects on hormone synthesis and secretion and steroidogenesis in mammalian cells and tissues.

[1] In a review of the *in vitro* methods available for assessing oestrogenic substances, Zacharewski (1997) has stated that cell proliferation observed in the “E-Screen” assay can be due to other non-oestrogenic factors.

Table 4.3 Summary of the *in vitro* data in isolated mammalian cells and tissues relating to different mechanisms of action of BADGE

Mechanism of endocrine disruption	Responses observed in *in vitro* systems
Oestrogenicity/anti-oestrogenicity	Data indicates an absence of induction of oestrogen-sensitive gene products and no or weak binding of BADGE to the human oestrogen receptor. Minimal cell proliferation in the E-Screen assay was evident.
Androgenicity/anti-androgenicity	A single study (with no experimental details given) reports some binding of BADGE to the androgen receptor
Thyroid effects	No data identified
Effects on hormone synthesis or secretion	No data identified
Effects on steroidogenesis	No data identified

4.4.1.2 In vivo studies

Tables 4.4 to 4.6 summarise the information on potential endocrine mediated responses in laboratory mammals following oral exposure (Table 4.4), dermal exposure (Table 4.5) and intra-peritoneal injection (Table 4.6) of BADGE.

A. Effects on endocrine glands and hormone sensitive tissues

The effect of BADGE on the uterus and vagina of ovariectomoized ICR mice has been investigated by Ogata *et al* (2001) in a screening assay. The mice received subcutaneous injections of BADGE at doses up to 1mg kg body weight^{-1} for 3 consecutive days. All the mice were then killed 24 hours after receiving the final injection. The uterus and vagina were removed, weighed and subjected to histopathological examination. No differences in organ weights or tissue histopathology were found between the negative control (DMSO treated) animals and the BADGE treated mice (see Table 4.6). In contrast, positive control mice treated with 100 μg kg body weight^{-1} of 17β-oestradiol showed positive signs of oestrogenic effects characterized by increased uterine weights and high grade stratification/cornification of the vaginal epithelium. Based on these data the authors concluded that BADGE does nor exert oestrogenic responses on the uterus and vagina in ovariectomized mice at relatively low doses.

Stebbins and Dryzga (2001) reported on a two year oral gavage toxicity study of BADGE in Fischer 344 rats in which 65 male and 65 female rats per dose level were exposed to 0, 50, 250 and 1000 mg kg body weight^{-1} of BADGE. The study (carried out to GLP) was terminated on days 99 (males) and 101 (females) of the study due to excessive toxicity. Full toxicological parameters consistent with a sub-chronic oral study conducted to OECD Test Guideline 408 were evaluated in a subset of 10 rats per sex per dose group. Treatment-related changes in clinical chemistry, urine analysis and organ weights indicative of renal toxicity were seen in the 250 and 1000 mg kg body weight^{-1} day^{-1} treatment groups. The only treatment related clinical pathology alteration in male and female rats given 50 mg kg body weight^{-1} day^{-1} was

increased serum cholesterol levels. The increased cholesterol was interpreted to be non-adverse because rats are relatively resistant to the induction of hyperlipidema and atherosclerosis even when serum cholesterol levels are elevated. The only gross pathological change was an increase in size of the caecum seen in the male rats in the 250 and 1000 mg kg body weight^{-1} day^{-1} groups and females receiving 1000 mg kg body weight^{-1} day^{-1}.

Treatment related histopathologic changes (see Table 4.4) were seen in a number of tissues in the 250 and 1000 mg kg body weight^{-1} day^{-1} treatment groups including:

- a slight decrease in the vacuolisation of the adrenal cortex in males at 250 and 1000 mg kg body weight^{-1} day^{-1};
- a slight atrophy of the endometrium and myometrium of the uterus at 1000 mg kg body weight^{-1} day^{-1};
- a very slight vacuolisation of proximal convoluted tubules of kidneys of females and slight decreased hyaline droplet formation in proximal convoluted tubules of kidneys of males exposed to 250 and 1000 mg kg body weight^{-1} day^{-1}. In addition two males and one female given 1000 mg kg body weight^{-1} day^{-1} that died prior to study termination had moderate to severe acute necrosis of renal proximal tubules. The tubular necrosis was interpreted to be treatment related and contributed to the deaths of the animals.
- increased eosinophilia of centrilobular hepatocytes of the liver of males given 250 and 1000 mg kg body weight^{-1} day^{-1} and females given 1000 mg kg body weight^{-1} day^{-1}. Five of ten males and one of the ten females given 1000 mg kg body weight^{-1} day^{-1} had a single eosinophilic focus of altered hepatocytes.
- degeneration of seminiferous tubules in male rats in the 1000 mg kg body weight^{-1} day^{-1} male rats. The degeneration was graded as very slight, involving less than 1% of the seminiferous tubules in eight rats and moderate involving approximately 40-50% of the seminiferous tubules in the other two surviving rats.

Based on alterations in body weights and serum cholesterol in rats given 50 mg kg body weight^{-1} day^{-1}, a no observed effect level (NOEL) was not determined. However, since these alterations were not associated with detrimental effects, the dose of 50 mg kg body weight^{-1} day^{-1} was interpreted to be the no observed adverse effect level (NOAEL). The NOEL for responses in endocrine organs or hormone sensitive tissues in both male and female rats was 250 mg kg body weight^{-1} day^{-1}.

B. Reproduction and fertility studies

Smith *et al* (1989) conducted a one-generation reproduction study in Sprague-Dawley rats, (carried out to OECD Guideline 415 and GLP) in which a BADGE-based epoxy resin (Araldite GY 250 or TK 10490) was administered by oral gavage at dose levels of 0, 20, 60, 180 and 540 mg kg body weight^{-1} day^{-1}. The vehicle used was an aqueous solution of 0.5 percent carboxymethyl-cellulose, 0.1 percent Tween 80.

No statistically significant adverse effects on mating performance, gestation period, or the ability of females to rear their offspring successfully to weaning were observed even at the highest exposure dose (540 mg kg body weight^{-1} day^{-1}). There were no treatment-related changes, (macroscopic appearance, weight measurements or histopathological examination), to the reproductive and alimentary tracts (top dose only) in either sex of the F_0 generation (see

Table 4.4). No exposure dose had an adverse effect on reproductive performance although there was a slight reduction in mean pup weight in the 540 mg kg body weight^{-1} day^{-1} group. If the small reduction in pup weight is considered to be a toxicological effect rather than a physiological effect, (the litter size was higher than controls), the NOEL for offspring is 180 mg kg body weight^{-1} day^{-1}.

Treatment of rats at 180 mg kg body weight^{-1} day^{-1} produced post-dosing salivation and a slight decrease in food consumption in females during the first week of treatment. Treatment at the highest dose, (540 mg kg body weight^{-1} day^{-1}), produced post-dosing salivation in all animals, slightly reduced food consumption in females, (first week of treatment only), reduced body weights in males and slight reduction in mean pup weights on days 12 and 21 post partum which was attributed to the higher litter size. The study concluded the parental NOEL, based on lower mean body weights in male rats, was 180 mg kg body weight^{-1} day^{-1}. In the study post-dosing salivation was not regarded as being indicative of a toxic response.

A two-generation reproduction study conducted to OECD Test Guideline 416 (TSCA Part 798) and to GLP has been carried out with pure BADGE (~ 99 % diglycidyl ether of Bisphenol A) (Hanley *et al* 1996). In the study Sprague-Dawley rats were administered dose levels of 0, 50, 540 and 750 mg kg body weight^{-1} day^{-1} by oral gavage. Body weights for adult males were decreased by approximately 8 to 11 % at dose levels of 540 and 750 mg kg body weight^{-1} day^{-1} in both the F_1 and F_2 generations. In females, body weights were also affected in both generations, but only at the highest dose level (750 mg kg body weight^{-1} day^{-1}). Secondary changes in absolute and/or relative liver and kidney weights were also observed in these dose groups. There were no treatment-related effects on body weights among males given 50 mg kg body weight^{-1} day^{-1} or among females given 50 or 540 mg kg body weight^{-1} day^{-1} in either generation.

There were no statistically significant treatment-related effects on reproductive performance and no treatment-related histopathological changes in any dose group (see Table 4.4). In addition to assessing reproductive endpoints, the study included the microscopic examination of all of the tissues from ten females and ten males of the first generation adults. No histopathological changes were found in any of the tissues with the exception of some irritation and inflammation in nasal tissue, which could be attributed to gastric reflux of the dosing solution as result of repeated gavage administration (Crissman 1997). In the study the NOEL for adult males was considered to be 50 mg kg body weight^{-1} day^{-1} and the NOEL for adult females was 540 mg kg body weight^{-1} day^{-1}. The NOEL for reproductive effects was 750 mg kg body weight^{-1} day^{-1}.

C. Developmental and teratogenicity studies

Developmental/teratogenicity studies have been carried out with administration of BADGE via the oral exposure route in female rats (Smith *et al* 1988a,b) (see Table 4.4) and the dermal route in New Zealand White rabbits (Breslin *et al* 1986, 1988)(see Table 4.5).

Smith *et al* (1988a) dosed groups of pregnant female rats with BADGE daily at levels of 0, 60, 180 and 540 mg kg body weight^{-1} day^{-1} through days 6 to 15 of gestation. The method followed OECD Test Guideline 414 (OECD 1981) and was carried out to GLP. The study showed no adverse effects on mean litter size or pre- and post-implantation losses even at the highest dose (540 mg kg body weight^{-1} day^{-1}). In addition, there were no adverse effects on embryo and foetal development as assessed by overall incidence and types of malformations, visceral and skeletal abnormalities at any dose level. The no observed effect level (NOEL) for foetotoxicity and developmental toxicity was reported as 540 mg kg body

weight^{-1} day^{-1}. Pregnant female rats in the 540 mg kg body weight^{-1} day^{-1} dose group showed signs of post-dosing salivation. However, on the basis of the assumption that the post-dosing salivation was not indicative of a toxic response *per se* the NOEL for maternal toxicity was reported as 540 mg kg body weight^{-1} day^{-1}.

Smith *et al* (1988b) dosed groups of pregnant female New Zealand White rabbits daily with BADGE by oral gavage at levels of 0, 20, 60 and 180 mg kg body weight^{-1} day^{-1} through days 7 to 19 of gestation. The method followed OECD Test Guideline 414 and was carried out to GLP. The study showed no adverse effects on mean litter size or pre- and post-implantation losses even at the highest dose (180 mg kg body weight^{-1} day^{-1}). In addition, there were no adverse effects on embryo and foetal development as assessed by overall incidence and types of malformations, visceral and skeletal abnormalities at any dose level. The no observed effect level (NOEL) for foetotoxicity and developmental toxicity was reported as 180 mg kg body weight^{-1} day^{-1}. The rabbits treated at 180 mg kg body weight^{-1} day^{-1} showed a decrease in food consumption and resulting weight loss as compared to controls. The no observed effect level (NOEL) for maternal toxicity (as weight reduction) was established at 60 mg kg body weight^{-1} day^{-1}.

Breslin *et al* (1986) conducted an initial assessment (to GLP) of the developmental effects of BADGE (99.3% purity) using New Zealand White rabbits. BADGE was applied dermally each day to pregnant females from gestation days 6 to 18 at doses of 0, 100, 300 and 500 mg kg body weight^{-1} day^{-1}. No embryo toxicity was observed among pregnant rabbits at any of the test doses. The no observed effect level (NOEL) for foetotoxicity and developmental toxicity was reported as 500 mg kg body weight^{-1} day^{-1}. The pregnant rabbits in the 100 mg kg body weight^{-1} day^{-1} dose exhibited minimal evidence of maternal toxicity. However, significant maternal toxicity was observed among pregnant rabbits in the 300 and 500 mg kg body weight^{-1} dose groups as evidenced by a dose-related increase in erythema, cracking (slight break), fissuring (linear open wound) and edema or crust at the site of application. The no observed effect level (NOEL) for maternal toxicity was established at 100 mg kg body weight^{-1} day^{-1}.

In a subsequent definitive teratogenicity study Breslin *et al* (1988) applied BADGE (>99.1% purity) to pregnant New Zealand White rabbits. BADGE was applied from gestation day 6 to 18 for 6 hours each day at doses of 0, 30, 100 and 300 mg kg body weight^{-1} to the clipped skin in a polyethylene glycol carrier. Foetuses were examined for external, visceral and skeletal alterations on day 28 of gestation[2].

There were generally no significant differences in reproductive parameters (including % females pregnant and numbers of litters) or foetal observations (including live foetuses per litter, foetal body weight and foetal sex ratios) between the control and treatment groups with the exceptions being:

[2] Observations carried out were for acephaly, omphalocele, forelimb flexure, hydrocephaly, multiple cardiac malformations, persistent trunchus arteriosus, retracaval ureter, skull malformations (delayed ossification of the hyoid, crooked hyoid) vertebrae malformations (delayed ossification of the atlas, extra ossification of the atlas, delayed ossification of the dentoid process of the axis, extra site of ossification of the axis and spur, fused hemi or scoliosis vertebrae) centrum malformation (extra site of ossification or delayed ossification) sternabrae malformations (fused or delayed ossification), rib malformations (extra cervical, fused, forked, calloused, assymmetry or delayed ossification) and flexed digits

- A statistically significant decrease in the pregnancy rate of rabbits in the 30 mg kg body weight^{-1} day^{-1} dose (77%) compared to the controls (100%). No effects relative to the controls were seen at the 100 and 300 mg kg body weight^{-1} day^{-1} doses (81% and 86% pregnancy rate respectively).

- A change in the foetal sex ratio in the in the 30 mg kg body weight^{-1} day^{-1} dose (40% males, 60% females) compared to the controls (50% males, 50% females). No effects relative to the controls were seen at the 100 and 300 mg kg body weight^{-1} day^{-1} doses (45% males, 55% females and 49% males, 51% females respectively).

In the paper these effects were considered random events in view of the lack of a monotonic dose response for these parameters.

No statistically significant increases in malformations or variations of foetuses were observed in any treatment group when compared with the control group. A total of 8 foetuses out of 540 examined for external and skeletal malformations and 312 examined for visceral malformations throughout the dose groups exhibited malformations. One control group foetus (from 141 foetuses examined for external and skeletal malformations and 79 examined for visceral malformations) exhibited omphalocele. In the 30 mg kg body weight^{-1} day^{-1} dose group one foetus (from 136 foetuses examined for external and skeletal malformations and 80 examined for visceral malformations) exhibited omphalocele and one exhibited multiple cardiac malformations consisting of cardiac inversion with associated malposition of cardiac vessels, persistent trunchus arteriosus and hypoplasia of the ventricles. This foetus also exhibited acephaly with an associated rudimentary cranial ossification, thoracic scoliosis and a misshapen atlas. In the 100 mg kg body weight^{-1} day^{-1} dose group one foetus (from 136 foetuses examined for external and skeletal malformations and 80 examined for visceral malformations) exhibited multiple cardiac malformations consisting of cardiac hypertrophy and distension with associated hypertrophy of the tricuspid valve and ventricular septal defect. This same foetus also exhibited a fused and hemivertebra, a forked rib and callosed ribs. A second foetus in this dose group exhibited persistent trunchus arteriosus. In the 300 mg kg body weight^{-1} day^{-1} dose group three foetuses (from 128 foetuses examined for external and skeletal malformations and 73 examined for visceral malformations) exhibited malformation: one foetus exhibited hydrocephaly, a second foetus exhibited persistent trunchus arteriosus and a third foetus exhibited a hemi-vertebra, fused ribs and a forked rib. All the malformations observed within a dose group occurred at low frequencies. Furthermore most of the malformations had also been observed at low frequencies in historical control data for New Zealand White rabbits generated in the test laboratory.

Significant maternal toxicity was observed among pregnant rabbits in the 300 mg kg body weight^{-1} day^{-1} dose group as evidenced by moderate to severe erythema, fissures, haemorrhage and slight edema at the exposure site. Similar, but less severe skin lesions were observed in pregnant rabbits in the 100 mg kg body weight^{-1} day^{-1} dose group. Skin effects (slight erythema) observed in pregnant rabbits in the 30 mg kg body weight^{-1} day^{-1} dose group were not considered toxicologically significant since this response was indistinguishable from the erythema caused by the occlusive bandage jackets which were used hold the test material in place.

The no observed effect level (NOEL) for both foetotoxicity and developmental toxicity and reproductive parameters was considered to be 300 mg kg body weight^{-1} day^{-1}.

D. Carcingenicity and oncogenicity studies

Various skin painting studies have been conducted in which pure BADGE or BADGE-based liquid resins have been applied (undiluted or 50 % diluted in acetone) repeatedly to the skin of mice over the animals' lifetime. Microscopic examination of the skin and internal tissues has in the main found no evidence for a carcinogenic effect (Gardiner *et al* 1992). A review of available carcinogenic data by the International Agency for Research on Cancer (IARC) concluded that BADGE showed limited evidence as an animal carcinogen and was not classifiable as to its carcinogenicity to humans (IARC 1999).

Table 4.4 Summary of the data on potential endocrine mediated responses in laboratory mammals following oral exposure

Species	Life stage of the test organism at start of test	Exposure route and dose series	Description of endocrine disruption measurement parameter(s) and effect doses	Reference	Test Relevance	Study Validity
Rat (Fischer 344)	Adult males and females	Oral gavage at 0, 50, 250 and 1000 mg kg body weight^{-1} day^{-1} for 100 days	Treatment related histopathological effects (relative to the controls) observed including: ▪ a slight increase in vacuolisation of the adrenal cortex in the 250 and 1000 mg kg body weight^{-1} day^{-1} groups ▪ slight atrophy of the endometrium of the uterus in the 1000 mg kg body weight^{-1} day^{-1} group ▪ degeneration of the seminiferous tubules in the testes in the 1000 mg kg body weight^{-1} day^{-1} group *(NOEL for effects on endocrine glands and hormone sensitive tissues = 250 mg kg body weight^{-1} day^{-1})*	Stebbins and Dryzga (2001)	Medium	Valid
Rat (Sprague Dawley)	Adult males and females	Oral gavage at 0, 20, 60, 180 and 540 mg kg body weight^{-1} day^{-1} over one generation	No significant effects (relative to the controls) on mating performance, gestation period or the ability to rear offspring to weaning at any test dose No significant effects (relative to the controls) on the reproductive tract in either sex of the F_0 generation at any test dose *(NOEL for reproductive effects = 540 mg kg body weight^{-1} day^{-1})*	Smith *et al* (1989)	Medium	Valid
	Adult males and females	Oral gavage at 0, 50, 540 and 750 mg kg body weight^{-1} day^{-1} over two generations	No significant effects (relative to the controls) on reproductive endpoints or histopathology of tissues at any test dose *(NOEL for reproductive effects = 750 mg kg body weight^{-1} day^{-1})*	Hanley *et al* (1996)	High	Valid
Rat	Pregnant females	Oral gavage at 0, 60, 180 and 540 mg kg body weight^{-1} day^{-1} on gestation days 6 to 15	No significant effects (relative to the controls) on embryo and foetal development at any test dose *(NOEL for foetotoxicity and developmental toxicity = 540 mg kg body weight^{-1} day^{-1})*	Smith *et al* (1988a)	Medium	Valid
Rabbit (New Zealand White)	Pregnant females	Oral gavage at 0, 60, 180 and 540 mg kg body weight^{-1} day^{-1} on gestation days 7 to 19	No significant effects (relative to the controls) on embryo and foetal development at any test dose *(NOEL for foetotoxicity and developmental toxicity = 540 mg kg body weight^{-1} day^{-1})*	Smith *et al* (1988b)	Medium	Valid

Table 4.5 Summary of the data on potential endocrine mediated responses in laboratory mammals following dermal exposure

Species	Life stage of the	Exposure route and	Description of endocrine disruption	Reference	Test	Study

	test organism at start of test	dose series	measurement parameter(s) and effect doses		Relevance	Validity
Rabbit (New Zealand White)	Pregnant females	Exposure by dermal application to 0, 100, 300 and 500 mg kg body weight^{-1} day^{-1} on gestation days 6 to 18	No significant effects (relative to the controls) on embryo and foetal development at any test dose *(NOEL for foetotoxicity and developmental toxicity = 500 mg kg body weight^{-1} day^{-1})*	Breslin *et al* (1986)	Medium	Valid
	Pregnant females	Exposure by dermal application to 0, 30, 100 and 300 mg kg body weight^{-1} day^{-1} on gestation days 6 to 18	No significant effects (relative to the controls) on embryo and foetal development at any test dose *(NOEL for foetotoxicity and developmental toxicity = 300 mg kg body weight^{-1} day^{-1})* Significant effects (relative to the controls) on pregnancy rate and foetal sex ratio at 30 mg kg body weight^{-1} day^{-1} (but not higher doses)	Breslin *et al* (1988)	Medium	Valid

Table 4.6 Summary of the data on potential endocrine mediated responses in laboratory mammals following intra-peritoneal injection

Species	Life stage of the test organism at start of test	Exposure route and dose series	Description of endocrine disruption measurement parameter(s) and effect doses	Reference	Test Relevance	Study Validity
Mice (ICR)	Ovariectomised females	Sub-cutaneous injections of doses up to 1 mg kg body weight^{-1} for 3 consecutive days	No significant effects (relative to the controls) in the weights or histopathology of the uterus or vagina	Ogata *et al* (2001)	Medium	Valid

E. General conclusions on potential endocrine mediated responses in laboratory mammals in in vivo studies

A series of definitive oral and dermal exposure studies (see Table 4.7) conducted to OECD Test Guidelines and GLP (including a multi-generation reproduction study) have provided no evidence that BADGE affects reproduction or developmental endpoints which may be endocrine mediated even in the presence of (high dose) toxicity to parental animals. Exposure of BADGE to pregnant rats and rabbits during the period of organogenesis does not induce any embryo or foetotoxicity or malformations at doses equal to or above those causing maternal toxicity.

Table 4.7 Summary of the potential endocrine mediated responses reported in *in vivo* studies with laboratory mammals

Type of study	Species and exposure route used	Dose series used	NOEL (mg kg body weight^{-1} day^{-1})		Reference
			Potential endocrine mediated responses	Systemic toxicity	
Sub-chronic oral toxicity (OECD 408)	Rat (Fischer 344) – oral exposure	0, 50, 250 and 1000 mg kg body weight^{-1} day^{-1}	250 (Histopathology)	50 (NOAELs - Males and females)	Stebbins and Dryzga (2001)
Reproduction – One generation (OECD 415)	Rat (Sprague Dawley) – oral exposure	0, 20, 60, 180 and 540 mg kg body weight^{-1} day^{-1}	540 (Reproduction)	180 (Males)	Smith *et al* (1989)
Reproduction – Two generation (OECD 416)	Rat (Sprague Dawley) – oral exposure	0, 50, 540 and 750 mg kg body weight^{-1} day^{-1}	750 (Reproduction)	50 (Males) 540 (Females)	Hanley *et al* (1996)
Development/ Teratogenicity (OECD 414)	Rat (Sprague-Dawley – oral exposure	0, 60, 180 and 540 mg kg body weight^{-1} day^{-1}	540 (Foetotoxicity and developmental toxicity)	540 (Maternal toxicity)	Smith *et al* (1988a)
	Rabbit (New Zealand White) – oral exposure	0, 60, 180 and 540 mg kg body weight^{-1} day^{-1}	540 (Foetotoxicity and developmental toxicity)	60 (Maternal toxicity)	Smith *et al* (1988b)
	Rabbit (New Zealand White) – dermal exposure	0, 100, 300 and 500 mg kg body weight^{-1} day^{-1}	500 (Foetotoxicity and developmental toxicity)	100 (Maternal toxicity)	Breslin *et al* (1986)
	Rabbit (New Zealand White) – dermal exposure	0, 30, 100 and 300 mg kg body weight^{-1} day^{-1}	300 (Foetotoxicity and developmental toxicity)	30 (Maternal toxicity)	Breslin *et al* (1988)
Combined chronic toxicity/ Oncogenicity (OECD 453)	No data	-	-	-	-

Significant effects on pregnancy rate and foetal sex ratios in New Zealand White rabbits exposed dermally to BADGE were found in a teratogenicity study at a dose of 30 mg kg body

weight^{-1} day^{-1} though effects were not evident at higher doses (100 and 300 mg kg body weight^{-1} day^{-1})(Breslin *et al* 1988). However, no effects on litter size or sex ratios were found in other studies in rats and rabbits at similar exposure doses (Smith *et al* 1988b, 1989).

A study by Stebbins and Dryzga (2000) recorded a NOEL of 250 mg kg body weight^{-1} day^{-1} for histopathological effects on the rat testis and uterus, though it is not clear whether these changes were endocrine mediated or result of direct toxic action. The available studies provide no consideration of changes in endocrine function (for example changes in hormone levels).

The lowest doses tested in the oral and dermal exposure studies were in the range of 20-30 mg kg body weight^{-1} day^{-1} and no effects which may be endocrine mediated were evident at these doses. Available evidence from a Uterotrophic screening assay (Ogata *et al* 2001) indicates that no effects on uterine weight occurred after sub-cutaneous injections of 1 mg kg body weight^{-1}.

The International Agency for Research on Cancer (IARC) again reviewed the extensive number of studies conducted on BADGE in 1999. IARC judged that these studies do not indicate that BADGE causes cancer. The importance of this is that in these studies there was not a single biological response indicative of endocrine-mediated changes (for example, an increase in incidence of breast cancer).

4.4.1.3 Human studies

No information on endocrine mediated responses of workers or consumers following exposure to BADGE have been identified. The information that is available primarily relates to skin sensitisation and irritation (Draft OECD SIDS dossier, OECD 1999).

4.4.2 Studies relevant to the assessment of potential endocrine disrupting effects in wildlife

4.4.2.1 In vitro studies

No data has been located on the conduct of *in vitro* studies using cells and tissues from wildlife species.

4.4.2.2 In vivo studies

A. Studies on aquatic organisms

The only data that has been located which is relevant to the assessment of potential endocrine disrupting effects of BADGE on aquatic species is a 21-day reproduction study in *Daphnia magna* (Thorpe 1984). In the study, neonates (<24 h old) were exposed to nominal concentrations of EPIKOTE 828 of 0, 0.03, 0.1, 0.3, 1.0 and 3.0 mg l^{-1}. The study evaluated the day when the first brood was produced, the number of live young produced per surviving adult on day 21, the adult mean length at day 21 and mortality (%) over the 21 days. The results showed that reproduction, adult growth and survival were all significantly reduced at 1 mg l^{-1} of EPIKOTE 828, but were unaffected at 0.3 mg l^{-1}. The reduction in numbers of offspring at 1 mg l^{-1} was 18% compared to that for the control organisms. No significant effect of EPIKOTE 828 on mean time to first brood was evident at any exposure concentration. The

NOEC for reproduction was reported as 0.3 mg l^{-1}. However, there is no information on the mechanism of action responsible for the effects on *Daphnia magna* reproduction.

The effects observed in the *Daphnia magna* reproduction test are probably not caused by direct oestrogenic effects since other studies have shown an absence of reproductive impairment at 0.39 mg l^{-1} when animals are exposed to the synthetic steroid 17α-ethinylestradiol (Schweinfurth *et al* 1996).

B. Studies on terrestrial organisms

No data has been located on the potential endocrine disrupting effects of BADGE on terrestrial organisms. The potential for BADGE to bind to organic carbon (see Section 4.1) means that the absence of data on terrestrial organisms represents an area of uncertainty with regard to the potential endocrine effects of the substance on wildlife. However, it should also be recognised that there are currently no internationally agreed methods specifically developed to assess endocrine disrupting effects in terrestrial organisms.

C. Studies on aerial organisms

No data has been located on the potential endocrine disrupting effects of BADGE on aerial organisms. However, given that BADGE is not volatile (see Section 4.1) the absence of data on aerial organisms does not represent a key area of uncertainty with regard to the potential endocrine effects of the substance. It should also be recognised that there are currently no internationally agreed methods specifically developed to assess endocrine disrupting effects in aerial organisms.

D. General conclusions on potential endocrine mediated responses in in vivo studies with wildlife species

The data that has been located on the potential endocrine disrupting effects of BADGE on wildlife is limited to one study on the effects on the reproduction on the water flea *Daphnia magna* (see Table 4.8), though there is no information on the mechanism of action for the effects. There is an absence of data for other wildlife species particularly in relation to reproduction and development in fish and terrestrial organisms.

Table 4.8 Summary of the studies assessing potential endocrine mediated responses in wildlife

Environmental compartment	Taxonomic group	Type of study	Species and exposure route used	Concentration series used	Lowest reported NOEC	Reference
Aquatic	Amphibians	No data	-	-	-	-
	Fish	No data	-	-	-	-
	Invertebrates	Reproduction (OECD TG 211)	*Daphnia magna* – aqueous exposure	No data	0.3 mg l^{-1} (a)	Thorpe (1984)
Terrestrial	Birds	No data	-	-	-	-
	Invertebrates	No data	-	-	-	-
Aerial	Invertebrates	No data	-	-	-	-

a – No information is available on the mechanism of action

4.5 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity

The general toxicity data in this Section has been taken from the Draft OECD SIDS dossier (OECD 1999) and has been taken as accurate. Individual source documents have not been checked unless they are considered to be key studies which have a major influence on the outcome of the review. All information taken from the Draft OECD SIDS dossier has been referenced as being from that source and individual references have not been given in the references.

4.5.1 Studies relevant to the assessment of general toxicity in humans

Table 4.9 summarises the general toxicity data from acute and repeat dose studies with BADGE.

4.5.1.1 Acute studies

A. Oral exposure

In single-dose oral toxicity tests with BADGE LD_{50} values of greater than >1000 mg kg body weight^{-1} have been recorded in studies with rats, mice and rabbits (see Table 4.9). In full dose tests oral LD_{50} values for a commercial BADGE-based epoxy resin were reported to be 11400 mg kg body weight^{-1} in rats, 15600 mg kg body weight^{-1} in mice, and 19800 mg kg body weight^{-1} in rabbits (Hine *et al* 1958). Using a commercial BADGE-based epoxy resin Weil *et al* (1963) reported an oral LD_{50} of 19.6 ml kg body weight^{-1} (~19600 mg kg body weight^{-1}) for rats. All of the various acute oral toxicity studies which have been undertaken with both pure BADGE and commercial BADGE-based resins have produced consistent results. While none of the studies meet all the conditions of a GLP guideline investigation many of them meet the scientific principals for assessment of an oral LD_{50} in rats.

B. Dermal exposure

A number of studies have investigated the acute toxicity in rats, mice and rabbits following dermal exposure to pure BADGE and commercial BADGE based resins (see Table 4.9). The values recorded range from >1200 to > 2400 mg kg body weight^{-1} for rats, > 800 mg kg body weight^{-1} for mice and > 3000 to > 20000 mg kg body weight^{-1} for rabbits.

C. Inhalation exposure

No data has been located on the acute toxicity of BADGE to laboratory mammals following inhalation exposure. Gardiner *et al* (1992) stated that the inhalation toxicity of DGEBPA or EGEBPA resins had not been studied at that time for two reasons: a) the inhalation exposure is unlikely and b) the material has a low vapour pressure. Nolan *et al* (1981) reported difficulty in generating an atmosphere at a respirable temperature (approximately 22 ^{o}C) that would contain sufficient DGEBPA to conduct a rodent inhalation study, even when large surface areas and high temperatures were used to initially generate an atmosphere before cooling to respirable temperatures.

D. Other routes of exposure

No data has been located on the acute toxicity of BADGE to laboratory mammals following exposure by sub-cutaneous or intra-peritoneal injection.

4.5.1.2 Repeat dose studies

A large number of repeat dose studies are given in the Draft OECD SIDS dossier with varying degrees of information on the findings being reported. Table 4.9 summarises these studies but this section focuses on the results with the lowest NOELs or information on mechanisms of action which are relevant to this review.

A. Oral exposure

In a sub-chronic dietary study male rats were fed pure BADGE in their diets for three months at concentrations of 0, 0.1, 0.3, 1.0 or 3.0% (Wolf 1958). Rats at the highest level rejected the diets and failed to gain weight; these rats showed effects upon gross and histopathological examination that were consistent with under-nutrition. Liver and kidney weights were higher, (as compared to controls), in the animals on the 3% diet. Animals in the 1% dose group exhibited slight enlargement of the kidneys with no adverse effects being seen in the 0.3 and 0.1% dose groups. No evidence of systemic toxicity, characterized by changes in haematology, clinical chemistry, gross pathology or histopathology of the spleen, heart, liver, kidney or adrenal glands, was found in any of the treated animals.

In another sub-chronic study a commercial low molecular weight BADGE epoxy resin (EPON 828) was fed to male Long-Evans rats at dietary concentrations of 0.2, 1.0 and 5.0 percent for 26 weeks (Hine *et al* 1958). All rats at the highest dose died by the end of 2 weeks, but gross and histopathological examination did not reveal additional evidence of systemic toxicity at any dose. There was no statistical increase in liver:body weight ratios, but there was a significant increase in kidney weights in groups fed 0.2 and 1.0% of EPON 828.

In an oral toxicity GLP guideline study, (based on the EEC Directive 79/831, Annex V/Part 4.2.1 Sub-acute toxicity oral), 5 male and 5 female rats, (RA1f SPF), were treated, by oral gavage, with a commercial grade low molecular weight BADGE epoxy resin, (Araldite GY 250) for 28 consecutive days (Basler *et al* 1984). The doses used were 0, 50, 200 and 1000 mg kg body weight^{-1} day^{-1}. These treatments had no effect on body weights, food and water consumption, food conversion, mortality, eye and hearing tests, haematology, blood chemistry, organ weights and pathology. There were no clinical symptoms and there were no compound-related macroscopic or histopathological changes of the spleen, heart, liver, kidney or adrenal gland. The no observable effect level (NOEL) was reported as 1000 mg kg body weight^{-1}.

B. Dermal exposure

In addition to the sub-chronic oral studies a number of investigations, in both the rat and mouse, have been undertaken to examine toxicity (and irritation) following dermal exposures.

In a study in rats (Fischer 344) Redmond *et al* (1995b) exposed groups of male and females 3 or 5 times per weeks (7 or 12 applications) to 0, 10, 100, 500 and 1000 mg DGEBPA (purity 99.7%) in acetone kg body weight^{-1} application^{-1} in a study conducted to OECD Test Guideline 410 and GLP. Repeated dermal application of DGEBPA caused very slight erythema at the site of dosing in male rats dosed 3 or 5 days per week with 1000 mg kg^{-1} application^{-1} on test

day 15. No significant dermal irritation was observed at dosages up to 500 mg kg^{-1} $application^{-1}$ in male rats or 1000 mg kg^{-1} $application^{-1}$ in female rats. In addition, no evidence of toxicity was observed at any dosage based on clinical observations, body weights, or gross pathology. Histopathologically, chronic dermatitis was induced in males treated with 100, 500 or 1000 mg kg^{-1} $application^{-1}$ and females treated with 500 or 1000 mg kg^{-1} $application^{-1}$ DGEBPA, following either the 3 or 5 times per week dosing regimens.

In the study with rats (Redmond *et al* 1996b) no apparent systemic toxicity with the exception of decreased body weight and body weight gain in male and females at 1000 mg kg^{-1} $application^{-1}$. Food consumption was also slightly lower for both males and females throughout the duration of the study. Serum cholesterol values were increased in a dose-related manner in mid- and high dose level rats of both sexes, but considered of questionable toxicological significance since no correlated histopathological changes were observed. Female rats dosed with 1000 mg kg^{-1} $application^{-1}$ three times per week (high-dose satellite group) for the same duration showed no signs of systemic toxicity. Histopathologic examination of tissues conducted on all test animals resulted in treatment-related effects only at the site of dermal application of test material. Epidermal hyperplasia with chronic inflammation, characterized as chronic dermatitis, was observed histopathologically at all dose levels in male rats and at dosages of 100 and 1000 mg kg^{-1} $application^{-1}$ in female rats (including the high-dose satellite group).

Hend *et al* (1977a) exposed male and female mice (CF1) dermally to samples of commercial low molecular weight BADGE epoxy resin (EPON 828) dissolved in acetone (0, 0.5, 1, 5 and 10% w/v) or toluene (0, 1, 5, 10 and 20 % w/v). Animals were exposed twice weekly for a period of 4 weeks. Male and female mice exhibited severe irritancy after 2 weeks exposure to toluene solvent alone. By the end of the study the degree of the irritancy had decreased and hair growth had recommenced. Test material, when applied as 1%, 5%, 10% and 20% w/v solutions in toluene, produced skin irritation after 2 weeks which was still present at 4 weeks. Using acetone solvent no skin irritation was seen during any part of the study when material was administered as 0%, 0.5%, 1%, 5% or 10% w/v solutions.

In a study in male mice (B6C3F1) conducted to OECD Test Guideline 410 and GLP groups of males were exposed 3 or 5 times per weeks (7 or 12 applications) to 0, 10, 100, 500 and 1000 mg DGEBPA (purity 99.7%) in acetone kg body $weight^{-1}$ $application^{-1}$ (Redmond *et al* 1995a). No grossly observable dermal irritation was found at the site of dosing in male mice dosed up to 1000 mg kg^{-1} $application^{-1}$ following either the 3 or 5 day regimen. In addition, no evidence of toxicity was observed based on in-life clinical observations, body weights, or gross pathology. Histopathologically, DGEBPA caused chronic active dermatitis in male B6C3F1 mice at all dose levels regardless of the dosing regimen (3 or 5 times per week). The occurrence of follicular spongiosis at 1000 mg kg^{-1} day^{-1} indicates that the maximum tolerated dose (MTD) had been exceeded. The MTD for dermal effects was between 100 and 500 mg kg^{-1} $application^{-1}$ when administered 3 times/week and was exceeded at 10 mg kg^{-1} $application^{-1}$ when administered 5 days per week.

In subsequent 13 week studies (Redmond *et al* 1996a,b) dermally exposed groups of male mice (B3C6F1) and rats (Fischer 344) to DGBEPA (purity 99.7%) in acetone in studies carried out to OECD Test Guideline 411 and GLP. The mice were exposed 3 days per week to doses of 0, 1, 10 and 100 mg DGEBPA in acetone kg body $weight^{-1}$ $application^{-1}$, while rats were exposed 5 days per week to 0, 1, 10 and 100 mg DGEBPA in acetone kg body $weight^{-1}$ $application^{-1}$. An additional 1000 mg kg body $weight^{-1}$ dose was used in the study with rats with applications 3 times per week.

In the study with mice (Redmond *et al* 1996a) no systemic toxicity was observed but mild to moderate chronic active dermatitis with a weak dose-response was observed histopathologically at dosages up to 100 mg kg^{-1} $application^{-1}$. Spongiosis and epidermal micro abscess formation indicated that the maximum tolerated dose (MTD) was met in mice administered 100 mg kg^{-1} $application^{-1}$.

Overall dermal applications of BADGE, up to 1000 mg kg body $weight^{-1}$ $application^{-1}$ 5 days a week for 13-weeks does not appear to cause systemic toxicity. Most of the studies on rats or mice have however reported dermal irritation/dermatitis with a NOEL in the range of 10 mg kg body $weight^{-1}$ $application^{-1}$ (Hend *et al* 1977a, Redmond *et al* 1995a,b, 1996a,b)

C. Inhalation exposure

No data has been located on the repeat dose toxicity of BADGE to laboratory mammals following inhalation exposure (see Section 4.5.1.1 C).

D. Other routes of exposure

No data has been located on the repeat dose toxicity of BADGE to laboratory mammals following exposure by sub-cutaneous or intra-peritoneal injection.

4.5.1.3 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity in mammals

The lowest NOEL identified from the review of data on potential endocrine mediated responses in laboratory mammals was 250 mg kg body $weight^{-1}$ day^{-1} based on an assessment of histopathological effects in endocrine glands and hormone sensitive tissues (Stebbins and Dryzga 2001), though these effects may have resulted from direct toxic action. In a one and two generation reproduction studies (Smith *et al* 1989, Hanley *et al* 1996) and developmental/teratogenicity studies (Smith *et al* 1988a,b; Breslin *et al* 1986, 1988) no effects on reproductive or developmental endpoints which may be endocrine mediated have been identified even at the highest doses tested. In these studies lower NOELs were identified for maternal toxicity (see Table 4.7).

In acute and repeat-dose studies the general systemic toxicity data for laboratory mammals indicates that the threshold in rats for an absence of effects which are not directly endocrine mediated occurs at a dose of approximately 10 mg kg body $weight^{-1}$ day^{-1} (Redmond *et al* 1996b). Most of the studies on rats or mice have reported dermal irritation/dermatitis with a NOEL in the range of 10 mg kg body $weight^{-1}$ $application^{-1}$ (Hend *et al* 1977a, Redmond *et al* 1995a,b, 1996a,b). In an oral exposure study in rats by Stebbins and Dryzga (2001) a dose of 50 mg kg body $weight^{-1}$ day^{-1} was interpreted to be the no observed adverse effect level (NOAEL). As a result it appears that on the basis of the available data that endocrine mediated responses are probably not the mechanism responsible for the most toxic effects observed in laboratory mammals.

Table 4.9 Summary of general mammalian toxicity data (Information from the Draft OECD SIDS dossier, OECD 1999)

Test type	Test species	Exposure period	Test dose series used	Endpoint	Effect dose	Reference	Study validity
Acute Oral Toxicity	Rat	Not relevant	No data	Median lethal dose (LD_{50})	5477 mg kg body weight^{-1}	Rowe (1948)[1]	Use with care[2]
	Rat	Not relevant	10% suspension in corn oil	Median lethal dose (LD_{50})	>2000 mg kg body weight^{-1}	Wolf (1956)[1]	Use with care[2]
	Rat (Long Evans)	Not relevant	No data	Median lethal dose (LD_{50})	11400 mg kg body weight^{-1}	Hine *et al* (1958)[1]	Valid[2]
	Rat	Not relevant	No data	Median lethal dose (LD_{50})	>3890 mg kg body weight^{-1}	Olson (1958)[1]	Use with care[2]
	Rat	Not relevant	No data	Median lethal dose (LD_{50})	~19600 mg kg body weight^{-1}	Weil *et al* (1963)[1]	Use with care[2]
	Rat (Long Evans)	Not relevant	No data	Median lethal dose (LD_{50})	>15000 mg kg body weight^{-1}	Hine *et al* (1958)[1]	Use with care[2]
	Rat (Wistar)	Not relevant	20% w/v solution in acetone	Median lethal dose (LD_{50})	>1000 mg kg body weight^{-1}	Hend *et al* (1977)[1]	Use with care[2]
	Rat (Wistar albino)	Not relevant	No data	Median lethal dose (LD_{50})	>1000 mg kg body weight^{-1}	Clark and Cassidy (1978)[1]	Use with care[2]
	Rat (Sprague Dawley)	14 day	No data	Median lethal dose (LD_{50})	>2000 mg kg body weight^{-1}	Lockwood and Taylor (1982)[1]	Use with care[2]
	Rat (Fischer 344)	Not relevant	No data	Median lethal dose (LD_{50})	>5000 mg kg body weight^{-1}	Price (1986)[1]	Use with care[2]
	Mice (Webster)	Not relevant	No data	Median lethal dose (LD_{50})	15600 mg kg body weight^{-1}	Hine *et al* (1958)[1]	Valid[2]
	Mice (CFI)	Not relevant	No data	Median lethal dose (LD_{50})	>500 mg kg body weight^{-1}	Hend *et al* (1977)[1]	Use with care[2]
	Rabbits	Not relevant	No data	Median lethal dose (LD_{50})	19800 mg kg body weight^{-1}	Hine *et al* (1958)[1]	Valid[2]

Table 4.9 Continued

Test type	Test species	Exposure period	Test dose series used	Endpoint	Effect dose	Reference	Study validity
Acute Dermal Toxicity	Rat (Wistar)	Not relevant	Commercial low molecular weight BADGE resin as 20% w/v solution in acetone	Median lethal dose (LD_{50})	>1200 mg kg body weight^{-1}	Hend *et al* (1977) [1]	Use with care[2]
	Rat (Wistar albino)	Not relevant	Solution of commercial low molecular weight BADGE resin	Median lethal dose (LD_{50})	>1600 mg kg body weight^{-1}	Clark and Cassidy (1978) [1]	Use with care[2]
	Rat (Wistar)	Not relevant	Samples of purified DGEBPA as 20% w/v solution in acetone	Median lethal dose (LD_{50})	>1600 mg kg body weight^{-1}	Hend *et al* (1978) [1]	Use with care[2]
	Rat (Fischer 344)	Not relevant	Single 24-hour percutaneous exposure to commercial low molecular weight BADGE resin	Median lethal dose (LD_{50})	>2000 mg kg body weight	Price (1986) [1]	Use with care[2]
	Mice (CF1)	Not relevant	Commercial low molecular weight BADGE resin as 20% w/v solution in acetone	Median lethal dose (LD_{50})	>800 mg kg body weight^{-1}	Hend *et al* (1977) [1]	Use with care[2]
	Mice (CF1)	Not relevant	Samples of purified DGEBPA as 20% w/v solution in acetone	Median lethal dose (LD_{50})	>800 mg kg body weight^{-1}	Hend *et al* (1978) [1]	Use with care[2]
	Rabbit	Not relevant	Solution of commercial low molecular weight BADGE resin	Median lethal dose (LD_{50})	>20000 mg kg body weight (<20 ml kg^{-1})	Weil *et al* (1963) [1]	Use with care[2]
	Rabbit (New Zealand albino)	Not relevant	Single 24-hour percutaneous exposure to commercial low molecular weight BADGE resin	Median lethal dose (LD_{50})	>3000 mg kg body weight (<3 ml kg^{-1})	Hine *et al* (1958) [1]	Use with care[2]

Table 4.9 Continued

Test type	Test species	Exposure period	Test dose series used	Endpoint	Effect dose	Reference	Study validity
Acute Dermal Toxicity	Rabbit (New Zealand albino)	14 day	Solution of commercial low molecular weight BADGE resin	Median lethal dose (LD_{50})	>2000 mg kg body weight^{-1}	Lockwood *et al* (1982) [1]	Use with care[2]
Acute Inhalation Toxicity	No data	-	-	-	-	-	-
Acute Toxicity (Intra-peritoneal injection	No data	-	-	-	-	-	-
Repeat Dose Toxicity (Oral)	Rats - males	90 days	0, 0.1, 0.3, 1.0 and 3.0 % pure BADGE daily in diet	NOEL	0.3 % dose	Wolf (1958)	Valid
	Rat (Long Evans) - males	182 days	0, 0.2, 1.0 and 5% commercial low molecular weight BADGE epoxy resins in diet	NOEL	1.0% dose	Hine *et al* (1958)	Valid
	Rat (Ralf, SPF) – males and females	28 days	0, 50, 200 and 1000 mg kg^{-1} day^{-1} in 0.1% Tween 80 daily by oral gavage	NOEL	1000 mg kg^{-1} day^{-1}	Basler *et al* (1984)	Valid

Table 4.9 Continued

Test type	Test species	Exposure period	Test dose series used	Endpoint	Effect dose	Reference	Study validity
Repeat Dose Toxicity (Dermal)	Rat (Fischer 344) – males and females	14 days	Dermal application of 0, 10, 100, 500 and 1000 mg DGEBPA in acetone kg body weight^{-1} application^{-1} 3 or 5 days a week	MTD	Not determined	Redmond *et al* (1995b)	Valid
	Rat (Fischer 344) – males and females	90 days	Dermal application of 0, 10 and 100 mg DGEBPA in acetone kg body weight^{-1} application^{-1} 3 or 5 days a week	NOEL (female) NOEL (male)	10 mg kg body weight^{-1} application^{-1} Not determined	Redmond *et al* (1996b)	Valid
	Mouse (CF1)	28 days	Dermal application of 0, 0.5%, 1%, 5% and 10% in acetone or 0, 1%, 5% 10% or 20% toluene	Not reported	Not reported	Hend *et al* (1977a)	Valid
	Mice (B6C3F1) – males and females	14 days	Dermal application of 0, 10, 100, 500 and 1000 mg DGEBPA in acetone kg body weight^{-1} application^{-1} 3 or 5 days a week	MTD (3 doses per week) MTD (5 doses per week)	>100 mg kg body weight^{-1} application^{-1} <10 mg kg body weight^{-1} application^{-1}	Redmond *et al* (1995a)	Valid
	Mice (B6C3F1) – males and females	90 days	Dermal application of 0, 10 and 100 mg DGEBPA in acetone kg body weight^{-1} application^{-1} 3 days a week	MTD	100 mg kg body weight^{-1} application^{-1}	Redmond *et al* (1996a)	Valid

[1] – Cited in Draft OECD SIDS dossier (OECD 1999), [2] – Assessment made on basis of data in Draft OECD SIDS dossier (OECD 1999)

4.5.2 Studies relevant to the assessment of general toxicity in wildlife

4.5.2.1 Studies on aquatic organisms

Table 4.10 summarises the general toxicity data for aquatic organisms exposed to BADGE.

A. Fish

Acute toxicity

BADGE has been assessed in a number of static acute fish toxicity studies following OECD Test Guideline 203 but not conducted to GLP and without analytical monitoring of exposure concentrations. The 96-hour LC_{50} values (based on nominal concentrations) for fathead minnows *(Pimephales promelas),* rainbow trout *(Oncorhynchus mykiss)* and zebrafish *(Danio rerio)* were calculated as 3.1 mg l^{-1}, 1.5 mg l^{-1}, and 2.4 mg l^{-1} respectively, (Draft OECD SIDS 1999). The corresponding 96-hour LC_0 and 96-hour LC_{100} values were 1.0 and 3.0 mg l^{-1} for rainbow trout and 1.8 and 3.2 mg l^{-1} for zebrafish.

Chronic toxicity

No chronic toxicity data for fish following exposure to BADGE has been located.

B. Invertebrates

Acute toxicity

BADGE has been assessed in a series of static *Daphnia magna* immobilisation and lethality studies following OECD Test Guideline 202 but not conducted to GLP and without analytical monitoring of exposure concentrations. A study by Ciba-Geigy (1985) found 24-hour LC_0, LC_{50} and LC_{100} values (based on nominal concentrations) of 1.7, 3.6 and 7.8 mg l^{-1}. Bailey and Rhinehart (1976) investigated the toxicity of a commercial low molecular weight BADGE epoxy resin (DER 331) and found 48-hour LC_{10}, LC_{50} and LC_{90} values (based on nominal concentrations) of 0.16, 0.95 and 5.6 mg l^{-1}. Thorpe (1984) tested the toxicity of a commercial low molecular weight BADGE epoxy resin (EPIKOTE 828) and recorded 48-hour EC_{50} values of 1.4 – 1.7 mg l^{-1} (based on nominal concentrations).

Chronic Toxicity

A 21-day study examining mortality and juvenile reproduction in *Daphnia magana* reported a 21-day NOEC values for survival (and reproduction) of 0.3 mg l^{-1} (Thorpe 1984).

4.5.2.2 Studies on terrestrial organisms

No general toxicity data for terrestrial organisms following exposure to BADGE has been located.

4.5.2.3 Studies on aerial organisms

No general toxicity data for aerial organisms following exposure to BADGE has been located.

Table 4.10 Summary of the general toxicity data for aquatic organisms (Information from the Draft OECD SIDS dossier, OECD 1999)

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study validity
Acute Fish Toxicity	Fathead minnows (*Pimephales promelas*)	96 hours	No data	LC_{50}	3.1 mg l^{-1}	Bailey and Rhinehart (1976)[1]	Valid[2]
	Rainbow trout (*Oncorhynchus mykiss*)	96 hours	No data	LC_0	1.0 mg l^{-1}	Ciba Geigy (1982a) [1]	Valid[2]
		"	"	LC_{50}	1.5 mg l^{-1}		
		"	"	LC_{100}	3.0 mg l^{-1}		
	Zebrafish (*Danio rerio*)	96 hours	No data	LC_0	1.8 mg l^{-1}	Ciba Geigy (1982b) [1]	Valid[2]
		"	"	LC_{50}	2.4 mg l^{-1}		
		"	"	LC_{100}	3.2 mg l^{-1}		
Chronic Fish Toxicity	No data	-	-	-	-	-	-
Acute Invertebrate Toxicity	Water flea (*Daphnia magna*)	24 hours	No data	LC_0	1.7 mg l^{-1}	Ciba Giegy (1985) [1]	Valid[2]
			"	LC_{50}	3.6 mg l^{-1}		
			"	LC_{100}	7.8 mg l^{-1}		
		24 hours	No data	LC_{10}	0.16 mg l^{-1}	Bailey and Rhinehart (1976) [1]	Valid[2]
			"	LC_{50}	0.95 mg l^{-1}		
			"	LC_{90}	5.6 mg l^{-1}		
		48 hours	No data	EC_{50} (Immobilisation)	1.4 - 1.7	Thorpe (1984)	Valid
Chronic Invertebrate Toxicity	Water flea (*Daphnia magna*)	21 days	No data	NOEC (Survival)	0.3 mg l^{-1}	Thorpe (1984)	Valid

[1] – Cited in Draft OECD SIDS dossier (OECD 1999), [2] – Assessment made on basis of data in Draft OECD SIDS dossier (OECD 1999)

4.5.2.4 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity in wildlife

Comparison of the limited data on potential endocrine mediated responses in the aquatic invertebrate *Daphnia magna* with the acute and chronic data for this species indicates that the threshold concentration for mortalities (0.3 mg l^{-1}) was the same as the threshold concentration for effects on reproduction (0.3 mg l^{-1}). However, there is no information on the mechanism of action for the effects on *Daphnia magna* reproduction. No comparisons could be made for fish due to the absence of data on endocrine mediated responses in this taxonomic group, which represents an area of uncertainty.

4.6 Current classification of the BADGE against European Commission and national regulations

Table 4.11 summarises the current classification of the substance against Council Directives in order to assess the regulations to which BADGE is subject. Under Directive 67/548/EEC the R phrases indicate that BADGE is toxic to aquatic organisms (R51) and may cause long-term adverse effects in the aquatic environment (R53).

BADGE is an authorised starting substance in food contact materials and regulated under the Commission Directive 2001/61/EC with a Specific Migration Limit of 1 mg kg^{-1} for BADGE and chlorinated derivatives having been recommended by the European Scientific Committee for Food. In Switzerland regulations indicate a 20 μg kg^{-1} detection limit for BADGE. However, it is currently understood that enforcement will be based on a 1 mg kg^{-1} for BADGE plus derivatives level.

Table 4.11 Current classification of BADGE against Council Directives

Directive	Status (listed or not)
67/548/EEC - Classification, packaging and labelling of dangerous substances	Classified: Xi, C R phrases: 36/38-43-51/53
90/128/EEC (20001/61/EC) - Plastic materials and articles intended to come into contact with foodstuffs	Listed (Specific Migration Limit = 1 mg kg^{-1})

No national environmental quality standards have been derived in any European country for the protection of aquatic or terrestrial ecosystems.

4.7 Exposure data

4.7.1 Worker exposure data

Data on concentrations of BADGE to which workers are potentially exposed during production and use was sought from the relevant CEFIC Sector Group and information was provided for the production of both liquid and solid epoxy resins.

4.7.1.1 Liquid epoxy resins

Liquid, (unmodified bisphenol A – epichlorhydrin based), epoxy resins, containing approximately 80-85% of BADGE, are manufactured in closed systems. Potential worker exposure to BADGE is most likely to occur in the manufacturing and industrial application of

formulated epoxy systems with dermal contact being the likely route of exposure. As epoxy resins are however classified as skin/eye irritants and skin sensitizers workers handling epoxy resins wear protective gloves to avoid skin contact. Safety literature from the epoxy resin suppliers provides guidance on the safe handling of epoxy resins including recommended protective equipment. As liquid epoxy resins are highly viscous, non-volatile materials worker exposure through inhalation of BADGE vapours, even at high temperatures, is unlikely to occur.

No in-house monitoring data are available for BADGE from downstream users.

4.7.1.2 Solid epoxy resins

Solid epoxy resins contain a certain level of BADGE as a natural fraction of the standard molecular weight distribution. Depending on average molecular weight, the levels of BADGE in solid epoxy resins might vary between <1% for the highest MW "9" type epoxy resin up to 20% for the lowest MW "1" type epoxy resin.

Solid epoxy resins are manufactured in closed systems. Due to the low volatility of solid epoxy resins vapour inhalation is unlikely. Exposure to dusts can, however, occur in various operations e.g. during bag filling in epoxy manufacturing plants or charging of epoxy resins from bags in paint manufacturing process. In such operations dust exposure is minimized through the installation of local exhaust ventilation and operators wear adequate respiratory protective equipment. To prevent dust exposures low molecular weight "1" type resins are to a large extent supplied in solutions.

In-house monitoring data from German epoxy resin manufacturers showed that the average dust exposure, from 114 measurements, was <4 mg/m^3 and is below the national general dust limit of 4 mg/m^3.

Dust exposure can also occur in some applications including spray application of epoxy powder paints. A total of 210 in-house measurements for epoxy powder applications have been carried out by the United Kingdom powder manufacturing industry and are shown in Table 4.12. The range of personal exposures across four companies for all activities was 0.3 to 10 mg/m^3, as 8h Time Weighted Averages (TWA) for total inhalable particulate. Based on the assumption that epoxy powder paints typically contain between 25-30% of epoxy resins and that powder coating grade epoxy resins contain between 5-10% of BADGE, this would correspond to a theoretical BADGE exposure of 0.009 - 0.3 mg/m^3 which is well below the Dutch exposure limit for BADGE of 10 mg/m^3 as 8h TWA and 5 mg/m^3 as 8h TWA for inhalable and respirable dust, respectively. It should also be remembered that possible exposure to BADGE containing dust is minimised as spraying is normally done in ventilated spray booths with workers wearing adequate respiratory protective equipment.

Due to the skin rash/sensitisation potential of solid epoxy resins, workers handling these materials typically wear protective gloves and exposure through skin contact is minimised. Safety literature from the epoxy resin suppliers provides guidance on the safe handling of solid epoxy resins including recommended protective equipment.

Table 4.12 In-house monitoring data for epoxy dust in epoxy powder coating manufacture (Source: British Coatings Federation - 17th January 2001 submitted to the EU Risk Assessment Programme on Bisphenol A)

Company A

Sample reference	Type of sampling	Activity	Dust exposure (mg/m^3 as 8 h TWA)
1	Personal sampler	Weighing and mixing	3.8
2	Personal sampler	Weighing and mixing	4.7
3	Static point	Weighing	1.6
4	Personal sampler	Pre-mix activities	2.7 – 4.8
5	Static point	Loading chute	0.9
6	Personal sampler	Extrusion	2.2
7	Personal sampler	Milling	0.8 – 4.8
8	Personal sampler	Filling by hand	7.4
9	Personal sampler	Laboratory weighing	1.0
10	Personal sampler	Laboratory extrude	1.3

Company B

Sample reference	Type of sampling	Activity	Dust exposure (mg/m^{-3} as 8 h TWA)	
			Range	Mean
1	-	Raw material assembly	0.5-5.0	2.3
2	-	Weighing	0.3-6.3	2.8
3	-	Premix	1.5-10.0	4.1
4	-	Extrusion	2.4-6.7	4.2
5	-	Milling	0.5-8.2	4.0

Company C (A total of 210 measurements carried out, average 5.0 mg/m^3)

Sample reference	Type of sampling	Activity	Dust exposure (mg/m^3 as 8 h TWA)
1	Personal sampler	Pre weighing	3.0
2	Personal sampler	Extrusion	1.5
3	Personal sampler	Micronising	6.5

Company D

Sample reference	Type of sampling	Activity	Dust exposure (mg/m^3 as 8 h TWA)	
			Range	Mean
1	Personal sampler	Mixing (4 mixers)	0.82-2.6	
2	Static point	Mixing (1 mixer)	0.38	
3	Personal sampler	Milling (8 mills)	1.1-9.4	
4	Static point	Milling (4 mills)	0.55-2.8	
5	-	Milling	0.5-8.2	4.0

4.7.1.3 Summary

The information provided by the CEFIC Sector Group is consistent with that of Gardiner *et al* (1992) in which it was concluded that "The manufacture of glycidyloxy compounds usually occurs in closed systems designed with engineering controls to minimise or prevent human exposure. It is during the use of these materials that there is somewhat greater potential for human exposure. However, since most of the epoxy resin systems have very low volatility vapour inhalation is usually not a concern. There is a possibility of aerosol formation during spray and high-speed applications to webs or continuous filaments. In most instances, however, the primary route of exposure is dermal". Skin contact during the manufacture of BADGE based resin products presents the greatest potential exposure problems as most formulations are viscous sticky liquids which are difficult to remove from the skin. However, due to the skin rash/sensitisation potential of liquid and solid epoxy resins, workers handling these materials typically wear protective gloves and exposure through skin contact is minimised.

Although no epidemiology studies on BADGE-based resins have been conducted, Ruhe *et al* (1975) reported the results of a health hazard evaluation at a manufacturing site using a DGEBPA–based epoxy resin. Based on the results of environmental air measurements, medical questionnaires, pulmonary function tests and skin patch tests, it was concluded that the resin did not represent a health hazard at the concentrations measured during normal operating conditions.

4.7.2 Consumer exposure data

Data on the concentrations of BADGE to which consumers are potentially exposed during the use of the products containing the substance was sought from the relevant CEFIC Sector Group. Information was provided which indicated that negligible consumer exposure is expected from the products to which consumers may be exposed. The major consumer exposure to BADGE is from food and drink cans lined with epoxy based coatings. BADGE is also used in epoxy resins for water distribution pipes and a potential for consumer exposure exists following leaching from the resins. Minor volumes of liquid epoxy resins are used in two-component epoxy glues sold to the public in retailer shops but this potential route of exposure is extremely limited given the volumes typically used.

4.7.2.1 Food and drink cans

For over 40 years epoxy resins have been widely used in a variety of can coating applications. Epoxy resins incorporating BADGE have achieved wide acceptance in protective coatings, including coatings for food and drink cans because of their combination of properties such as toughness, adhesion and chemical resistance.

BADGE migration levels are governed by a variety of parameters such as coating composition, coating weight, curing conditions, sterilisation time and temperature and type of foodstuff. Carbonated soft drinks are the predominant type of beverage distributed in cans. These cans are typically filled at room temperature, and stored at or below room temperature. Canned foods are mostly sterilised at high temperatures, up to 135°C. The sterilisation time will vary, with shorter residence times for higher temperatures. Typically, sterilisation at 120°C is performed for 90 minutes. Approximate coating weights for typical beverage cans are 250 mg per 330 ml for a tinplate can (coating weight 1.06 mg cm^{-2}) and 125 mg per 330 ml for an aluminium can (coating weight 0.53 mg cm^{-2}). For food cans, coating weights may vary between 0.4 and 2.5 mg cm^{-2}.

In 1996 in Switzerland BADGE was detected in oil from canned fish products packaged in easy-open cans at levels above the Swiss regulatory threshold of 20 μg kg^{-1}. As a result of these findings canned products were removed from distribution by authorities and the retail trade. Easy open can coatings do differ from most food and drink can coatings in that the latter are hardened with curing agents. Information shows that, if the coating is fully cured, there are relatively low levels of free BADGE left in the coating and limited opportunities for BADGE migration at measurable levels into the can contents.

Following this incident there have been on-going discussions between European[3] and United States trade associations representing the entire supply chain involved in the manufacture of food and drink coated can and regulators in Europe and the United States to address issues related to BADGE migration in cans.

In recent years a number of studies have investigated the migration of BADGE from coated food and drink cans. In 2001 the United Kingdom Food Standards Agency (FSA) conducted a market survey of BADGE in canned food. The migration levels of BADGE detected in the canned foodstuff were <0.1 mg kg^{-1} food which is well below the regulatory limit of 1 mg kg^{-1} food (see Table 4.13).

Using information on European consumption pattern for canned food, the total surface areas of cans and the United Kingdom FSA market survey data, Dionisi and Oldring (2002) calculated the *per capita* exposure to BADGE is 3 to 8 μg per person per day. The difference depends upon whether surface area migration data or the measured amount of BADGE in food were used in the calculations. For a 60 kg individual this equates to a daily intake of approximately 0.05-0.13 μg kg body $weight^{-1}$ day^{-1}. A summary of data including, food can production numbers, *per capita* consumption of food from cans and the calculated *per capita* exposure to BADGE from various food types based on migration data is shown in Table 4.11.

Using a Monte Carlo Simulation based on the measured average content of BADGE in various canned foods Landenberger, (International Life Science Institute (ILSI) Workshop, Ispra, October 2001), calculated an average BADGE exposure of 0.004 μg kg body $weight^{-1}$ day^{-1} for a 60 kg individual. The maximum exposure was calculated to be of 0.19 μg kg body $weight^{-1}$ day^{-1} for a 60 kg individual while the minimum was calculated as 0.000005 μg kg body $weight^{-1}$ day^{-1} for a 60 kg individual.

[3] The associations are as follows: Epoxy Resin Committee of the Association of Plastic Manufactures in Europe (ERC APME), European Confederation of Paint, Printing Ink and Artists Colours Manufacturers Associations (CEPE), European Secretariat of Manufacturers of Light Metal Packaging (SEFEL) and the Society of the Plastics Industry, Including the Epoxy Resins System Task Group (SPI ERSTG)

Table 4.13 Exposure to BADGE from *per capita* consumption of canned food

Type of food	*Per capita* consumption (kg person^{-1} year^{-1})	Average Migration levels of BADGE and derivatives reported by FSA (mg kg^{-1})	*Per capita* exposure to BADGE (mg person^{-1} year^{-1})
Fruit	2.9	0.03 (0.015)	0.09 (0.04)
Meat	1.3	0.03 (0.015)	0.04 (0.02)
Fish	2.2	0.14 (0.08)	0.31 (0.18)
Ready Meals	3.2	0.03 (0.015)	0.10 (0.05)
Vegetables	9.5	0.06 (0.05)	0.55 (0.48)
Soups	0.8	0.03 (0.015)	0.02 (0.01)
Milk/Cream	1.7	0.03 (0.015)	0.05 (0.02)
Others	1.1	0.03 (0.015)	0.03 (0.02)
Total	22.6		1.19 (0.82)

When a Margin of Safety[4] (MOS) approach is used in which the lowest potential NOEC for endocrine mediated responses in laboratory mammals (250 mg kg body weight^{-1} day^{-1}) is compared with exposure levels from migration from canned food (0.05-0.13 µg kg body weight^{-1} day^{-1}) the outcome for adults is a value of 1.92 x 10^6 to 5 x 10^6. Based on the fact that a value of 100 indicates that the risk is acceptable then exposure of consumers to BADGE via migration from epoxy resins in cans does not appear to represent a risk in terms of causing effects on reproduction and development in humans which may be endocrine mediated.

4.7.2.2 Lining of water distribution pipes

BADGE is a component of epoxy resins which are used to line pipes used in water distribution systems. This may involve pipes which are pre-lined in the factory or the application of the epoxy resins *in situ* where pipes are being repaired rather than replaced. In certain European countries pre-lined pipes require testing and certification before they are used in water distribution systems. In the case of both pre-lined and repaired pipes they should be flushed for a sufficient period to ensure that residual levels of BADGE will be below acceptable levels for drinking water before the pipes are again used to supply water to consumers (including children). Leaching of BADGE is perceived to be a greater problem where the epoxy resins are used *in situ* and current (but confidential) data indicates that where they are applied correctly and leaching is carried out before re-use in the distribution system residual levels of BADGE are low.

4.7.3 Environmental exposure data

No recent measured environmental (aquatic, terrestrial or aerial) exposure data has been obtained based on searches of the COMMPS database and literature sources.

Environmental exposure from both epoxy resin manufacturing sites and down stream use plants is considered to be very small. An environmental release of BADGE from end-use applications is unlikely to occur as epoxy resins are reacted with hardeners/curing agents into

[4] Margin of safety (MOS) = (Lowest NOEL for endocrine mediated responses)/Exposure concentration

crosslinked systems which are stable against thermal and hydrolytic breakdown. This taken with the fact that BADGE is inherently biodegradable reduces concerns about any possible aquatic environmental effects.

Given the potential for BADGE to bind to organic carbon the absence of data on potential terrestrial exposure routes represents an area of uncertainty with regard to the potential endocrine effects of the substance on wildlife.

Given that BADGE is not considered to be volatile and the rate constant for vapour phase reaction with photo-chemically produced hydroxy radicals corresponds to a half life of 1.92 hours the potential for aerial organisms to be exposed to the substance is limited.

4.8 Overall Conclusions on BADGE

The following conclusions have been drawn from a review of the data for BADGE:

4.8.1 Data from studies assessing potential endocrine disrupting effects

4.8.1.1 Human related studies

- *In vivo* sub-chronic studies in rats using oral exposure up to doses of 1000 mg kg body weight^{-1} day^{-1} generally resulted in no histopathological effects on endocrine glands and hormone sensitive tissues. The exception were slight effects on the testes and uterus of rats at doses of 1000 mg kg body weight^{-1} day^{-1} in a 100 day oral exposure study, resulting in a NOEL of 250 mg kg body weight^{-1} day^{-1} for histopathological effects on endocrine glands and hormone sensitive tissues. However, these effects could result from direct toxic action. The available studies provide no consideration of changes in endocrine function (for example changes in hormone levels).

- One and two generation reproduction studies carried out in rats using oral exposure showed no evidence of effects on reproductive parameters at the highest doses tested (540 and 750 mg kg body weight^{-1} day^{-1} respectively).

- *In vivo* developmental studies in rats and rabbits indicated no foetotoxic and developmental effects at the highest doses tested (300 – 540 mg kg body weight^{-1} day^{-1}) even though maternal toxic effects were evident at lower doses[1]. Significant effects on pregnancy rate and foetal sex ratios in New Zealand White rabbits exposed dermally to BADGE were found in a teratogenicity study at a dose of 30 mg kg body weight^{-1} day^{-1} though effects were not evident at higher doses (100 and 300 mg kg body weight^{-1} day^{-1}). However, no effects on litter size or sex ratios were found in other studies in rats and rabbits at similar exposure doses.

- Available evidence from a Uterotrophic screening assay (Ogata *et al* 2001) indicates that no effects on uterine weight occurred after sub-cutaneous injections of 1 mg kg body weight^{-1}.

- *In vitro* screening studies using mammalian cells and tissues have shown an absence of induction of oestrogen-sensitive gene products and no or weak binding of BADGE to the human oestrogen receptor in mammalian cells and tissues. Minimal cell proliferation in the E-Screen assay was evident. A single study (with no experimental details given) reports

some binding of BADGE to the androgen receptor. No information was available on the effects of BADGE on thyroid function and hormone synthesis and secretion and steroidogenesis in mammalian cells and tissues.

4.8.1.2 Wildlife studies

- The data on potential endocrine disrupting effects in wildlife is limited to a reproduction study in the water flea *Daphnia magna* which showed a NOEL of 0.3 mg l^{-1}, though there is no information on the mechanism of action for the effects observed. No data was available on the effects of BADGE on the reproduction and development of fish.

- No data on potential endocrine mediated responses in terrestrial or aerial species could be located. Given the potential for BADGE to bind to organic carbon the absence of data on terrestrial organisms represents an area of uncertainty with regard to the potential endocrine effects of the substance on wildlife.

- No *in vitro* data was available from assays using cells and tissues from wildlife species.

4.8.2 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity

4.8.2.1 Human related studies

- In acute and repeat-dose studies the general systemic toxicity data for laboratory mammals indicates that the threshold in rats for an absence of effects which are not directly endocrine mediated occurs at a dose of approximately 10 mg kg body $weight^{-1}$ day^{-1} (Redmond *et al* 1996b). Most of the studies on rats or mice have reported dermal irritation/dermatitis with a NOEL in the range of 10 mg kg body $weight^{-1}$ $application^{-1}$ (Hend *et al* 1977a, Redmond *et al* 1995a,b, 1996a,b). In an oral exposure study in rats by Stebbins and Dryzga (2001) a dose of 250 mg kg body $weight^{-1}$ day^{-1} was interpreted to be the no observed adverse effect level (NOAEL). As a result it appears that on the basis of the available data that endocrine mediated responses are probably not be the mechanism responsible for the most toxic effects observed in laboratory mammals.

4.8.2.2 Wildlife studies

- Comparison of the limited data on potential endocrine mediated responses in the aquatic invertebrate *Daphnia magna* with the acute and chronic data for this species indicates that the threshold concentration for mortalities (0.3 mg l^{-1}) was the same as the threshold concentration for effects on reproduction (0.3 mg l^{-1}). However, there is no information on the mechanism of action for the effects on *Daphnia magna* reproduction. No comparisons could be made for fish due to the absence of data on endocrine mediated responses in this taxonomic group, which represents an area of uncertainty.

4.8.3 Exposure data

4.8.3.1 Workers

- Liquid, (unmodified bisphenol A – epichlorhydrin based), epoxy resins (containing approximately 80-85% of BADGE) and solid epoxy resins are manufactured in closed systems. Potential worker exposure to BADGE is most likely to occur in the manufacturing and industrial application of formulated epoxy systems with dermal contact being the likely route of exposure. As epoxy resins are however classified as skin/eye irritants and skin sensitisers workers handling epoxy resins wear protective gloves to avoid skin contact. Safety literature from the epoxy resin suppliers provides guidance on the safe handling of epoxy resins including recommended protective equipment. As liquid epoxy resins are highly viscous, non-volatile materials worker exposure through inhalation of BADGE vapours, even at high temperatures, is unlikely to occur. Due to the low volatility of solid epoxy resins vapour inhalation is unlikely. Exposure to dusts can however occur in various operations e.g. during bag filling in epoxy manufacturing plants or charging of epoxy resins from bags in paint manufacturing process. In such operations dust exposure is minimised through the installation of local exhaust ventilation and operators wear adequate respiratory protective equipment. To prevent dust exposures low molecular weight “1” type resins are to a large extent supplied in solutions.

4.8.3.2 Consumers

- Epoxy resins are primarily used in industrial applications and negligible consumer exposure is expected from the products in which BADGE is used. The major consumer exposure to BADGE is from food and drink cans lined with epoxy based coatings. BADGE is also used in epoxy resins for water distribution pipes and a potential for consumer exposure exists following leaching from the resins. Minor volumes of liquid epoxy resins are used in two-component epoxy glues sold to the public in retailer shops but this potential route of exposure is extremely limited given the volumes typically used.

- When a Margin of Safety (MOS) approach is used in which the lowest NOEC for potential endocrine mediated responses in laboratory mammals (250 mg kg body weight^{-1} day^{-1}) is compared with exposure levels from migration from canned food (0.05-0.13 µg kg body weight^{-1} day^{-1}) the outcome is a value of 1.92 x 10^6 - 5 x 10^6. Based on the fact that a value of 100 indicates that the risk is acceptable then exposure of consumers to BADGE via migration from epoxy resins in cans does not appear to represent a risk in terms of causing effects on reproduction and development in humans which may be endocrine mediated.

4.8.3.3 Environment

- No national environmental quality standards have been derived in any European country for the protection of aquatic or terrestrial ecosystems and no measured environmental (aquatic, terrestrial or aerial) exposure data has been obtained based on searches of the COMMPS database and literature sources.

- Environmental exposure from both epoxy resin manufacturing sites and down stream use plants is considered to be very small. An environmental release of BADGE from end-use applications is unlikely to occur as epoxy resins are reacted with hardeners/curing agents into crosslinked systems which are stable against thermal and hydrolytic breakdown. This taken with the fact that BADGE is inherently biodegradable reduces concerns about any possible aquatic environmental effects.

- Given the potential for BADGE to bind to organic carbon the absence of data on potential terrestrial exposure routes represents an area of uncertainty with regard to the potential endocrine effects of the substance on wildlife.

- Given that BADGE is not considered to be volatile and the rate constant for vapour phase reaction with photo-chemically produced hydroxy radicals corresponds to a half life of 1.92 hours the potential for aerial organisms to be exposed to the substance is limited.

4.9 Summary of the weight of evidence for endocrine disrupting effects in humans and wildlife and associated uncertainties

The summary of the weight of evidence for endocrine disrupting effects of BADGE in humans and wildlife along with associated uncertainties are given in Table 4.14.

Table 4.14 Summary of the weight of evidence conclusion and uncertainties associated with the assessment of the endocrine disrupting effects of BADGE

	Target group	
	Humans	Wildlife
Weight of evidence	The available data from *in vivo* studies in laboratory mammals (using oral or dermal exposure routes) indicates that BADGE does not cause adverse effects on reproductive and developmental endpoints (which may be endocrine mediated) at exposure levels where general systemic toxic effects are observed. The lowest recorded NOEL from the *in vivo* mammalian studies was 250 mg kg body weight^{-1} day^{-1} for histopathological effects in endocrine glands and hormone sensitive tissues, though the observed effects at higher doses may have resulted from direct toxic action. The available exposure data indicates that current exposure patterns to BADGE do not represent a risk to workers or consumers.	The available aquatic effects data shows that the threshold exposure concentration of BADGE above which reproduction of the invertebrate *Daphnia magna* is reduced (NOEC = 0.3 mg l^{-1}) is similar to the threshold level for general toxic effects (i.e. lethality). However, there is no information on the mechanism of action for the effects on reproduction observed in *Daphnia magna*.
Uncertainties	There are no major uncertainties with regard to the evaluation of potential adverse effects of BADGE on reproductive and developmental endpoints since data is available from a definitive multi-generation study as well as supporting reproduction and developmental studies. Mechanistic uncertainties exist because the available studies provide no direct measurement of changes in endocrine function (for example changes in hormone levels)	There are uncertainties with regard to potential adverse effects of BADGE on reproduction and development in wildlife due to the absence of key data for: • A wider range of aquatic taxa, particularly fish and sediment dwelling invertebrates • Terrestrial organisms The absence of data on aerial organisms is not a major uncertainty since BADGE is not volatile and the potential for these organisms to be exposed is limited. No environmental exposure data for BADGE in the aquatic, terrestrial and aerial compartments has been located

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5. REVIEW OF DATA FOR CARBON DISULPHIDE

Table of Contents

Notes:

This section contains information collected and collated from a range of sources including published papers, reports of studies conducted by industrial companies or sector groups and data compilations such as BUA (1991) and IUCLID (2000). The data from IUCLID has been taken as accurate and individual source documents have not been checked unless they are considered to be key studies which have a major influence on the outcome of the review. All information taken from IUCLID has been referenced as being from that source and individual references have not been given in the references.

This review has been carried out in accordance with the evaluation framework described in Section 2. In the review the International Programme for Chemical Safey (IPCS) definition of an endocrine disrupter has been adopted, namely that it is "*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*".

In the context of the review it is recognised that there are various laboratory-based *in vivo* and *in vitro* methods utilising a range of (eco)toxicological endpoints that are claimed by different sources to be relevant to the assessment of endocrine disruption in humans and wildlife. However, since this field is still in an early stage of development there is uncertainty regarding the significance of many of the current findings.

From the numerous recent reviews of potential test methods (such as the Detailed Review Paper prepared by OECD in 1997) there is a clear consensus in terms of the hierarchy of the relevance of test methods. In this hierarchy longer-term *in vivo* studies considering effects on reproduction and/or development (and including mechanistic information) are of greater relevance than short-term *in vivo* screening tests which are of greater relevance than *in vitro* assays. The greater relevance of chronic *in vivo* tests or those assessing effects during critical windows of sensitivity is also evidenced by the fact that these are the key (eco) toxicological methods being developed in the OECD Endocrine Disruption Testing and Assessment (EDTA) Programme. This hierarchy approach to data relevance has been adopted in the review along with a weight of evidence consideration of the available data.

The review has been carried out to address three key questions:

1. Does the available data indicate there is evidence that a chemical causes endocrine disrupting effects in target groups of humans and/or wildlife?

2. Do endocrine disrupting effects of the chemical in target groups of humans and/or wildlife occur at lower concentrations than those causing effects on general systemic toxicological endpoints?

3. Are particular target groups of workers, consumers or organisms in the environment likely to be exposed to concentrations of chemicals which exceed effects thresholds due to current emission patterns.

It should be recognised that this review is not designed to be a full Risk Assessment of a substance under the Existing Substances Regulation 793/93.

5.1 Physio-chemical data for carbon disulphide

5.1.1 Summary details on the substance

CAS Number	75-15-0
EINECS Number	200-843-6
IUPAC Name	Carbon disulphide
Other names	Carbon bisulphide, dithiocarbonic anhydride, sulphocarbonic anhydride
Molecular weight	76.14
Chemical formula	CS_2
Chemical structure	S = C = S

5.1.2 Physico-chemical properties and environmental fate information (from IUCLID 2000)

The data on the physico-chemical properties of carbon disulphide and its environmental fate (see Table 5.1) indicate that the substance is expected to exist solely as a vapour in the ambient atmosphere since it is highly volatile with a Henry's Law Constant of 1.42 x 10^3 Pa-m^3 mol^{-1} (1.44 x 10^{-2} atm-m^3 mol^{-1}) at 24 ^oC exceeding a value range of 1 -100 Pa-m^3 mol^{-1} that indicates volatility. However, carbon disulphide is degraded by reaction with photo-chemically produced hydroxyl radicals, the half life for the reaction being estimated to be 5.5 days. It should be recognised that as well as being released by industrial processes, carbon disulphide is present in the environment as a result of natural processes (see Section 5.2).

In the aquatic environment photolysis is not considered to be a significant loss mechanism but carbon disulphide can be hydrolysed to carbon dioxide and hydrogen sulphide in alkaline (pH 9) solutions with a half-life of 1.1 years. Carbon disulphide has been shown to be readily biodegradable and is not expected to sorb to organic carbon based on a low organic carbon water partition coefficient (log Koc) of 1.79.

Soils represent a natural sink for atmospheric carbon disulphide which can be degraded in soil by specialised bacteria (BUA 1991).

Table 5.1 Physico-chemical properties and environmental fate data (from BUA 1991 and IUCLID 2000)

Physico-chemical property	Value (and comments)
Physical state at ambient temperature	Clear, colourless or faintly yellow liquid
Water solubility	2.1 g l^{-1} at 20 ^{o}C
Octanol-water partition coefficient (log Kow)	1.94 – 2.14
Organic carbon water partition coefficient (log Koc)	1.79
Henry's Law Constant	1.42 x 10^{3} Pa-m^{3} mol^{-1} (1.44 x 10^{-2} atm-m^{3} mol^{-1}) at 24 ^{o}C
Type of degradation	
Aquatic - abiotic	Photolysis is not considered to be a significant loss mechanism but carbon disulphide can be hydrolysed to carbon dioxide and hydrogen sulphide in alkaline (pH 9) solutions with a half-life of 1.1 years. Volatilisation from water surfaces is rapid.
Aquatic - biotic	Carbon disulphide has been shown to be readily biodegradable (80% loss after 28 days) in a closed bottle test.
Terrestrial	Soils represent a natural sink for atmospheric carbon disulphide which can be degraded in soil by specialised bacteria.
Atmospheric	Carbon disulphide is expected to exist solely as a vapour in the ambient atmosphere and is degraded by reaction with photo-chemically produced hydroxyl radicals, the half life for the reaction being estimated to be 5.5 days.

A Mackay Level 1 fugacity model has shown that for a discharge of 1000 tonnes of carbon disulphide 99.6% of the substance will partition into the air (Table 5.2), with amounts present in other compartments being minimal.

Table 5.2 Summary of the results of a Mackay Level 1 fugacity model

Compartment	Volumes of different compartments	% of substance present in different compartments
Water	2 x 10^{11}	0.34
Suspended sediment	10^{6}	2.3 x 10^{-5}
Bottom sediment	10^{8}	7.3 x 10^{-4}
Fish	2 x 10^{5}	1.85 x 10^{-6}
Air	10^{14}	99.6
Aerosol	2000	3 x 10^{-7}
Soil	9 x 10^{9}	0.033

5.2 Production and Uses

5.2.1 Production Patterns

The information provided by the ad-hoc CS_2 user/producer group on estimated production patterns in 2001 indicates that approximately 115000 tonnes was produced in the EU with a further 50000 tonnes being imported. It was estimated that <5000 tonnes of carbon disulphide was exported resulting in a total volume of 160000 – 170000 tonnes being used in the EU.

However, the production of carbon disulphide in industrial facilities has to be considered in the light of natural production of the substance. Carbon disulphide is produced naturally by soil and sediment micro-organisms, vegetation, forest and grass fires and volcanic activity. The ocean appears to be a major global source of carbon disulphide. Current data suggest that coastal areas and other areas of high biological productivity have greater fluxes of carbon disulphide than the open ocean. Emissions from the oceans have been estimated to be 6 x 10^5 tonnes yr^{-1} (3.53 - 3.75x the total volume used in the EU in 2001). The microbial reduction of sulphates in soil produces fluxes of carbon disulphide. The annual global emission from this source has been estimated to be 9 x 10^5 tonnes yr^{-1} (5.29 -5.63x the total volume used in the EU in 2001). Other natural sources include volcanic emissions, estimated to be 2 x 10^4 tonnes yr^{-1}, and marshlands, estimated emissions 1 x 10^5 tonnes yr^{-1}. Worldwide, at least 40%, and possibly, as much as 80% of the releases are a result of natural or biogenic activity (IPCS 2002).

5.2.2 Use Patterns

Carbon disulphide is an organic solvent and its most important use is in the production of viscose rayon fibres and sausage skins (see Table 5.3). It is also used in the manufacture of rubber chemicals, pharmaceutical and fine chemicals and agricultural chemicals

Table 5.3 Summary of the use patterns of carbon disulphide

Uses	Proportion of total tonnage (%)	Volume used (tonnes)	Number of producers
Cellulose regeneration Viscose rayon, cellophane sausage skins and cellulose sponges	50	~ 80000	25 (13 for viscose rayon, 4 for sausage skins)
Use as Intermediates Rubber chemicals Pharmaceuticals and fine chemicals Agricultural chemicals	 20 10 20	 ~ 32000 ~ 16000 ~ 32000	 ~10 ~10 ~10
Total	**100**	**~160000**	-

5.3 Toxicokinetics, metabolism and bioaccumulation

There is a considerable body of information on the toxicokinetics, metabolism and bioaccumulation of carbon disulphide and the information in this section has been derived

from a series of review documents (WHO 1979, Beachamp *et al* 1983, BUA 1991, BRE 1993, Cox *et al* 1996, Amaranth *et al* 2001)

5.3.1 Toxicokinetics and metabolism

5.3.1.1 Laboratory mammals

Carbon disulphide is taken up rapidly into the blood after oral treatment, intraperitoneal and intracardial injection as well as after inhalative exposure. For all animal species, a steady state concentration of free carbon disulphide in the blood is attained within 30 to120 minutes, while the acid-labile carbon disulphide, which is probably protein-bound, further increases with prolonged exposure and attains equilibrium only after days, depending on the dosage or exposure concentration

Approximately the same amounts of free and acid-labile carbon disulphide have been found in the blood of rats at inhalation concentrations of 15-120 mg/m^3 CS_2. With repeated exposure, there is an overproportional increase of acid-labile carbon disulphide in blood in comparison with the amount of free carbon disulphide.

The elimination kinetics of both the free and acid-labile carbon disulphide in rat blood occurs at two phases with half-lives of 6-9 minutes and 50-80 minutes (free) as well as 2 hours and 40 hours (acid-labile), respectively. Acid-labile carbon disulphide is bound mainly to erythrocytes and largely to haemoglobin.

In tissues, steady-state concentrations of free carbon disulphide are attained within 4 to 5 hours, whereas those of acid-labile carbon disulphide increase continuously. After 8 hours exposure to 2,000 mg/m^3 CS_2, the highest concentrations of free carbon disulphide were found in the adipose tissue, adrenal gland and liver of rats. Acid-labile concentrations of carbon disulphide were highest in the adrenal gland and essentially lower in adipose tissue and the liver. The elimination of acid-labile carbon disulphide from tissue took place considerably slower than that of free carbon disulphide.

Unaltered carbon disulphide is eliminated via the lung after dermal, oral, intraperitoneal as well as inhalative exposure of various animal species. The quantitative data varied between 1% and 80%. For example, in rats 55% and 80% of intraperitoneal doses of 1 and 100 mg kg body weight^{-1} applied to rats were exhaled unaltered.

The acid-labile carbon disulphide is formed by the direct reaction of the substance with amino groups and/or sulfhydryl groups of amino acids. Carbon disulphide probably also reacts with catechol amines.

Carbon disulphide is metabolized mainly in the endoplasmic reticulum of the liver cell by the mixed-function oxidase cytochrome P-450. The responsible isoenzyme is probably identical to the alcohol dehydogenase. Carbon disulphide and atomic sulphur, which is probably generated in the reactive singlet state are formed as reaction products. Carbon disulphide or monothiocarbonate, the hydration product, is transformed to carbon dioxide and hydrogen sulphide by carboanhydrase. Carbon disulphide is transformed into sulphate via several reaction steps, while carbon dioxide enters the endogenous pool and thus is exhaled only partially. The reactive sulphur binds onto sulphydryl group of proteins, a reaction which is held responsible for the destruction of cytochrome P-450. During the incubation of liver microsomes with carbon disulphide, cytochrome P-450 is transformed to inactive cytochrome P-420. The destruction or inactivation of the enzyme responsible for the metabolism of carbon

disulphide has also been detected *in vivo* Carbon disulphide is reported to induce liver UDP glucuronosyltransferase and induction of this enzyme has been associated with the hepatic-thyroid axis effects in rats treated with a range of compounds. Male rats exposed via the inhalation route for 4 hours to atmospheres containing 1-4 mg l^{-1} CS_2 have shown alterations in adrenal dopamine metabolism indicative of inhibited dopamine β-hydroxylase.

After intraperitoneal application to rats of 1 mg ^{14}C-CS_2 kg body $weight^{-1}$, only 2.2% of the radioactivity is recovered within 24 h in the rat urine. Dithiocarbamates as well as bivalent sulphur were detected only indirectly. Carbon dioxide and small amounts of carbonyl sulphide (together up to 40% of the dose taken up) were identified clearly as carbon disulphide metabolites in the exhaled air of exposed experimental animals. For example, after intraperitoneal application to rats of 1 and 100 mg kg body $weight^{-1}$, respectively 25% and 5% of the applied dose was exhaled as CO_2.

Quantitative statements on the metabolism of carbon disulphide are problematic on the basis of currently available studies, because either the total radioactivity was measured or unspecific detection methods were used such as measuring the increase of bivalent sulphur. Furthermore, data which supports an accumulation of metabolites - formed via the oxidative degradation pathway – cannot be interpreted concerning the tissue distribution of radioactively labelled carbon disulphide, because the radioactive carbon (^{14}C) as well as the radioactive sulphur (^{35}S) can be taken up into endogenous pools.

5.3.1.2 Humans

In a similar manner to animal experiments, rapid uptake was shown after test individuals inhaled carbon disulphide. Equilibrium between the inhaled and exhaled carbon disulphide was established within about 2 hours, although values of 5 to 8 hours have also been recorded. Acid-labile carbon disulphide is primarily found in blood and mainly in the erythrocytes. The distribution of free and acid-labile carbon disulphide is human tissue has not been examined. The calculated retained quantities of 20% to 90% of the inhaled carbon disulphide indicate, however, that similar conditions are present as in animal studies. The amount of carbon disulphide which is retained in the body and is not exhaled again is decreased by physical effort as well as longer exposure to this substance. Nonetheless, strong individual deviations have been recorded.

Carbon disulphide is also taken up by the skin, which was proven by exhalation of carbon disulphide via the lung. The elimination of free carbon disulphide occurs mainly via the lung with the half-lives of 1 minute (initial phase) and about 100 minutes (slow 2^{nd} phase). The quantitative data on the exhalation of carbon disulphide range between 6% and 40% of the absorbed amount and are subject to very strong variations among individuals. Carbon disulphide can still be found in exhaled air 4 days after exposure. As was found in animal experiments, practically no unaltered carbon disulphide is excreted via the kidneys and the faeces. The elimination of carbon disulphide through the skin is specified as 0.5-1% of the amount taken up.

Besides sulphate, the metabolites thiourea, 2-thio-5-thiazolidinone, 2-thiothiazolidine-4-carboxylic acid and recently a new metabolite, 2-thioxothiazolidine-4-ylcarbonylglycerine (TTCG), have been identified in human urine. There is a linear correlation between the exposure concentration and the urinary excretion of 2-thiothiazolidine-4-carboxylic acid. At a maximum concentration in the workplace value of 30 mg/m^3, a 2-thiothiazolidine-4-carboxylic acid concentration of 5-8 mg l^{-1} (approximately 5 mg g^{-1} creatinine) is excreted in the urine of test subjects as well as occupationally exposed workers. The amount of 2-thiothiazolidine-4-

carboxylic acid is less than 5% of the carbon disulphide uptake. This amount decreases after exposure with a half-life of around 2 hours. Carbon disulphide also inhibits the exogenous substance-metabolising enzyme system in humans.

Importantly, carbon disulphide has been shown to be capable of penetrating the placenta and has been detected in the urine of infants of exposed mothers and in the breast milk.

5.3.2 Bioaccumulation

No measured values for the bioaccumulation of carbon disulphide in biota have been located. The low octanol water partition coefficient (log Kow) of 1.94 – 2.14 for carbon disulphide indicates that the substance is not expected to bioaccumulate in biota.

5.4 Studies relevant to the assessment of potential endocrine disrupting effects

5.4.1 Studies relevant to the assessment of potential endocrine disrupting effects in humans

5.4.1.1 In vitro studies

A. Receptor competitive binding assays

No data has been located on responses of carbon disulphide in competitive binding assays using mammalian cells and tissues.

B. Recombinant yeast assays

No data has been located on responses of carbon disulphide using recombinant yeast assays.

C. Mammalian cell growth assays

No data has been located on responses of carbon disulphide using mammalian cell growth assays.

Summary of *in vitro* data

There appear to be no data on the potential of carbon disulphide to cause endocrine disruption in *in vitro* studies utilising mammalian cells or tissues which are assessing (anti)oestrogenic and/or (anti)androgenic effects. No data has been identified on the effects of carbon disulphide on thyroid function and effects on hormone synthesis and secretion and steroidogenesis in mammalian cells and tissues.
Le and Fu (1996) conducted chromosomal analysis of human sperm and found that in an *in vitro* system a concentration of 10 μmol l^{-1} (761 μg l^{-1}) CS_2 produced increased aberrations.

5.4.1.2 In vivo studies

Tables 5.4 to 5.6 summarise the data on potential endocrine disruption effects of carbon disulphide in laboratory mammals following exposure via the oral (Table 5.4), inhalational (table 5.5) and intra-peritoneal injection (Table 5.6) routes.

A. Effects on endocrine glands and hormone sensitive tissues

Gondzik (1971) studied the effects of carbon disulphide on testicular tissues of male rats. Three experiments were conducted using 85 'mongrel' rats, 2-5 months old and weighing 200-260 g. In the first experiment, 12 rats were injected intra-peritoneally every second day for 60 days with 12.5 mg kg^{-1} of distilled carbon disulphide dissolved in peanut oil; 5 were given pure peanut oil and 5 were untreated. In the second experiment, 15 animals were given intra-peritoneal doses of 25.0 mg kg^{-1} every other day for 60 days; 10 rats were given pure peanut oil and 9 were untreated. In the third experiment, 10 rats were given 25.0 mg kg^{-1} by intra-peritoneal injection every other day for 120 days; 10 were injected with peanut oil and 9 were untreated. Animals exposed to the 12.5 mg kg^{-1} dose showed no effects compared with those observed in the controls. Rats exposed at the highest dose (25 mg kg^{-1}) had thickened vascular walls, blood cell engorged vessels, disorganized seminiferous epithelium, and decreased numbers of spermatozoa. Rats injected with carbon disulphide for a 120-day period, however, showed marked testicular damage comprising advanced regressive lesions involving all parts of the testicles of the usually round and smooth tubular basement membrane. Spermatogonia were few and sometimes non-existent in the seminiferous tubules, and spermatogenesis was absent. Leydig cells showed degeneration and atrophy. However, it needs to be recognised that the criteria used for irreversibility of germ cell loss were ambiguous and also that the route of exposure is an important element of mammalian susceptibility to testicular injury.

Mihalache and Mihalache (1989) found that CS_2 impairs reproduction and fertility in *male* rats treated intraperitoneally once per week for 6 months with 5, 10, 15 or 25 mg kg^{-1}. Carbon disulphide exposed animals showed histopathology in the testis comprising interstitial oedema, stasis, congestion, thickening of the seminiferous tubule basement membrane and suppression of spermatogenesis. As with the study of Gondzik *et al* (1971) the route of exposure is probably important in terms of the results observed.

Thomas *et al* (1985) stated that "*In rats carbon disulphide causes progressive testicular atrophy after repeated parenteral administration. The testis from these animals exhibit vasodilation, haemorrhage and fluid exudation into the testicular parenchyma, suggesting a possible vascular origin for the atrophy. Testicular damage is not observed after inhalation exposure*".

In an *in vivo* study of mid gestation rats, CS_2 (doses of 300 or 600 mg kg^{-1} day^{-1} administered for 10 days) increased contractility of the uterus to pharmacological challenges and the mechanism involved calcium pathways (Tsai *et al* 2000).

B. Reproduction and fertility studies

Acadzhanova (1978) exposed rats to carbon disulphide to concentrations of 1, 10 and 100 mg/m^3 for 4 months and reported that carbon disulphide affects the reproductive cycles in *female* rats. Prolongation of the oestrous cycles following 4 month inhalational exposure to 10 mg/m^3 and above was found, the effect being particularly evident in the 3rd and 4th months of exposure. This resulted in an NOEL of 1 mg/m^3. Lesions were detected in the ovary and also adrenals, thyroid and pituitary and some effects were consistent with trophic hormone stimulation in the anterior pituitary. However, there are issues with the accuracy of the exposure doses reported.

Tabacova *et al* (1983) conducted a non-regulatory two generation reproduction study in pregnant albino rats (30-32 *females* per group) via the inhalational route at dose levels of 0.03 and 10 mg/m^3 (which are reportedly non-teratogenic doses) and 100 and 200 mg/m^3 (which are reportedly teratogenic doses). Inhalation exposure of pregnant albino rats to 0.03 and 10 mg/m^3 did not produce congenital malformations or functional biochemical changes in neonates but affected postnatal development at 10 mg/m^3 causing impairment of viability, retardation of morphological and sensory development and behavioural deviations in the F_2 generation compared with controls and the F_1 generation (see also section on developmental toxicity). The two highest dose levels were both teratogenic and maternally neurotoxic.

The results of the study were taken as evidence for "intrauterine sensitization" to carbon disulphide. This response was defined as a greater incidence of maternal toxicity in an F_1 generation and teratogenicity and embryotoxicity in an F_2 generation resulting from gestational exposure of the F_1 generation rats when they had experienced a previous *in utero* exposure to carbon disulphide during gestational exposure of F_0 rats. The potential mechanism for "intrauterine sensitisation" is unclear, particularly given the absence of known mutagenic effects associated with carbon disulphide exposure.

Limitations of the study include a lack of information on chemical purity and exposure methods, lack of concurrent controls, lack of a clear dose-response trend and incomplete reporting on the statistical significance of reported behavioural effects.

Tepe and Zenick (1984) evaluated the effects on *male* rats (Long Evans) of inhalational exposures of 0, 1106 or 1896 mg/m^3 (0, 350 or 600 ppm) for 5 hours per day, 5 days per week for 10 weeks and reported that high dose (1896 mg/m^3) animals showed lower epididymal sperm counts and reduced plasma testosterone. Rats also showed alterations to sexual behaviour and decreased ejaculated sperm counts at 1896 mg/m^3 (600 ppm). Subsequently Zenick *et al* (1984) reported that inhalational exposure of *male* rats to 600 ppm or 1896 mg/m^3 for 6 hours per day, 5 days per week for 10 weeks resulted in altered copulatory behaviour and decreased ejaculated sperm counts by the fourth and seventh weeks of exposure respectively. Exposure had no effects on epididymal sperm counts or follicle stimulating hormone (FSH) or lutenising hormone (LH) levels[1]. Neuroendocrinologic evaluations at the 1896 mg/m^3 exposure level included a normal increase in gonadotropins following injection of GnRH and a normal increase in testosterone following injection of human chorionic gonadotropin (HCG). The mechanism for the abnormal copulatory behaviour may involve alterations in the contraction of the smooth muscles of the vas deferens and/or epididymus and the overall neurological control of the ejaculatory process.

C. Developmental and teratogenicity studies

A number of studies have been conducted which have assessed the developmental and teratogenic effects of carbon disulphide resulting from oral or inhalation exposure.

Oral exposure

[1] In male mammals gonadotropin releasing hormone (GnRH) from the hypothalmic-pituitary axis stimulates pituitary gonadotrophs to secrete follicle stimulating hormone (FSH) and lutenising hormone (LH) . FSH acts on the Sertoli cells to produce androgen-binding globulin. Inhibin has a negative feedback effect on FSH. LH activates Leydig cells to produce testosterone via the cAMP second messenger pathway. Feedback inhibition of GnRH by testosterone regulates this pathway. Prolactin (PRL) has an inhibitory effect on testosterone production if present at high levels.

Jones-Price *et al* (1984a) investigated the effects of carbon disulphide on the development of rats following oral exposure of pregnant rats to 600 to 1800 mg kg^{-1} daily during gestation days 6-15. Animals were sacrificed on day 20. The study showed retarded body weight gains and increased liver weights for the dams. There were dose-dependent reductions in foetal weight and questionable non-dose dependent evidence of teratogenicity.

In a corresponding study in rabbits Jones-Price *et al* (1984b) investigated the effects of CS_2 development following oral exposure of pregnant rats to 0, 25, 75 and 150 mg kg^{-1} daily during gestation days 6-19. Animals were sacrificed on day 30. Retarded body weight gains and increased liver weights were measured in the dams at 75 and 150 mg kg^{-1}. At 150 mg kg^{-1} there were reductions in foetal number and weight and increases in resorptions. Foetal malformations were only observed following exposure to 150 mg kg^{-1} day^{-1}.

Inhalation exposure

Yaroslavskiy (1969) exposed female albino *rats* to carbon disulphide vapour to study its effects on the course and duration of pregnancy. Groups of 12-20 rats were exposed to carbon disulphide at a concentration of 2000 mg/m^3 (642 ppm) for 2 hours per day during the entire pregnancy. Two identical series of tests were performed on rats. In the first experiment, the 16.8% pre-implantation embryonic mortality rate which occurred in the 12 exposed animals was statistically significantly different from the rate of 3.3% in the 12 controls. In the second experiment, the pre-implantation mortality rate was 22.6% in 12 exposed rats and was statistically significantly different from the 6.5% in the 14 controls. The reproductive success of each CS_2 exposed group was also statistically lower than that of its control group in both experiments (6.8 versus 9.7 foetuses per rat in study 1 and 8.0 versus 9.3 foetuses per rat in study 2). There were seven post-implantation deaths in the foetuses of exposed rats and none in those of the controls. There were no significant differences between experimental and control rats in the mean corpus luteum counts or in mean foetal weights. In a repeat study with *mice* carried out at 2000 mg/m^3 Yaroslawskiy (1969) found similar effects as for rats as well as post-implantation losses.

Tabacova *et al* (1978) exposed Wistar albino rats (32 animals per group) to 50, 100 and 200 mg/m^3 (16, 32 and 64 ppm) CS_2 by inhalation for 8 hours per day throughout gestation. There were no statistically significant results in the 50 mg/m^3 group. In the 100 and 200 mg/m^3 groups, there were statistically significant reductions in foetal body weights and post-natal body weights for 21 days, which subsequently disappeared. There was an increase in external malformations (hydocephalus, club foot and tail deformations) at the two higher doses. Subsequently, Tabacova *et al* (1983) reported impaired viability and morphological development in offspring from rats exposed to 10 mg/m^3 in a reproduction study in which Wistar albino rats were exposed to low concentrations (0.03 and 10 mg/m^3 or 0.01 and 3 ppm) prenatally for 8 hours per day throughout gestation (see previous section). No congenital malformations or significant prenatal effects were found in the 9-11 litters evaluated at each dose. No significant effects were seen in maternal females at exposures of $\leq$ 100 mg/m^3. However, it should be recognised that the experimental details for the study are limited and there are concerns regarding the accuracy of the exposure range reported.

Beliles *et al* (1980) conducted reproduction studies in rats and rabbits in which females were exposed to carbon disulphide by inhalation at 0, 60-120 mg/m^3 for 7 hours a day, 5 days a week from 3 weeks before mating to gestation day 18 for rats and day 21 for rabbits. No significant teratogenic effects and no effects on course of pregnancy were detected at any test dose.

Hardin *et al* (1981) conducted developmental toxicity studies in the rat and rabbit. Rats and rabbits were exposed to inhalational dose levels of 62.3 mg/m^3 and 124.6 mg/m^3 (20 ppm or 40 ppm) during the entire length of the pregnancy period and also 34 weeks before breeding to simulate occupational exposure. The exposure levels were equivalent to 5 and 10 mg kg^{-1} day^{-1} in rats and 11 and 22 mg kg^{-1} day^{-1} in rabbits. No effects were observed at the dose levels and 11 mg kg^{-1} day^{-1} was taken as the reference dose (RfD).

Lehotsky *et al* (1985) investigated behavioural effects in the offspring of Lati:CFY rats (8 per group) exposed to 0, 10, 700, or 2000 mg/m^3 CS_2 (3, 230, or 640 ppm) for 6 hours per days over days 7 to 15 of gestation. The two high doses caused significant perinatal mortality. It was reported that there was a dose-related change in avoidance conditioning[2] among male pups over the first 15 days. While the magnitude of the effect on avoidance conditioning was greater at all doses relative to controls, and at 2000 mg/m^3 compared with 700 mg/m^3, the effect was virtually identical between the 10 and 700 mg/m^3. This lack of dose-response effect raises some question about the significance of this finding.

Saillenfait *et al* (1989) exposed rats via inhalation to 0, 310, 620, 1240 and 2480 mg/m^3 CS_2 (0, 100, 200, 400 and 800 ppm) for 6 hours per day during days 6 – 20 of gestation. Animals were sacrificed on day 21. Lower exposures (310 and 620 mg/m^3 or 100 and 200 ppm) were not associated with maternal toxicity or adverse effects on the developing embryo or foetus. Higher concentrations (1240 and 2480 mg/m^3 or 400 and 800 ppm) resulted in a significant reduction of maternal weight gain as well as reductions of foetal body weight and a low incidence of club foot. Significant increases in unossified sternebrae were reported following exposure to 2480 mg/m^3 (800 ppm).

A US EPA guideline standard teratogenicity and developmental toxicity study has been conducted in rabbits to GLP that showed embryo-lethality and teratogenicity (Pathology Associates 1991). In this study, groups of 24 time-mated New Zealand white rabbits were exposed inhalationally to CS_2 at dose levels of 0, 190, 316, 948, 1896 and 3792 mg/m^3 (0, 60, 100, 300, 600 and 1200 ppm) for 6 hours per day from days 6-18 of pregnancy. The NOEL for developmental and maternal toxicity was 948 mg/m^3 (300 ppm). At 1896 mg/m^3 (600 ppm) there was a statistically increased incidence of postimplantation loss and early and late resorptions and reduced number of live foetuses indicating embryolethality. This effect also occurred at 3792 mg/m^3 (1200 ppm) to a more marked degree where there was also a significantly increased incidence of late resorptions. Both 1896 mg/m^3 (600 ppm) and 3792 mg/m^3 (1200 ppm) resulted in reduced foetal weights but maternal toxicity only occurred at 3792 mg/m^3 (1200 ppm) indicating selective embryo-foetal toxicity at 1896 mg/m^3 (600 ppm). At 3792 mg/m^3 (1200 ppm) there was a significantly increased incidence of total visceral malformations and an increase in hydrocephalus (litter incidence of 28.6%, foetal incidence of 5.7% compared with 0% in controls, though it should also be noted that this may underestimate the true malformation rate since so few foetuses survived to term examination). There was also a low incidence of the frequently associated finding of enlarged brain ventricles (internal hydrocephalus) at both 1896 and 3792 mg/m^3 (600 and 1200 ppm).

Skeletal variations of note at 3792 mg/m^3 (1200 ppm) included increased incidence of extra lumbar vertebrae and hypoplastic pubis and the total number of skeletal malformations was

[2] Avoidance conditioning was tested using a bell as a conditional stimulus prior to an electric shock. The animals learned to avoid the shock by jumping onto a pole at the sound of the bell. The latency to jump onto the pole and errors were measured as a means to evaluate avoidance conditioning in the treated versus control animals.

also significantly increased. Despite reduced foetal bodyweights no data on skeletal ossification was presented.

Nemec *et al* (1993) reported no teratogenicity or maternal, developmental or reproductive toxicity among pregnant CD rats and their offspring following exposure to 388 or 775 mg/m^3 (125 or 250 ppm) CS_2 from 2 weeks prior to mating through to gestation day 19. At 1550 mg/m^3 (500 ppm) dams had decreased body weight gain and food consumption and decreased litter viability, but no teratogenic effects were noted.

D. Carcinogenicity and oncogenicity studies

No evidence of any carcinogenic potential of CS_2 has been found either in animal or in epidemiological studies (BUA 1991, IPCS 2002).

Table 5.4 Summary of the data on potential endocrine mediated responses in laboratory mammals following oral exposure

Species	Life stage of the test organism at start of test	Exposure route and dose series	Description of endocrine disruption measurement parameter(s) and effect doses	Reference	Test Relevance	Study Validity
Rat	Adult females	Oral gavage at 0, 600, 1200 and 1800 mg kg body weight^{-1} daily during gestation days 6 - 15	Non-dose dependent evidence of teratogenicity and dose dependent reductions in foetal weight	Jones-Price *et al* (1984a)	Medium	Use with care
Rabbit	Adult females	Oral gavage at 0, 25, 75 and 150 mg kg body weight^{-1} daily during gestation days 6 - 19	Foetal malformations at 150 mg kg body weight^{-1} and dose-dependent reductions in foetal numbers and weights	Jones-Price *et al* (1984b)	Medium	Valid

Table 5.5 Summary of the data on potential endocrine mediated responses in laboratory mammals following inhalation exposure

Species	Life stage of the test organism at start of test	Exposure route and dose series	Description of endocrine disruption measurement parameter(s) and effect doses	Reference	Test Relevance	Study Validity
Rat	Adult males	Inhalation at 0, 1106 and 1800 mg/m^3 for 5 hours a day, 5 days per week for 10 weeks	Significant reduction (relative to the controls) of the spermatocyte count in the epididymis and ejaculate and altered mating behaviour at 1896 mg/m^3 Significant reduction of plasma testosterone at 1896 mg/m^3	Tepe and Zenick 1984	Medium	Valid
Rat	Adult males	Inhalation at 0, 1106 and 1800 mg/m^3 for 5 hours a day, 5 days per week for 10 weeks	Significant reduction (relative to the controls) of the spermatocyte count in the epididymis and ejaculate and altered mating behaviour No changes (relative to the controls) of endocrine parameters (testosterone, FSH, LH and response to HCG) at any test dose	Zenick *et al* (1984)	Medium	Valid

Table 5.5 Continued

Species	Life stage of the test organism at start of test	Exposure route and dose series	Description of endocrine disruption measurement parameter(s) and effect doses	Reference	Test Relevance	Study Validity
Rat	Adult females	Inhalation at 0 and 2000 mg/m^3 for 2 hours per day during pregnancy	No effects (relative to the controls) on the course of pregnancy or foetal weight	Yaroslavskiy (1969)	Medium	Use with care
Rat	Adult females	Inhalation at 0, 1, 10 and 100 mg/m^3 for 4 months	Significant prolongation of the oestrous cycle (relative to the controls) at 10 and 100 mg/m^3	Acadzhanova (1978)	Medium	Use with care
Rat	Adult females	Inhalation at 0, 60-120 mg/m^3 for 7 hours a day, 5 days a week from 3 weeks before mating to gestation day 18	No significant teratogenic effects and no effects on course of pregnancy (relative to the controls) at any test dose	Beliles *et al* (1980)	Medium	Valid
Rat	Adult females	Inhalation at 0, 0.03, 10, 100 and 200 mg/m^3 over two generations	Significant embryotoxic and teratogenic effects (relative to the controls) at $\geq$100 mg/m^3 Generational differences in response recorded with enhanced sensitivity in F_2 generation	Tabacova *et al* (1983)	Medium	Use with care
Rat	Adult females	Inhalation at 0, 310, 620, 1240 and 2480 mg/m^3 for 6 hours per day on gestation days 6 -20	Significant embryotoxic and teratogenic effects (relative to the controls) at $\geq$1240 mg/m^3	Saillenfait *et al* (1989)	Medium	Valid
Rat	Adult females	Inhalation at 0, 388 and 775 mg/m^3 from 2 weeks prior to mating through to gestation day 19	No significant teratogenic effects (relative to the controls) at any test dose	Nemec *et al* (1993)	Medium	Valid
Rat and rabbit	Adult females	Inhalation at 0, 62.3 and 124.6 mg/m^3 from 34 weeks before breeding and during pregnancy	No significant teratogenic effects and no effects on course of pregnancy (relative to the controls) at any test dose for either species	Hardin *et al* (1985)	Medium	Valid
Mouse	Adult females	Inhalation at 0 and 2000 mg/m^3 for 2 hours per day during the entire pregnancy	No effects (relative to the controls) on the course of pregnancy or foetal weight	Yaroslavskiiy (1969)	Medium	Use with care

Table 5.5 Continued

Species	Life stage of the test organism at start of test	Exposure route and dose series	Description of endocrine disruption measurement parameter(s) and effect doses	Reference	Test Relevance	Study Validity
Rabbit	Adult females	Inhalation at 0, 60-120 mg/m^3 for 7 hours a day, 5 days a week from 3 weeks before mating an to gestation day 21	No significant teratogenic effects and no effects on course of pregnancy, embryos, number and weight of foetuses (relative to the controls) at any test dose	Beliles *et al* (1980)	Medium	Valid
Rabbit	Adult females	Inhalation at 0, 190, 316, 948, 1896 and 3792 mg/m^3 for 6 hours per day on gestation days 6 - 18	Significant embryo-foetal toxicity (relative to the controls) at $\geq$1896 mg/m^3	Pathology Associates (1991)	Medium	Valid

Table 5.6 Summary of the data on potential endocrine mediated responses in laboratory mammals following intra-peritoneal injection

Species	Life stage of the test organism at start of test	Exposure route and dose series	Description of endocrine disruption measurement parameter(s) and effect doses	Reference	Test Relevance	Study Validity
Rat	Adult males	Injection of 0, 12.5 and 25 mg kg^{-1} every day for 60 and/or 120 days	No significant effects (relative to the controls) on histology of the testis at 12.5 mg kg^{-1} after 60 days Significant reductions in the spermatocyte count after 60 days and degeneration and atrophy of the testicular tubules after 120 days exposure to 25 mg kg^{-1} day^{-1}.	Gondzik *et al* (1971)	Medium	Use with care
Rat	Adult males	Injection of 0, 5, 10, 15 or 25 mg kg^{-1} once per week for 6 months	Dose dependent effects on the histopathology of the testis	Mihalache and Mihalache (1989)	Medium	Use with care

E. General conclusions on potential endocrine mediated responses to carbon disulphide in laboratory mammals in in vivo studies

There appears a lack of good quality regulatory studies of sub-acute (e.g. OECD 407; USEPA 870.3050) or subchronic (OECD 408/409; USEPA 870.3100/ 870.3150) exposure by the conventional oral/dietary route, or reproduction (OECD 416, 421; USEPA 870.3800/ 870.3550) or chronic toxicity/carcinogenicity (OECD 451/ 452/ 453; USEPA 870.4100/ 870.4200/ 870.4300) studies. However, a series of non-regulatory studies (see Table 5.7) have been conducted generally using the inhalation route of exposure although the reports are often unclear on the actual mg kg^{-1} doses achieved.

Carbon disulphide can affect male fertility in rats through changes in sperm count and mating behaviour and the NOEL in laboratory animals for effects is at > 1000 mg/m^3. There is some evidence that the observed effects are caused by a toxic effect on the testis or by indirect effects on the ejaculation process. However, BUA (1991) stated that the influence of the hypothalamus-pituitary gland-gonad axis is improbable.

A number of studies have shown that carbon disulphide possesses embryotoxic effects at high doses but levels that are lower than those causing maternal toxicity. Rabbits appear to be more sensitive than rats. Teratogenic effects were described exclusively at maternally toxic doses. The NOEL for embryotoxic effects for rabbits was in the region of 900 mg/m^3 and was higher for teratogenic effects. Initial neurotoxic effects already occur at these concentrations in the 90 day tests. The results of Tabacova *et al* (1978, 1983) and Tabacova and Balabaeva (1980) describe embryotoxic effects from carbon disulphide at 0.03 mg/m^3 and teratogenic effects at 10 mg/m^3. However, there are issues with the quality of these studies because of the poor test descriptions, accuracy of the exposure doses and unverifiable calculations of statistical significance of the results.

BUA (1991) concluded that "*In laboratory animals CS_2 is embryotoxic at high doses or concentrations, which, however, are still below the maternal toxicity threshold. Teratogenic effects can be observed only at maternally toxic doses*.".

The Environmental Hazard Assessment prepared for the United Kingdom Department of the Environment (BRE 1993) concluded that "*Exposure to carbon disulphide has been shown to cause adverse effects on fertility in male and female rats. Embryo/foetotoxicity and teratogenicity also occurred at exposure concentrations not producing maternal toxicity.".*

Table 5.7 Summary of the potential endocrine mediated responses reported for carbon disulphide in *in vivo* studies with laboratory mammals

Type of study	Species and exposure route used	Dose series used	NOEL (mg kg body weight^{-1} day^{-1})		Reference
			Potential endocrine mediated responses	Systemic toxicity	
Sub-chronic toxicity	Rat (Intra-peritoneal injection)	0, 25 mg kg^{-1}	25 mg kg^{-1} (LOAEL - testicular pathology)	No data in this study	Gondzik (1971)
Reproduction – One generation	Rat (Inhalation)	0, 1106 and 1896 mg/m^3 (0, 350 and 600 ppm)	1106 mg/m3 (350 ppm) LOAEL - altered male sexual behaviour and sperm counts = 1896 mg/m^3)	No data in this study	Tepe and Zenick (1984), Zenick *et al* (1984)
Reproduction – Two generation	Rat (Inhalation)	0, 0.03, 10, 100 and 200 mg/m^3	0.03 mg/m^3 (NOAEL) 10 mg/m^3 (LOAEL - postnatal viability and development)	No data in this study	Tabacova *et al* (1983)
Development/ teratogenicity	Rat (Inhalation)	0, 2000 mg/m^3 (0, 642 ppm)	2000 mg/m^3 (642 ppm) (LOAEL - embryotoxicity)	No data in this study	Yaroslavskiy (1969)
	Rat (Inhalation)	0, 310, 620, 1240 and 2480 mg/m^3 (or 0, 100, 200, 400 and 800 ppm)	1240 mg/m^3	1240 mg/m^3	Sallenfait *et al* (1989)
	Rat (Inhalation)	0, 388 and 775 mg/m^3 daily (0, 125 and 250 ppm)	775 mg/m^3 (250 ppm) (Developmental)	388 mg/m^3 (Maternal toxicity)	Nemec *et al* (1993)
	Rat and rabbit (Inhalation)	Inhalation at 0, 60-120 mg/m^3	120 mg/m^3 in both species (Developmental)	120 mg/m^3 (Maternal toxicity)	Beliles *et al* (1980)
	Rat and rabbit (Inhalation)	0 62.3 and 124.6 mg/m^3 (or 0, 20 and 40 ppm (~ 5 and 10 mg kg^{-1} day^{-1} orally in rats and 11 and 22 mg kg^{-1} day^{-1} in rabbits)	124.6 mg/m^3 (40 ppm) in rats and rabbits (NOAEL)	124.6 mg/m^3 (Maternal toxicity)	Hardin *et al* (1981)
	Rabbit (Inhalation)	(0, 190, 316, 948, 1896 and 3792 mg/m^3 0, 60, 100, 300, 600 and 1200 ppm)	948 mg/m^3 (300 ppm) (Embryo-foetal toxicity)	948 mg/m^3 (300 ppm) (Maternal toxicity)	Pathology Associates (1991)
	Rabbit (Oral)	0, 25, 75 and 150 mg kg body weight^{-1} day^{-1}	75 mg kg body weight^{-1} day^{-1} (LOAEL - Embryotoxicity)	25 mg kg body weight^{-1} day^{-1} (Maternal toxicity)	Jones-Price *et al* (1984b)

5.4.1.3 Human studies

Acute intoxication from carbon disulphide occurred more frequently at the start of the 20th century at viscose rayon and rubber plants (Henschler 1975, WHO 1979)(see Table 5.8). Coma and deaths were observed at exposure concentrations of ≥10000 mg/m^3. Short-term exposure to CS_2 at concentrations of 3000 – 5000 mg/m^3 led to acute neuropsychological effects, extreme irritability and aggressiveness, mood swings, delirium, hallucinations, paranoid and suicidal tendencies.

Table 5.8 Intoxication symptoms in humans after short-term exposure to carbon disulphide

Concentration (mg/m^3)	Exposure period	Symptoms
500 - 700	No data	No subjective symptoms
1000 – 1200	8 hours	Headaches, drowsiness
1500 – 1600	4 hours	Headaches, vasomotor disturbances
2500	1.5 – 3 hours	Severe headaches
3600	0.5 hours	Dizziness
	1.5 – 3 hours	Sensibility, disturbances
6400 - 10000	0.5 hours	Narcotic state, severe headaches

In chronic studies a primary target of carbon disulphide (CS_2) toxicity is the nervous system and the major neurotoxic action of carbon disulphide is the development of mental disturbances. These include change of personality, irritability, and forgetfulness, often with accompanying neurophysiological and neuropathological changes after prolonged exposure. Such changes include decreased peripheral nerve impulse conduction, motor and/or sensory neuropathies, cerebral or cerebellar atrophy, and neuropsychological organic changes (Aaserud *et al* 1988, 1990; Foa *et al* 1976; Hirata *et al* 1992; Ruijten *et al* 1990, 1993). Alterations in behavioral indices have historically been associated with high levels of CS_2, often in excess of 63.2 mg/m^3 or 20 ppm (Foa *et al* 1976; Hanninen *et al* 1978). Vascular atherosclerotic changes are also considered a major effect of chronic carbon disulphide exposure in humans. Several occupational studies have demonstrated an increase in the mortality due to ischemic heart disease in CS_2 exposed workers (Hernberg *et al* 1970; MacMahon and Monson 1988; Tiller *et al* 1968; Tolonen *et al* 1979, Swaen *et al* 1994). A 2.5-fold excess in mortality from coronary heart disease in workers exposed to CS_2 was first reported by Tiller *et al* (1968). A subsequent prospective study by Hernberg *et al* (1970) found a 5.6-fold increased risk in coronary heart disease mortality and a 3-fold increased risk of a first nonfatal myocardial infarction in CS_2 exposed workers.

However, there are numerous reports of effects on the endocrine and reproductive system following occupational exposures to carbon disulphide. These include menstrual disorders, pregnancy complications and abortions in women (compare with section on developmental toxicity and incidence of embryofoetal loss), increased fasting blood sugar (diabetogenic effect), reduced serum hormones and lower sperm counts with abnormal sperm development (see Table 5.9). The exact exposure concentrations for these studies are unknown, but they were probably orders of magnitude above current levels (BUA 1991).

Gonad and hormone effects in males

Cavalleri *et al* (1966a,b) studied urinary excretion of total 17-ketosteroids and 17-hydroxysteroids in male Serbian CS_2 workers in an artificial fibres plant. Average exposures

ranged from 183-525 mg/m^3 and workers were divided into 5 groups depending on duration of exposure (workers were in the age range 22-30 years and this was suggested to provide an age controlled homogenous group). Urinary 17-ketosteroids were decreased in an exposure related manner with lowest levels in the groups with longest and most marked exposure histories (results expressed in mg/24 hours: control 11.27, 10.6 in those exposed up to 1 year, 9.41 in those exposed up to 4 years, 8.34 for workers exposed up to 8 years and 6.68 in invalids identified through previous intoxication incidents). Excretion of urinary 17-hydroxysteroids was also decreased. Levels of adrenocortical hormones and metabolites (cortisol, cortisone and its metabolite 17-ketosteroid) have also been found to be reduced in workers in a viscose rayon mill in China (Yang *et al* 1998). Together these studies indicate an effect on adrenocortical function related to CS_2 exposure.

Wink (1970) studied urinary excretion of 17-ketosteroids in workers with an average exposure of 30 mg/m^3 CS_2 and found a statistically and biologically non-significant trend for increased excretion compared with non-exposed controls. Wink (1970) pointed out differences with previous studies reporting decreased excretion of corticosteroids and 17-ketosteroids and the differences in study designs and subject selection, history and exposure.

Lancranjan *et al* (1969) in a study of 33 exposed viscose rayon workers with intoxication symptoms and 31 control subjects found that concentrations of 40-80 mg/m^3 (with peaks up to 780 mg/m^3) resulted in reduced sperm counts and altered sperm morphology and decreased excretion of 17-ketosteroids in males. In a later study of 109 subjects chronically exposed to carbon disulphide (reportedly 15-20 times the maximum allowable concentration of 15 mg/m^3 at the time of the study with peaks of up to 780 mg/m^3) and showing frank signs of neuropathy from "sulfocarbonism", there was no evidence of thyroid dysfunction (Lancranjan *et al* 1972) although the available methodology given in this study was limited. Lancranjan (1972) also reported that occupational exposure to CS_2 produces adverse effects on spermatogenesis.

Cirla *et al* (1978) found reduced thyroxine in 80 heavily exposed (120-240 mg/m^3 with last three years < 60 mg/m^3) male rayon workers compared with 55 non-exposed controls. This study also reported reductions in FSH and LH and increased incidence of sexual impotency (although testosterone was not affected) in the heavily exposed group compared with non-exposed controls. The authors concluded the problem was not one of clinical impotence but rather of sexual behavioural difficulties not correlated with hormone failure or age.

Wagar *et al* (1981) conducted a study of 15 male workers exposed to CS_2 at levels below the Finnish threshold limit value of 30 mg/m^3 (or 10 ppm) and reported serum follicle stimulating hormone (FSH) and lutenising hormone (LH) was elevated but prolactin, cortisol and thyroid function did not differ with age matched controls.

Vanhoorne *et al* (1993, 1994) investigated responses of viscose rayon workers in Belgium. In a study of 117 viscose rayon workers exposed to CS_2 (personal monitoring of 17 individuals showed exposures varied from 4 to 112 mg/m^3) reduced prolactin was reported (Vanhoorne *et al* 1993) while male workers exposed to CS_2 also reported reduced libido and potency (Vanhoorne *et al* 1994).

In a cross sectional study of 432 male rayon manufacturing workers in Japan exposed to CS_2 the endocrinologic parameter of glycosylated haemoglobin (diabetogenic indicator) was increased (Takebayashi *et al* 1998).

Hormone and pregnancy effects in females

Zhou *et al* (1988) investigated pregnancy outcomes and menstrual disturbances in 265 women occupationally exposed to CS_2 in five facilities and 291 controls. The CS_2-exposed women had a significantly higher incidence of menstrual disturbances (34.9%) compared to the control group (18.2%). CS_2 levels varied between the five facilities (exposure category means of low = 3.1 mg/m^3, intermediate = 6.5 mg/m^3, and high = 14.8 mg/m^3), but all workers from these CS_2 facilities had significantly higher incidences of menstrual disturbance. Irregularity of menstruation was the most common disturbance, followed by abnormal bleeding. No evidence was observed to indicate an adverse effect on the term and outcome of pregnancy. However, this study has a number of design flaws including differences between the 'matched' groups which raise issues as to the validity of the conclusions.

Bao *et al* (1991) using historical prospective epidemiological methods monitored the pregnancy outcome of 682 female workers in China occupationally exposed to CS_2 at least 6 months before pregnancy and compared findings with 745 matched (age, area, healthcare provision, education) controls. An increased rate of birth defects (2.6%) among 682 exposed women was noted compared to 745 women in the control group (1.3%). The most common defects were congenital heart defects, inguinal hernia, and CNS defects. However, there was no significant difference in birth defects between those with estimated exposures greater than 10 mg/m^3 compared to those with lower exposures. There were no differences in rates of stillbirth, low birth weight, or neonatal or perinatal deaths among any of the groups.

In a study of 199 women in Poland with symptoms of menopause who either worked in a clothing factory without exposure to CS_2 (80 individuals) or in a synthetic fibres factory where exposure to CS_2 was in the range of 9.36 - 23.4 mg/m^3, numerous endocrine and reproductive effects were detected (Pieleszek 1997). Women exposed to CS_2 showed earlier menopause, menstrual disturbances, increased abortions, decreased serum concentrations of oestrone, oestradiol, progesterone, 17-hydroxyprogesterone, plasma dopamine and urinary adrenaline and noradrenaline and increased serum serotonin, testosterone and dehydroepiandrosterone sulphate and plasma prolactin. No differences were detected in FSH or LH levels to account for the changes in the sex steroids (compare with Wagar *et al* 1981 and Vanhoorne *et al* 1994).

Table 5.9 Potential endocrine mediated effects in humans after chronic exposure to carbon disulphide in viscose rayon plants (after BUA 1991)

Study	Exposure concentration (mg/m^3)	Exposure period (years)	Significant findings	Reference
Effects on male hormones and gonads				
70 exposed viscose workers and 26 workers as controls	185 - 525 (Max ≤ 900)	1 - 8	Decreased excretion of 17-keto and 17-hydroxy steroids dependent on exposure period	Cavalleri *et al* (1966a,b)
33 exposed viscose workers with intoxication symptoms and 31 controls	40 – 80 (Max ≤ 780)	2	Reduced sperm count and altered sperm morphology, decreased excretion of 17-keto-steroids	Lancranjan *et al* (1969)
15 exposed viscose workers and 15 workers as controls	30	23	No effects on 17-keto and 17-hydroxy steroid levels in urine	Wink (1970)
140 exposed viscose workers with intoxication symptoms and 50 controls	No data	3.5	Reduced sperm count and altered sperm morphology	Lancranjan 1972
68 exposed viscose workers and 15 workers as controls	185 – 525 (Max ≤ 900)	2 - 14	Reduced serum thyroxine levels	Cavalleri 1972
50 exposed viscose workers and 50 workers as controls (cohort study)	10 – 25 (Max ≤ 30)	3 - 12	No effects on serum thyroxine, FSH, LH and testosterone levels	Cirla and Graziano (1981)
15 exposed viscose workers and 16 controls	< 10 - < 30	10 - 36	Increased serum FSH and LH , but no increase in serum prolactin, cortisol, thyroxine and TSH and testosterone levels	Wagar *et al* (1981)
86 exposed viscose workers and 89 workers as controls	3 - 45 (72% < 10)	1 - 31	No effects on sperm count and sperm morphology	NIOSH (1984)
20 exposed viscose workers, 10 slightly exposed viscose workers as controls	50 - 100	> 10	No effects on serum FSH, thyroxine and testosterone levels	Vanhoorne (1981)
231 exposed viscose workers and 231 viscose workers as controls (cohort study)	10 - 25 (≤ 60 earlier in part)	14	Increased fertility (number of children) in the exposure group (as a result of greater social status)	Braun and Kolk (1985)
Effects on female hormones and pregnancy				
183 exposed viscose workers and 197 workers as controls	40 – 60 (Calculated)	1 - 6	Menstrual complaints were frequently reported	Cai and Bao (1981)
92 exposed viscose rayon workers and 108 viscose workers as controls	40 – 60 (Calculated)	1 - 6	Complications with pregnancy and birth frequently reported	"
Comparative retrospective study in Finland (643 spontaneous abortions and 650 births)	No data	No data	For women viscose rayon workers significantly increased spontaneous abortion rate, but also increased when only the husband worked at plant	Hemminki and Niemi (1982)
256 exposed viscose workers and 291 workers as controls	3 -15	1 - > 10	Menstrual complaints were frequently reported, but differences in pregnancy complications and birth numbers were not significant	Zhou *et al* (1988)

It needs to be recognised that these studies have been conducted over a number of years and older data does not necessarily reflect current working practices that have been adopted in Western Europe. It is also important to recognise that the carbon disulphide concentrations reported may not accurately reflect those to which subjects were actually exposed since in most cases these were spot samples rather than means of values taken over a period of time. Furthermore, in plants producing viscose rayon the levels of carbon disulphide to which subjects are exposed depends on the actual part of the process under consideration (see Section 5.7). A report on the chronic toxicity of carbon disulphide by the Californian Office of Environmental Health Hazard Assessment (OEHHA 2000) concluded that "*The possibility of determining LOAEL and/or NOAEL values for the major CS_2-related adverse effects from epidemiology studies, which predominately use workers from the viscose rayon industry, is limited. The limitations include incomplete historical exposure measurements, concurrent exposure to other chemicals (including hydrogen sulphide or methylene chloride), lack of personal exposure determinations, and a high variability of individual exposures due to decreases of plant CS_2 concentrations over time.*".

5.4.2 Studies relevant to the assessment of potential endocrine disrupting effects in wildlife

5.4.2.1 In vitro studies

No data has been located on the conduct of *in vitro* studies using cells and tissues from wildlife species.

5.4.2.2 In vivo studies

A. Studies on aquatic organisms

Ghate (1985) investigated the effects of carbon disulphide on the development and lethality of embryos of the frog *Microhyla ornata*. Embryos were exposed for 96 hours to a high[3] nominal CS_2 concentration of 126 mg l^{-1} (0.5 ml of a 2% CS_2 solution in alcohol were added to 100 ml of water) which resulted in a 90-100% occurrence of swelling of the notochord (pre-vertebrae) at the base as well as waviness along its length. The embryos showed slight oedema, but pigmentation was unaffected and eclosion did not occur.

Van Leewen *et al* (1986) investigated the effects of carbon disulphide on the embryo-larval development of rainbow trout (*Oncorhynchus mykiss*). The embryos were exposed to a high nominal CS_2 concentration of 100 mg l^{-1} from 0.25 to 48 hours. The resulting ET_{50} values (exposure time after which teratogenic or lethal effects occur in half the embryos) for 3 hour, 7 day, 10 day and 14 day old embryos were 0.7 hours, 14 hours, 25 hours and 15 hours respectively.

In a study carried out by Akzo Nobel (1991) the effects of carbon disulphide on the development and lethality of eggs of the zebrafish (*Danio rerio*) was determined. In the study, groups of eggs were exposed to nominal CS_2 concentrations of 0, 24, 76, 243 and 778 µg l^{-1} for 10 days in the first study and nominal CS_2 concentrations of 0, 1000, 2500 and 6250 µg l^{-1}

[3] Under Directive EC/79/831 a substance is classified as very toxic to aquatic organisms if acute toxicity levels for relevant tests are below 1 mg l^{-1}. while it would be is classified as toxic to aquatic organisms if acute toxicity levels are in the range 1 – 10 mg l^{-1}. A substance is classified as harmful to aquatic organisms if acute toxicity levels are in the range 10 – 100 mg l^{-1}.

for 8 days in the second study both under semi-static regimes with closed vessels. In the first study no effects were evident on the hatching of eggs and malformations and lethality of larvae at the highest test concentration (778 μg l^{-1}). In the second study no marked effects were evident on the eggs or larvae at 1000 μg l^{-1} but effects were evident at the two higher exposure concentrations, where the development of zebrafish eggs was affected. The most sensitive parameter was the hatch rate of the eggs, though the survival of eggs and larvae and normal development were also affected. In the 2500 μg l^{-1} treatment the rate of hatching of the larvae was markedly delayed such that 97% of the larvae had only hatched by day 8 whereas >95% of larvae had hatched in the controls by day 5. In the 6250 μg l^{-1} treatment malformations in the notochord were observed, which were abnormally stretched and twisted within 48 hours after fertilisation. Irregularities in the muscle tissues and organ dislocations were closely related to the notochordal anomalies. The affected animals were shorter and squater with aberrant pigmentation. Due to the defective chorda, swimming movements and feeding were impossible which ultimately resulted in lethality. Non-specific malformations which were also observed following CS_2 exposure included oedemous bodies a lumpy skin, incomplete fins and early death of the hatched larvae. In the second study the NOEC value for hatching was 1000 μg l^{-1} (LOEC = 2500 μg l^{-1}) while the NOECs for specific malformations and survival were 2500 μg l^{-1} (LOEC = 6250 μg l^{-1}).

B. Studies on terrestrial organisms

No data has been located on the potential endocrine disrupting effects of carbon disulphide on terrestrial organisms. Given that carbon disulphide is not expected to strongly sorb to organic carbon (see Section 5.1) the absence of data on potential endocrine disrupting effects in terrestrial organisms does not represent a key area of uncertainty. It should also be recognised that there are currently no internationally agreed methods specifically developed to assess potential endocrine disrupting effects in terrestrial organisms.

C. Studies on aerial organisms

No data has been located on the potential endocrine disrupting effects of carbon disulphide on aerial organisms. Given that carbon disulphide is highly volatile the absence of data on potential endocrine disrupting effects in aerial organisms represents an important area of uncertainty. It should also be recognised that there are currently no internationally agreed methods specifically developed to assess potential endocrine disrupting effects in aerial organisms.

D. General conclusions on potential endocrine mediated responses in in vivo studies with wildlife species

Only limited data have been located on the potential endocrine disrupting effects of carbon disulphide on aquatic, terrestrial or aerial organisms (see Table 5.10). In the aquatic environment carbon disulphide has been shown to affect the development of frog embryos

Table 5.10 Summary of the studies assessing potential endocrine disrupting effects in wildlife

Environmental compartment	Taxonomic group	Type of study	Species and exposure route used	Concentration series used	Lowest reported NOEC	Reference
Aquatic	Amphibians	96h Embryo-larval test	Frog (*Microhyla ornata*) – Aqueous exposure	0, 126 mg l^{-1}	< 126 mg l^{-1} (a)	Ghate (1985)
	Fish	8 day Embryo-larval test	Zebrafish (*Danio rerio*) – Aqueous exposure	0, 1.0, 2.5 and 6.25 mg l^{-1}	1.0 mg l^{-1} (a) (Hatching rate)	Akzo Nobel (1991)
	Invertebrates	No data	-	-	-	-
Terrestrial	Birds	No data	-	-	-	-
	Invertebrates	No data	-	-	-	-
Aerial	Invertebrates	No data	-	-	-	-

a – No information is available on the mechanism of action

with effects as clear malformations of the notochord being evident, though only at a high exposure concentration of 126 mg l^{-1}. In rainbow trout effects on embryo-larval development were also evident at an exposure concentration of 100 mg l^{-1}. In zebrafish effects on hatching rate were evident at nominal exposure concentrations of 2.5 mg l^{-1} (NOEC = 1 mg l^{-1}), though there is no information on the mechanism of action for the effects. Information is not available on the effects of carbon disulphide on the reproduction of aquatic organisms.

The volatility of carbon disulphide means that wildlife organisms that are exposed to carbon disulphide via inhalation represent those potentially most at risk from exposure. However, it should be recognised that this substance is released into the environment from natural sources (see Section 5.6).

5.5 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity

The general toxicity data in this section has largely been obtained from the IUCLID data set for carbon disulphide and has been taken as accurate. Individual source documents have not been checked unless they are considered to be key studies which have a major influence on the outcome of the review. All information taken from IUCLID as been referenced as being from that source and individual references have not been given in the references.

5.5.1 Studies relevant to the assessment of general toxicity in humans

Table 5.11 summarises the general toxicity data from acute and repeat dose studies with carbon disulphide which has largely been collated from the information in IUCLID (2000).

5.5.1.1 Acute studies

A. Oral exposure

Carbon disulphide is of low acute oral toxicity with LD_{50} values of 3188 mg kg^{-1}, 2780 – 3020 mg kg^{-1} and 2125 mg kg^{-1} reported for the rat, mouse and Guinea pig respectively (See Table 5.8).

B. Dermal exposure

No data has been located of the acute effects of carbon disulphide via dermal exposure.

C. Inhalation exposure

Acute inhalation studies have been carried out in rats, mice, rabbits and cats (see Table 5.8). Gibson and Roberts (1972) and Izmerov *et al* (1982) reported LD_{50} values of >0.7 and 10 mg l^{-1} in inhalation studies with rats. Henschler (1975) reported a 6 hour LC_{100} value of 16 mg l^{-1} in rabbits and 1 and 3 hour LC_{100} values of 122 and 23 mg l^{-1} respectively in cats. In the National Toxicology Programme inhalational LC_{50} values for rats and mice have been reported as 25 g m^{-3} h^{-1} and 10 g m^{-3} h^{-1} respectively (HSDB 2001).

D. Other routes of exposure

Gibson and Roberts (1972) reported an intra-peritoneal LD_{50} value of 1890 mg kg^{-1} in mice while in rabbits Henschler (1975) reported an LD_{50} value of 300 mg kg^{-1} following sub-cutaneous injection while Brieger (1949) reported an LD_{50} value of 315 mg kg^{-1} following intra-venous injection.

Green and Hunter (1985) estimated 24-hour LD_{50} values for carbon disulphide in 1, 5, 10, 20, 30 and 40-day-old rats after intra-peritoneal injection. Carbon disulphide was least toxic to 20 day old pups (LD_{50} = 1545 mg kg^{-1}) and most toxic to 1 day old pups (583 mg kg^{-1})(see Table 5.7). In the National Toxicology Programme the intra-peritoneal LD_{50} in Guinea pigs has been reported as 400 mg kg^{-1} (NTP).

5.5.1.2 Repeat dose studies

A. Oral exposure

There appear to be no regulatory standard sub-acute, sub-chronic or chronic toxicity studies by the oral route.

B. Dermal exposure

At present no data has been located on the repeat dose toxicity of carbon disulphide to laboratory mammals following exposure by dermal exposure.

C. Inhalation exposure

Cohen *et al* (1959) conducted a study to determine the biochemical changes due to carbon disulphide in 11 male New Zealand white rabbits. The rabbits were exposed to carbon disulphide by inhalation for 6 hours per day, 5 days per week, for up to 38 weeks. Concentrations of carbon disulphide were 775 mg/m^3 (250 ppm) during the first 16 weeks, 1555 mg/m^3 (500 ppm) for the next 5 weeks, and 2330 mg/m^3 (750 ppm) for the final 17 weeks. Total serum cholesterol increased in exposed rabbits when the carbon disulphide concentration was increased to 2330 mg/m^3 (750 ppm) and returned to normal after cessation of exposure. Increased urinary and faecal excretion of zinc by the exposed rabbits and a gradual decrease in the mean concentration of zinc in the blood serum was noted during the study.

Wronska-Nofer (1973) also showed a positive relationship between the level of triglycerides, the rate of cholesterol synthesis, and CS_2 exposure in Wistar rats exposed to 0, 233, 506, 1014 and 1725 mg/m^3 CS_2 (0, 73.8, 160, 321 and 546 ppm) for 5 hours a day, 6 days per week over 8 months. This study found a sub-chronic LOAEL of 233 mg/m^3 (73.8 ppm) for disturbances in lipid metabolism (increase in serum cholesterol and serum triglycerides).

Subsequently Lewis *et al* (1999) investigated the capacity of CS_2 to induce arterial fatty deposits by itself, and its ability to enhance the rate of fatty deposit formation induced by a high fat diet. Groups of 20 female C57BL/6 mice were exposed to 0, 158, 1580 and 2528 mg/m^3 CS_2 (0, 50, 500 and 800 ppm) by inhalation. Half the animals in each group were placed on an atherogenic high fat diet and half on a control diet. Mice were necropsied after 1, 4, 8, 12, 16, or 20 weeks of exposure, and the rates of fatty deposit formation under the aortic valve leaflets were evaluated. Exposure of mice on the control diet to 1580 and 2528 mg/m^3 (500 and 800 ppm) CS_2 induced a small but significant increase in the rate of fatty deposit formation over non-exposed controls. In the animals on the high fat diet there was marked enhancement of the rate of fatty deposit formation in mice exposed to 1580 and 2528 mg/m^3

(500 and 800 ppm) over the animals on the high fat diet alone. In addition, there was a small but significant enhancement in mice exposed to 158 mg/m^3 (50 ppm) over the rate of fatty deposit formation induced by the high fat diet alone. Thus CS_2 is atherogenic at high concentrations and in conjunction with other risk factors, CS_2 at relatively low concentrations can enhance atherogenesis in mice. A LOAEL of 158 mg/m^3 (50 ppm) was reported in the study. The reported atherosclerosis in the study is consistent with findings in humans. Antov *et al* (1980) found thickening of the wall of the aorta and heart after a 4 month inhalational exposure to CS_2 up to 112 mg/m^3.

Seppalainen and Linnoila (1976) report a reduction of conduction velocity in the sciatic nerve of rats exposed to 240 mg/m^3 for 22 weeks (6 hours per day, 5 days per week for 10 weeks followed by 3 times per week for 12 weeks). Recovery of effects in a similar shorter duration study was also reported. Knobloch *et al* (1979) in an inhalation study in Wistar rats observed impairment in the conduction velocity of the sciatic and tibial nerves after 6 and 12 months of intermittent exposure to or 913 mg/m^3 or 289 ppm CS_2 (LOAEL of 913 mg/m^3).

In a 90 day sub-chronic inhalation study, Sprague-Dawley and Fischer 344 rats exposed discontinuously (6 hours per day, 5 days per week) to CS_2 developed morphological alterations in nerves including axonal swelling and myelin degradation (Gottfried *et al* 1985). This study established a sub-chronic NOAEL of 158 mg/m^3 (50 ppm) and a LOAEL of 948 mg/m^3 (300 ppm) for morphological changes in nerves.

Okayama *et al* (1987) exposed female Wistar rats to 316 or 1896 mg/m^3 (100 or 600 ppm) of carbon disulphide for 6 hours per day for 5 days per week for 12 weeks to determine the effects on tissue Vitamin B6 concentrations. Liver, kidney, and brain tissue were assayed at the end of 12 weeks (12 rats per group and 12 controls exposed to fresh air) for five forms of B6: pyridoxine, pyroxidal, pyridoxamine, pyridoxal phosphate, and pyridoxamine phosphate. During the experiment, urine was assayed for 4-pyridoxic acid on day 5 of weeks 2, 4, 6, 10, and 12. By week eight there were statistically significant differences in the body weights of the 3 groups; 221 $\pm$ 5.2 g in the controls, 216 $\pm$ 9.2 g in the 316 mg/m^3 treatment and 208 $\pm$ 7.5 g in the 1896 mg/m^3 treatment. Excretion of 4-pyridoxic acid throughout the experiment after exposure to 316 mg/m^3 was essentially the same as the control, but excretion of 4-pyridoxic acid after exposure to 1896 mg/m^3 was statistically significantly decreased during the first 4 weeks of exposure and was continuously different for the 12 weeks. Pyridoxine was not detected in liver, kidney, or brain tissue and pyridoxamine was detected only in liver tissue.

Hayes and Laws (1991) reported on a study in which eight dogs were exposed to 1264 mg/m^3 (400 ppm) carbon disulphide in air 8 hours per day, 5 days per week, for 10-15 weeks. When removed from the chamber, the dogs were drowsy and staggered and stumbled as if drunk. They were very thirsty but did not eat for hours after leaving the chamber. Although they slept most of the time they were in the chamber, they were excited and noisy at night in their kennels. The dogs also developed many clinical and pathological signs analogous to those in workers: marked behavioural changes and toxic disease of nerve cells of the cortex of the brain were observed in all of them. Rigidity and tremor (Parkinsonism) and choreatic movements were seen frequently, as was disease of the nerve cells of the basal ganglia. Motor weakness, flaccid paralysis, and nerve tenderness were the most frequent signs observed; the peripheral nerves showed axonal degeneration while the myelin sheath was well preserved. Cardiovascular changes included electrocardiographic abnormalitities especially inversion of the T wave, retinal angiospasms, and atherosclerosis of the veins of the cortex of the brain.

These findings in experimental animals comprising neurotoxicity and cardiovascular effects mirror those found in humans.

D. Other routes of exposure

At present no data has been located on the repeat dose toxicity of carbon disulphide to laboratory mammals following exposure by sub-cutaneous or intra-peritoneal injection.

5.5.1.3 Comparison of data from studies assessing potential endocrine mediated responses and/or general toxicity in mammals

Carbon disulphide (CS_2) has a wide range of reported effects in laboratory animals and humans (principally derived from occupational monitoring) including toxicity to the nervous (including ocular and retinopathy) and cardiovascular systems, liver, kidney and other organs and tissues. Carbon disulphide can react with various biological molecules and observed toxic effects can be based on a reaction with sulfhydryl- and amino groups of proteins and amino acids but also of catechol amines. This can lead to an impairment or disturbance of various biochemical parameters. Depletion of lower-molecular substances, e.g. catechol amines, amino acids (cysteine) or enzyme co-factors are also possible. Besides this, the formation of thiocarbamates is primarily held responsible for the neurotoxicity, because thiocarbamates can complex with various essential metals, such as copper and zinc, and thus inactivate certain enzymes. It has not been clarified whether carbon disulphide also reacts with amino groups of nucleic acids. Besides the direct reaction of carbon disulphide with biological molecules, the formation of a reactive intermediate during oxidative metabolism is also probable as a toxic mechanism. In particular, liver toxicity is thought to be connected with the formation of reactive sulphur from carbon disulphide which can bind onto sulfhydryl groups and thus inactivate enzymes and other SH-proteins. It is believed that the reduction of cytochrome P-450 is caused by this reactive sulphur. However, it is still not clear whether the excretion products of carbon disulphide, e.g. dithiocarbamic acid derivatives, originate from the reaction of lower molecular weight compounds with carbon disulphide or stem from proteins which have reacted with carbon disulphide and then were degraded (WHO 1979, Beachamp *et al* 1983, BUA 1991, BRE 1993).

In BUA (1991) a NOEL for the most sensitive biological endpoints was estimated to be between 150 and 800 mg/m^3 (47.5 and 253 ppm) for laboratory animals and between 10 and 60 mg/m^3 for humans. It was stated that it had been established beyond doubt that the cardiovascular mortality rate of workers exposed to CS_2 is increased only at exposure concentrations of > 60 mg/m^3, though there are differences between countries. These values compare with data for effects on reproduction which show that the NOELs in laboratory animals for effects on male fertility in rats (through changes in sperm count and mating behaviour) and humans for effects on changes in sperm morphology were > 1000 mg/m^3 and > 30 mg/m^3 respectively. In laboratory animals CS_2 is embryotoxic at high doses, which, however are still below the maternal toxicity threshold. Teratogenic effects can only be observed at maternally toxic doses.

Table 5.12 Summary of general acute mammalian toxicity data (Information from BUA 1991 and IUCLID 2000)

Test type	Test species	Exposure period	Test dose series used	Endpoint	Effect dose	Reference	Study validity
Acute Oral Toxicity	Rat	Not relevant	No data	Median lethal dose (LD_{50})	3188 mg kg body weight^{-1}	Izmerov *et al* (1982)[1]	Use with care[2]
	Mouse	Not relevant	No data	Median lethal dose (LD_{50})	3020 mg kg body weight^{-1}	Gibson and Roberts (1972)[1]	Use with care[2]
	Guinea Pig	Not relevant	No data	Median lethal dose (LD_{50})	2125 mg kg body weight^{-1}	HSDB (2001)[1]	Use with care[2]
Acute Inhalation Toxicity	Mouse	1 hour	No data	Median lethal dose (LC_{50})	>0.7 mg l^{-1}	Gibson and Roberts (1972)[1]	Use with care[2]
		2 hours	No data	Median lethal dose (LC_{50})	10 mg l^{-1}	Izmerov *et al* (1982)[1]	Use with care[2]
	Rabbit	6 hours	No data	Median lethal dose (LD_{100})	16 mg l^{-1}	Henschler (1975)[1]	Use with care[2]
	Cat	3 hours	No data	Median lethal dose (LD_{100})	23 mg l^{-1}	Henschler (1975)[1]	Use with care[2]
		1 hour	No data	Median lethal dose (LD_{100})	122 mg l^{-1}		
Acute Toxicity (Intra-peritoneal injection)	Rat	No data	No data	Median lethal dose (LD_{50})	583 - 1545 mg kg body weight^{-1}	Green and Hunter (1985)[1]	Use with care[2]
	Mouse	No data	No data	Median lethal dose (LD_{50})	1890 mg kg body weight^{-1}	Gibson and Roberts (1972)[1]	Use with care[2]
Acute Toxicity (Sub-cutaneous injection)	Rabbit	No data	No data	Median lethal dose (LD_{100})	300 mg kg body weight^{-1}	Henschler (1975)[1]	Use with care[2]
Acute Toxicity (Intra-venous injection)	Rabbit	No data	No data	Median lethal dose (LD_{100})	315 mg kg body weight^{-1}	Brieger (1949)[1]	Use with care[2]

[1] – Cited in IUCLID (2000), [2] – Assessment made on basis of data in IUCLID (2000)

As a result it would appear that in humans and laboratory mammals the potentially endocrine mediated effects occur at exposure levels *above* those causing other effects by other mechanisms. However, it has to be recognised that carbon disulphide presents possible risks of impaired fertility and harm to the unborn child.

5.5.2 Studies relevant to the assessment of general toxicity in wildlife

5.5.2.1 Studies on aquatic organisms

Table 5.13 summarises the general aquatic toxicity data from acute studies with carbon disulphide which has largely been collated from the information in BUA (1991) and IUCLID (2000).

A. Fish

Acute toxicity

Acute toxicity tests have been carried out on a range of fish species (see Table 5.8). The 96-hour LC_{50} values reported were 65 mg l^{-1} for bleak (*Alburnus alburnus*), 94-265 mg l^{-1} for golden orfe (*Leuciscus idus*), 3 - 4 mg l^{-1} for guppy (*Poecilia reticulata*) and 135 mg l^{-1} for mosquito fish (*Gambusia affinis*). In the study on golden orfe, Juhnke and Lüdemann (1978) recorded LC_0 and LC_{100} values of 25-164 mg l^{-1} and 126-290 mg l^{1}. The higher LC_{50} values in studies using static open systems may result from volatilisation and consequent concentration losses of carbon disulphide.

Chronic toxicity

No chronic toxicity data for fish following exposure to carbon disulphide has been located.

B. Invertebrates

Acute toxicity

The only acute toxicity data for aquatic invertebrates is a 48 hour immobilisation study with *Daphnia magna* (Van Leewen *et al* 1986) which reported 24h EC_{50} and 48h EC_{50} values of 10 and 2.1 mg l^{-1}.

Chronic toxicity

No chronic toxicity data for fish following exposure to carbon disulphide has been located.

5.5.2.2 Studies on terrestrial organisms

Donner *et al* (1981) fed male dew flies (*Drosophila melanogaster*) with feed containing CS_2 in concentrations of 200 to 1000 mg kg^{-1} (ppm) for 24 hours (emulsion of CS_2 in 0.1 ml Tween 80 in 9.9 ml of a 5% sugar solution). No lethal effect was observed up to 650 mg kg^{-1}, while 80% and 100% of the animals died at concentrations of 800 and 1000 mg kg^{-1}.

In pigeons reduced performance in conditioned response tests was observed after both 4 and 8 hours exposure to a CS_2 concentration of 2000 mg/m^3 when, although the appropriate

Table 5.13 Summary of the non-mammalian toxicity data for carbon disulphide (Information from IUCLID 2000)

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study validity
Acute Fish Toxicity	Bleak *(Alburnus alburnus)*	96h	No data	LC_{50}	65 mg l^{-1}	Bengtsson and Tarkpea (1983)[1]	Use with care[2]
	Golden orfe *(Leuciscus idus)*	48h	Static	LC_0	25 - 164 mg l^{-1}	Juhnke and Lüdemann (1978) [1]	Use with care[2]
				LC_{50}	95 - 265 mg l^{-1}		
				LC_{100}	126 - 290 mg l^{-1}		
	Guppy *(Poecilia reticulata*	96h	Semi-static, closed vessels	LC_{50}	4 mg l^{-1}	Van Leeuwen *et al* (1985) [1]	Valid
		96h	Semi-static, closed vessels	LC_{50}	3 mg l^{-1}	Akzo Nobel (1991)	Valid
	Mosquito fish *(Gambusia affinis)*	96h	Static	LC_{50}	135 mg l^{-1}	Wallen *et al* (1957a & b) [1]	Use with care[2]
Acute Invertebrate Toxicity	*Daphnia magna*	24h	Semi-static, closed vessels	EC_{50}	10 mg l^{-1}	Van Leeuwen *et al* (1985)	Valid
		48h	"	EC_{50}	2.1 mg l^{-1}		

[1] – Cited in IUCLID (2000), [2] – Assessment made on basis of data in IUCLID (2000)

actions which the pigeons had learnt were carried out, there were longer periods between successive attempts (Levine 1976).

5.5.2.3 Studies on aerial organisms

No general toxicity data for aerial organisms following exposure to carbon disulphide has been located.

5.5.2.4 *Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity in wildlife*

The data on potential endocrine mediated responses in wildlife is limited to studies on the development of frog and fish embryos. In studies on the embryos of the frog *Microhyla ornata* and the rainbow trout (*Oncorhynchus mykiss*) malformations of the notochord were found at nominal exposure concentrations at or above 100 mg l^{-1}. In the zebrafish effects on hatching rate were found at an exposure concentration of 2.5 mg l^{-1} (NOEC = 1 mg l^{-1}), but whether the mechanism of action is endocrine disruption is unknown. The latter effect concentration is similar to the lowest exposure concentrations of 2 - 4 mg l^{-1} causing acute toxicity in invertebrates and fish.

5.6 Current classification of the substance against European Commission and national regulations

Table 5.14 summarises the current classification of the substance against Council Directives in order to assess the regulations to which carbon disulphide is subject.

Table 5.14 Current classification of carbon disulphide against Council Directives

Directive	Status (listed or not)
67/548/EEC - Classification, packaging and labelling of dangerous substances	Classified: F, T, E R phrases: 11-36/38-48/23-62-63

Under Directive 67/548/EEC the R phrases indicate that there is danger of serious damage to health by prolonged exposure through inhalation (R48/23), possible risk of impaired fertility (R62) and a possible risk of harm to the unborn child (R63).

No national environmental quality standards for carbon disulphide have been derived in any European country for the protection of aquatic or terrestrial ecosystems.

5.7 Exposure data

The draft IPCS CICAD for carbon disulphide which is in preparation has stated that “virtually all anthropogenic and natural releases are to air” (IPCS 2002). Furthermore, in considering the exposure of different target groups to carbon disulphide it needs to be recognised that this substance is produced naturally and the presence of low levels in different environmental compartments does not indicate an anthropogenic source.

5.7.1 Worker exposure data

Occupational exposure to carbon disulphide may occur through inhalation and dermal contact with this compound at workplaces where carbon disulphide is produced or used. BUA (1991) investigated the balance of environmental emissions in the Federal Republic of Germany in 1990 and reported that almost all (97%) of the known anthropogenic emissions entered the atmosphere and originated from the viscose rayon industry.

5.7.1.1 Emissions of carbon disulphide

Data for CS_2 emissions has been provided by the ad-hoc user/ producer panel. The largest *CS_2 production location* has an annual loss of ~ 1 tonne, relative to a capacity of 80,000 tonnes (actual production 60,000 tonnes). The other two facilities probably have larger losses, since they do not yet have complete vent return systems. Venting losses for CS_2 are known to be 1 kg/tonne.

For *viscose rayon production plants* the worst case loss estimate (based on published data from one viscose rayon factory are that annual losses to the atmosphere are 82 % of the amount bought. One very large production facility (B) is emitting small volumes of CS_2 per tonne of production, suggesting catalytic conversion, but this could not be confirmed. One facility (E) is emitting around 3250 tonnes annually, that is ~ 15% of estimated annual consumption.

For *cellulose films and sausage skin production plants* worst-case estimates are that 73% of the amount bought is annually lost to the environment (air only). Calculated maximum concentrations at ground level (plume dispersion models) around one facility are 0.012 mg/m^3 for an annual loss of around 2400 tonnes annually. Some facilities have catalytic thermal conversion, which is believed to decrease the environmental loss percentage to around 10% of consumption. There are four (two cellophane film only and two both film and sausage casings) production facilities in Europe which are equipped with catalytic thermal conversion but no information on their annual consumption and emission patterns.

For *cellulose sponge production facilities* worst-case estimates are for a 35% loss of consumption to the environment, whereas best case estimates are a reduction of emissions to near zero by use of catalytic thermal conversion. Total CS_2 consumption for this sector is estimated to be below 2500 tonnes annually.

For *plants producing intermediates* (agricultural chemicals, rubber chemicals and pharmaceuticals/fine chemicals) limited data indicates that the amount lost to the atmosphere is below 0.5 % of the amount consumed.

5.7.1.2 Exposure levels in viscose rayon plants

In the 1990s information on levels of carbon disulphide measured in viscose rayon plants information has been presented by Riihimaki *et al* (1992), Kitamura *et al* (1993) and Drexler *et al* (1994).

Riihimaki *et al* (1992) reported carbon disulphide levels in ambient air samples of 0.63 – 28.4 mg/m^3 (0.2 – 9 ppm), mean value = 12.6 mg/m^3 (4 ppm) while concentrations in personal air samples (on a time weighted average) ranged from 9.5 – 22.1 mg/m^3 (3 – 7 ppm). Kitamura *et al* (1993) reported mean carbon disulphide levels in air samples collected from personal tubes during the production of viscose rayon fibres ranged from 1 to 148 mg/m3. Carbon disulphide

concentrations in the air of the spinning department of viscose rayon fibre factory ranged from 0.32 – 26.9 mg/m^3 with a mean value of 9.5 mg/m^3 (3 ppm). In the pause cabin CS_2 levels ranged from 0.032 – 3.2 mg/m^3 (0.01 – 1 ppm) with a mean value of 0.13 mg/m^3 (0.04 ppm).

Drexler *et al* (1994) compared carbon disulphide levels from passive personal air monitoring, active personal air monitoring and stationary air monitoring in a series of departments of a viscose rayon plant including spool spinning, spinning of technical rayon, washing, post treatment and second aging (see Table 5.15). The results from all monitoring approaches indicated that highest CS_2 levels were measured in the technical rayon spinning and washing areas whereas lower CS_2 levels were recorded in the second aging area.

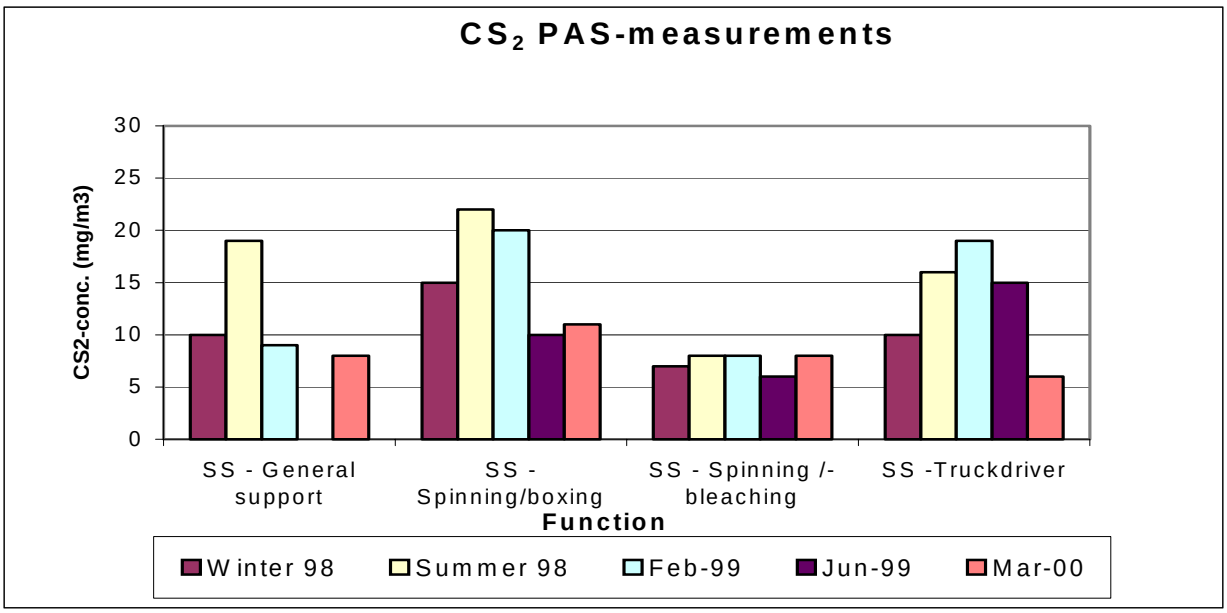
Table 5.15 Summary of air monitoring in different parts of a viscose rayon plant (after Drexler *et al* 1994)

Area of plant	Passive personal air monitoring		Active personal air monitoring		Stationary air monitoring	
	mg/m^3	ppm	mg/m^3	Ppm	mg/m^3	ppm
Spool spinning	10.8	3.42	13.2	4.18	2.9	0.91
Spinning of technical rayon	20.8	6.57	19.1	6.05	23.7	7.51
Washing	38.1	12.07	30.1	9.54	88.7	28.01
Post treatment	11.5	3.63	-	-	5.2	1.63
Second aging	6.1	1.94	6.3	2.0	7.3	2.3

Recent 1998-2000 data on exposure levels in a viscose rayon textile factory have been obtained from the CEFIC Sector Group (see Figure 5.1). Personal air sampling was carried out using passive diffusion badges (Drager Orsa V) with different groups within the factory (general support, spinning/boxing, spinning/bleaching and truckdrivers) being randomly sampled for 8 hours per day per person. Groups were sampled 2-3 times per year and samples were analysed by heat desorption and gas chromatography.

The highest exposure levels were recorded for workers in the continuous spinning area (>20 mg/m^3 on all sampling occasions) whereas levels measured in other parts of the factory were generally $\leq$ 15 mg/m^3.

Air quality standards for occupational exposure of carbon disulphide are in place in Europe (general value being 30 mg/m^3 with specific values being defined in certain countries for certain uses of the substance). Provided these values are complied with adverse effects on workers should be minimised.



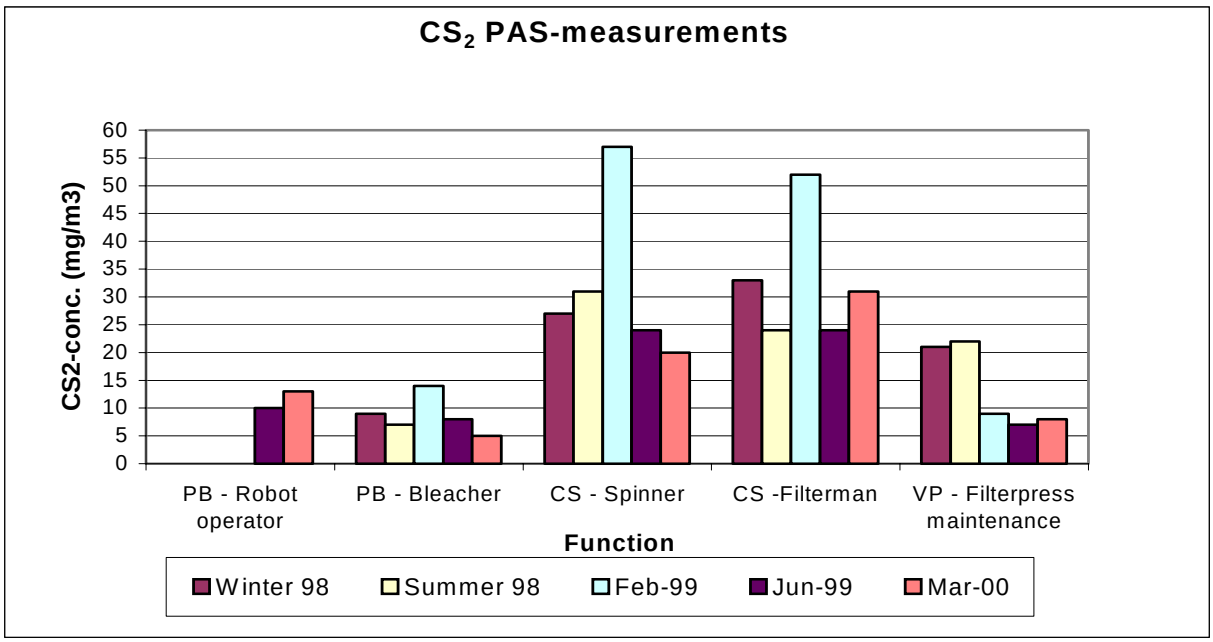


Figure 5.1 Summary of air monitoring in different parts of a viscose rayon plant in the period 1998 – 2000 (CS – Continuous spinning, SS – Spool spinning, PB – Pressure bleaching, VP – Viscose production)

5.7.2 Consumer exposure data

As carbon disulphide occurs ubiquitously in the environment, the general population is exposed to this compound. The primary route of exposure to carbon disulphide is through inhalation of ambient air although baseline atmospheric CS_2 concentrations are typically low with values of 60 - 320 ng/m^3 (6 x 10^{-5} to 32 x 10^{-5} mg/m^3) having been reported (BUA 1991).

In the sample risk characterisation in the draft CICAD for carbon disulphide (IPCS 2002), the estimated mean airbourne exposure to CS_2 for the general population, including populations in the vicinity of point sources, is considerably less than a tolerable concentration of 100 μg/m^3. This tolerable concentration was derived based on the benchmark concentration estimated for a 5% excess risk of an abnormal response (based on the 5th percentile of the unexposed workers in the critical study). In the risk characterisation the critical study related to peroneal motor nerve conduction velocity (MCV) in viscose rayon workers and the data was adjusted for continuous exposure (24 hours per day, 7 days per week). An overall uncertainty factor of 50 was applied to the effects data.

There also exists the possibility that consumers may be exposed to carbon disulphide present in food items. In the 1990s in Australia and the United States carbon disulphide was detected at low levels in fruit and vegetables, probably as a result of natural processes and a range of food products produced from grain which may have been fumigated with carbon disulphide (Daft 1991, Ahmed *et al* 1996).

In a United States Food and Drug Administration survey of 549 food items for fumigant residues, 7 were found to contain carbon disulphide with the average CS_2 residue concentration being 0.74 ppm (Daft 1991). Carbon disulphide is not generally used to fumigate grain in Western Europe but exposure may result from food imported from outside the region. The level of exposure will clearly be dependent on the type, source and amount of products consumed which contain carbon disulphide residues.

Table 5.16 summarises the levels of carbon disulphide measured by Ahmed *et al* (1996) in fruit and vegetables at a large Australian wholesaler in the period 1993 to 1995.

Table 5.16 Levels of carbon disulphide measured in fruit and vegetables from an Australian wholesaler (Ahmed *et al* 1996)

Fruit or vegetable	Concentration range (mg kg^{-1}) in period 1993-1995	Number of samples	% positive samples
Apples	0.06 – 1.74	40	82.5
Avocados	<0.02 – 0.12	16	6.3
Bananas	<0.02	13	0
Beans	0.07 – 0.68	25	44
Broccoli	0.08 – 1.09	21	100
Cabbage	0.04 – 1.37	25	80
Bok choy	<0.02 – 0.12	7	14.3
Capiscum	0.04 – 0.59	24	37.5
Carrots	0.07 – 1.17	56	21.4
Cauliflower	0.07 – 1.11	22	45.5
Celery	0.06 – 1.7	22	27.3
Cherries	0.02 – 0.91	21	38.1
Chinese cabbage	0.25 – 2.19	6	50
Citrus fruits	0.08 – 0.33	45	6.7
Cucumbers	0.03 – 3.05	14	71.4
Grapes	0.08 – 4.11	45	80
Lettuce	0.07 – 0.77	39	38.5
Mangos	0.08 – 0.4	16	37.5
Mushrooms	<0.02 – 0.12	17	5.9
Nectarines	<0.02 – 8.9	38	47.4
Onions	0.04 – 4.2	46	28.3
Peaches	0.07 – 3.3.1	36	63.0
Pears	0.07 – 1.35	27	63.0
Potatoes	0.01 – 0.93	51	37.3
Rock melons	0.02 – 0.1	24	16.7
Silver beets	0.03 – 4.68	20	55
Strawberries	0.004 – 2.5	24	29.2
Tomatoes	0.51 – 1.19	60	38.3
Zucchini	0.04 – 0.21	22	27.3

There is also a low but transient potential for exposure to carbon disulphide via release from some types of textile floor coverings. Sollinger *et al* (1994) identified carbon disulphide in head space emissions from new carpeting (75% olefin, 25% nylon polypropylene backing) at approximately 0.1 mg/m^3.

5.7.3 Environmental exposure data

The draft CICAD for CS_2 states that "Since most carbon disulphide is released to air, for environmental effects, it is terrestrial (and aerial) organisms in the vicinity of industrial sources that are at the greatest potential risk. Aquatic organisms close to discharge points might also be potentially affected. However, based on the sample risk characterisation, conservative comparisons of estimated exposure with no-effect values indicate that it is unlikely that carbon disulphide causes adverse effects on populations of terrestrial or aquatic organisms".

No national environmental quality standards have been derived in any European country for the protection of aquatic or terrestrial ecosystems and no measured environmental exposure

data has been obtained based on searches of the COMMPS database. Information from BUA (1991) and IUCLID (2000) indicated that typical aquatic CS_2 concentrations were < 10 ng l^{-1}.

5.8 Overall Conclusions on Carbon disulphide

The following conclusions have been drawn for the review of carbon disulphide:

5.8.1 Data from studies assessing potential endocrine disrupting effects

5.8.1.1 Human related studies

- Carbon disulphide can affect male fertility in rats through changes in sperm count and mating behaviour and the NOEL in laboratory animals for effects is > 1000 mg/m^3. There is some evidence that this toxicity is caused by a toxic effect on the testicles or by indirect effects on the ejaculation process. However, BUA (1991) stated that the influence of the hypothalamus-pituitary gland-gonad axis is improbable.

- A number of studies have shown that carbon disulphide possesses embryotoxic effects at high doses but levels that are lower than those causing maternal toxicity. Rabbits appear to be more sensitive than rats. Teratogenic effects were described exclusively at maternally toxic doses. The NOEL for embryotoxic effects for rabbits were in the region of 900 mg/m^3 and is higher for teratogenic effects. Initial neurotoxic effects already occur at these concentrations in the 90 day tests. The results of Tabacova *et al* (1978, 1983) and Tabacova and Balabaeva (1980) describe embryotoxic effects from carbon disulphide at 0.03 mg/m^3 and teratogenic effects at 10 mg/m^3. However, there are issues with the quality of these studies because of the poor test descriptions and unverifiable calculations of significance. BUA (1991) concluded that "*In laboratory animals CS_2 is embryotoxic at high doses or concentrations, which, however, are still below the maternal toxicity threshold. Teratogenic effects can be observed only at maternally toxic doses*.". The Environmental Hazard Assessment for carbon disulphide prepared for the United Kingdom Department of the Environment (BRE 1993) concluded that "*Exposure to carbon disulphide has been shown to cause adverse effects on fertility in male and female rats. Embryo/foetotoxicity and teratogenicity also occurred at exposure concentrations not producing maternal toxicity.".*

- In humans, there have been numerous studies of workers exposed occupationally that report effects on pituitary-gonadal function in men and women, as well as indications of adverse reproductive outcomes in women. In men spermatogenic, diabetogenic and adrenal effects have also been reported. These effects have generally been reported for workers in the viscose rayon industry at exposure levels considerably above current occupational exposure standards (< 30 mg/m^3)

- There is no *in vitro* data on the potential (anti)-oestrogenic, (anti)-androgenic or (anti)-thyroid effects of carbon disulphide. *In vivo* data indicates reproductive toxicity and endocrine disturbance in laboratory species and humans.

5.8.1.2 Wildlife studies

- Only limited data have been located on the potential endocrine disrupting effects of carbon disulphide on aquatic, terrestrial or aerial organisms (see Table 5.6). In the aquatic environment carbon disulphide has been shown to affect the development of frog embryos with clear effects on malformations of the notochord being evident, though only at a high

exposure concentration of 126 mg l^{-1}. In rainbow trout effects on embryo-larval development were also evident at an exposure concentration of 100 mg l^{-1}. In zebrafish effects on hatching rate were evident at a nominal exposure concentrations of 2.5 mg l^{-1} (NOEC = 1 mg l^{-1}), though there is no information on the mechanism of action for these effects. Information is not available on the effects of carbon disulphide on the reproduction of aquatic organisms.

- No data has been located on the potential endocrine disrupting effects of carbon disulphide on terrestrial or aerial organisms. The volatility of carbon disulphide means that wildlife organisms that are exposed to carbon disulphide via inhalation represent those most at risk from exposure. However, it should be recognised that this substance is released into the environment from natural sources.

- No *in vitro* data is available from tests using cells and tissues from wildlife species.

5.8.2 Comparison of data from studies assessing potential endocrine disruption effects and/or general toxicity

5.8.2.1 Human related studies

- CS_2 is well documented to cause neurotoxicity and cardiovascular effects in humans which are probably the most critical endpoints (BUA 1991) notwithstanding the adverse effects on human reproductive function.

- In BUA (1991) a NOEL for the most sensitive biological endpoints was estimated to be between 150 and 800 mg/m^3 (47.5 and 253 ppm) for laboratory animals and between 10 and 60 mg/m^3 for humans. It was stated that it had been established beyond doubt that the cardiovascular mortality rate of workers exposed to CS_2 is increased only at exposure concentrations of > 60 mg/m^3, though there are differences between countries. These values compare with data for effects on reproduction which show that the NOELs in laboratory animals for effects on male fertility in rats (through changes in sperm count and mating behaviour) and humans for effects on changes in sperm morphology were > 1000 mg/m^3 and > 30 mg/m^3 respectively. In laboratory animals CS_2 is embryotoxic at high doses, which, however are still below the maternal toxicity threshold. Teratogenic effects can only be observed at maternally toxic doses. As a result it would appear that in humans and laboratory mammals the most toxic effects of carbon disulphide are probably due to mechanisms other than those which are endocrine mediated. However, it has to be recognised that carbon disulphide presents possible risks of impaired fertility and harm to the unborn child.

5.8.2.2 Wildlife studies

- The data on potential endocrine mediated responses in wildlife is limited to studies on the development of frog and fish embryos. In studies on the embryos of the frog *Microhyla ornata* and the rainbow trout (*Oncorhynchus mykiss*) malformations of the notochord were found at nominal exposure concentrations at or above 100 mg l^{-1}. In the zebrafish effects on hatching rate were found at an exposure concentration of 2.5 mg l^{-1} (NOEC = 1 mg l^{-1}), but whether the mechanism of action is endocrine disruption is unknown. The latter effect concentration is similar to the lowest exposure concentrations of 2 - 4 mg l^{-1} causing acute toxicity in invertebrates and fish.

5.8.3 Exposure data

5.8.3.1 Workers

- Occupational exposure to carbon disulphide may occur through inhalation and, to a lesser degree, dermal contact with this compound at workplaces where carbon disulphide is produced or used. Workers at greatest risk appear to be those involved in the viscose rayon industry. However, if the existing air quality standards (currently 10ppm = 30mg/m^3 in EU countries) which have been derived are complied with, potential adverse effects on workers should be minimised.

5.8.3.2 Consumers

- As carbon disulphide occurs ubiquitously in the environment, the general population is exposed at low levels to this compound. The primary route of exposure to carbon disulphide is through inhalation of ambient air though baseline atmospheric CS_2 concentrations are typically low with values of 60 - 320 ng/m^3 (6 x 10^{-5} to 32 x 10^{-5} mg/m^3) having been reported (BUA 1991).

- In the sample risk characterisation in the draft CICAD for carbon disulphide (IPCS 2002), the estimated mean airbourne exposure to CS_2 for the general population, including populations in the vicinity of point sources, is considerably less than a tolerable concentration of 100 μg/m^3.

5.8.3.3 Environment

- The draft CICAD for CS_2 states that "Since most carbon disulphide is released to air, for environmental effects, it is terrestrial (and aerial) organisms in the vicinity of industrial sources that are at the greatest potential risk. Aquatic organisms close to discharge points might also be potentially affected. However, based on the sample risk characterisation, conservative comparisons of estimated exposure with no-effect values indicate that it is unlikely that carbon disulphide causes adverse effects on populations of terrestrial (aerial) or aquatic organisms".

- No national environmental quality standards have been derived in any European country for the protection of aquatic or terrestrial ecosystems and no measured environmental exposure data has been obtained based on searches of the COMMPS database. Information from BUA (1991) and IUCLID (2000) indicated that typical background aquatic CS_2 concentrations were below 10 ng l^{-1}.

5.9 Summary of the weight of evidence for endocrine disrupting effects in humans and wildlife and associated uncertainties

The summary of the weight of evidence for endocrine disrupting effects of carbon disulphide in humans and wildlife along with associated uncertainties are given in Table 5.17.

Table 5.17 Summary of the weight of evidence conclusion and uncertainties associated with the assessment of the endocrine disrupting effects of Carbon disulphide

	Target group	
	Humans	Wildlife

Weight of evidence	Carbon disulphide can affect male fertility in rats through changes in sperm count and mating behaviour and the NOEL in laboratory animals for effects is at > 1000 mg/m^3. There is some evidence that this toxicity is caused by a toxic effect on the testicles or by indirect effects on the ejaculation process. However, a BUA review stated that the influence of the hypothalamus-pituitary gland-gonad axis is improbable. A number of studies have shown that carbon disulphide possesses embryotoxic effects at high doses but levels that are lower than those causing maternal toxicity. Rabbits appear to be more sensitive than rats. Teratogenic effects were described exclusively at maternally toxic doses. The NOEL for embryotoxic effects for rabbits were in the region of 900 mg/m^3 and is higher for teratogenic effects. Initial neurotoxic effects already occur at these concentrations in the 90 day tests. Studies by Tabacova and co-workers describe embryotoxic effects from carbon disulphide at 0.03 mg/m^3 and teratogenic effects at 10 mg/m^3. However, there are issues with the quality of these studies. In humans, there have been numerous studies of workers exposed occupationally that report effects on pituitary-gonadal function in men and women, as well as indications of adverse reproductive outcomes in women. In men spermatogenic, diabetogenic and adrenal effects have also been reported. These effects have generally been reported for workers in the viscose rayon industry at exposure levels considerably above current occupational exposure standards (< 30 mg/m^3)	The available data on the effects of carbon disulphide on the development of aquatic organisms has been shown that the threshold exposure concentration above which the hatching rate of fish (*Danio rerio*) is affected is 1.0 mg l^{-1}. However, it is not clear whether these responses are endocrine mediated. This exposure level is only slightly lower than concentrations causing acute toxicity in fish (and invertebrates).
Uncertainties	Carbon disulphide has not been subjected to standard regulatory toxicity tests and this raises uncertainties as to the validity of certain data, particularly that for the only multi-generational reproduction study. This uncertainty is offset to a degree by the fact that there is a considerable body of relevant data for humans derived from studies on workers (principally those in the viscose rayon industry).	No data has been located on the potential endocrine disrupting effects of carbon disulphide on the reproduction of aquatic organisms or the reproduction/development of terrestrial or aerial organisms. The volatility of carbon disulphide means that wildlife organisms that are exposed to carbon disulphide via inhalation represent those most at risk from exposure. There is limited data on environmental concentrations of CS_2, however it needs to recognised that environmental levels are influenced by natural releases.

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6. REVIEW OF DATA FOR 4-CHLORO-3-METHYLPHENOL

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Section **Description** **Page**

Notes:

This section contains information collected and collated from a range of sources including published papers, reports of studies conducted by industrial companies or sector groups and data compilations such as IUCLID (2000). The data from IUCLID has been taken as accurate and individual source documents have not been checked unless they are considered to be key studies which have a major influence on the outcome of the review. All information taken from IUCLID has been referenced as being from that source and individual references have not been given in the references.

This review has been carried out in accordance with the evaluation framework described in Section 2. In the review the International Programme for Chemical Safety (IPCS) definition of an endocrine disrupter has been adopted, namely that it is "*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*".

In the context of the review it is recognised that there are various laboratory-based *in vivo* and *in vitro* methods utilising a range of (eco)toxicological endpoints that are claimed by different sources to be relevant to the assessment of endocrine disruption in humans and wildlife. However, since this field is still in an early stage of development there is uncertainty regarding the significance of many of the current findings.

From the numerous recent reviews of potential test methods (such as the Detailed Review Paper prepared by OECD in 1997) there is a clear consensus in terms of the hierarchy of the relevance of test methods. In this hierarchy longer-term *in vivo* studies considering effects on reproduction and/or development (and including mechanistic information) are of greater relevance than short-term *in vivo* screening tests which are of greater relevance than *in vitro* assays. The greater relevance of chronic *in vivo* tests or those assessing effects during critical windows of sensitivity is also evidenced by the fact that these are the key (eco)toxicological methods being developed in the OECD Endocrine Disruption Testing and Assessment (EDTA) Programme. This hierarchy approach to data relevance has been adopted in the review along with a weight of evidence consideration of the available data.

The review has been carried out to address three key questions:

1. Does the available data indicate there is evidence that a chemical causes endocrine disrupting effects in target groups of humans/mammals and/or wildlife?

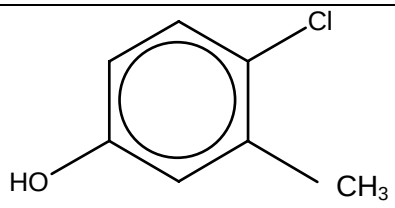
2. Do endocrine disrupting effects of the chemical in target groups of humans/mammals and/or wildlife occur at lower concentrations than those causing effects on general systemic toxicological endpoints?

3. Are particular target groups of workers, consumers or organisms in the environment likely to be exposed to concentrations of chemicals which exceed effects thresholds due to current emission patterns.

It should be recognised that this review is not designed to be a full Risk Assessment of a substance under the Existing Substances Regulation 793/93.

6.1 Physico-chemical data for 4-chloro-3-methylphenol

6.1.1 Summary details on the substance

CAS Number	59-50-7
EINECS Number	200-431-6
IUPAC Name	4-chloro-3-methylphenol
Other names	3-methyl-4-chlorophenol, para chlorometacresol, 4-chloro-3-cresol, 4-chloro-m-cresol, p-chloro-m-cresol, 2-chloro-5-hydroxytoluene, 4-chloro-1-hydoxy-3-methyl benzene, 6-chloro-3-hydroxytoluene
Molecular weight	142.58
Chemical formula	C_7H_7ClO
Chemical structure	

6.1.2 Physico-chemical properties and environmental fate information (from IUCLID 2000)

The data on the physico-chemical properties of 4-chloro-3-methylphenol and its environmental fate (see Table 6.1) indicate that the substance is readily biodegradable and is not expected to sorb to sludge in waste water treatment plants based on a low organic carbon water partition coefficient (1.25 – 1.7). In the aquatic environment photolysis is likely to be the most important abiotic degradation process for 4-chloro-3-methylphenol since the substance does not hydrolise significantly.

4-chloro-3-methylphenol does not strongly sorb to organic carbon in sediments and soils based on a low organic carbon water partition coefficient (log Koc = 1.25 – 1.7). In addition 4-chloro-3-methylphenol is degraded in soil with a half life of 1.4 to 21 days.

Volatilisation is unlikely to represent a major removal process from the aquatic environment based on the Henry's Law Constant of 4.52 x 10^{-2} to 2.72 x 10^{-1} Pa-m^3 mol^{-1} (4.58 x 10^{-7} to 2.76 x 10^{-6} atm-m^3 mol^{-1}) being lower than a value range of 1 - 100 Pa-m^3 mol^{-1} which is considered to indicate volatility.

Table 6.1 Physico-chemical properties and environmental fate data (from IUCLID 2000)

Physico-chemical property	Value (and comments)
Physical state at ambient	Solid
Water solubility	3600 to 3900 mg l^{-1} at 20°C
Octanol-water partition coefficient (log Kow)	3.1
Organic carbon water partition coefficient (log Koc)	1.25 to 1.7
Henry's Law Constant	4.52 x 10^{-2} to 2.72 x 10^{-1} Pa-m3 mol^{-1} (4.58 x 10^{-7} to 2.76 x 10^{-6} atm-m^3 mol^{-1})
Type of degradation	
Aquatic - abiotic	Photolysis is likely to be the most important abiotic degradation process for 4-chloro-3-methylphenol since the substance does not hydrolise significantly.
Aquatic - biotic	4-chloro-3-methylphenol was reported as readily biodegradable (100% loss after 28 days at 28°C) in a wide range of aerobic environments.
Terrestrial	For 4-chloro-3-methylphenol the half life for degradation in soil (T½) = 1.4 to 21 days
Atmospheric	For 4-chloro-3-methylphenol the half life for atmospheric degradation (T½) = 27 hours

A Mackay Level 1 fugacity model has shown that for a discharge of 1000 tonnes of 4-chloro-3-methylphenol 50.6% of the substance will partition into the soil (Table 6.2), with 45.4% partitioning into the water. Amounts present in other compartments are minimal. The high partitioning into soil predicted is a result of the log Koc of 2.86 calculated for the substance by the model which is markedly higher than the measured value of 1.25 – 1.7.

Table 6.2 Summary of the results of a Mackay Level 1 fugacity model

Compartment	Volumes of different compartments	% of substance present in different compartments
Water	2 x 10^{11}	45.4
Suspended sediment	10^6	0.035
Bottom sediment	10^8	1.12
Fish	2 x 10^5	0.0029
Air	10^{14}	2.83
Aerosol	2000	1.5 x 10^{-5}
Soil	9 x 10^9	50.6

6.2 Production and Uses

6.2.1 Production Patterns

Information from IUCLID (2000) indicates that approximately 1000 – 2000 tonnes of 4-chloro-3-methylphenol was produced in 1998 in the EU.

6.2.2 Use Patterns

The principle use of 4-chloro-3-methylphenol is as a general biocide to prevent micro-organisms degrading organic material, for example to preserve raw leather in tanneries or metal working fluids in the metal working industry. These materials which are used to lubricate and cool during metal grinding or in plant machinery, are rich in proteins which provide a source of nutrition for bacterial growth (Bayer, personal communication). 4-chloro-3-methylphenol is also used as a disinfectant for consumers.

Another use of 4-chloro-3-methylphenol is as a pharmaceutical preservative for materials (such as hand and body creams) which contain organic compounds in an aqueous phase (NIPA Laboratories, personal communication).

6.3 Toxicokinetics, metabolism and bioaccumulation

6.3.1 Toxicokinetics and metabolism

In a study by Bayer (1980) 5 male Wistar rats were exposed to 4-chloro-3-methylphenol by oral gavage. It was found that 54-95% of the test substance administered was excreted in the urine and 0.4% was recovered in the faeces within 24 hours. In another study conducted by Bayer (1981) 4-chloro-3-methylphenol was administered to male Wistar rats at a dose range of 0, 11, 37 and 110 mg kg body weight^{-1} day^{-1} for 13 weeks. No accumulation of test substance in the liver and adipose tissue was observed.

6.3.2 Bioaccumulation

In the toxicokinetic study of 4-chloro-3-methylphenol carried out in Wistar rats (see Section 6.3.1) found no accumulation of test substance in the liver and adipose tissue.

Studies have been carried out by Japan by MITI (1992) on the bioaccumulation of 4-chloro-3-methylphenol in fish (carp) over a 42 day period. The studies carried out to OECD TG 305C showed bioconcentration factors (BCFs) of 5.5 to 11 for carp (*Cyprinus carpio*) exposed to 0.002 mg l^{-1} and 6.7 to 13 for fish exposed to 0.02 mg l^{-1} (MITI 1992). The data indicate that 4-chloro-3-methylphenol does not bioaccumulate in aquatic organisms, which is consistent with the octanol water partition coefficient of 3.1 (see Section 6.1).

6.4 Studies relevant to the assessment of potential endocrine disrupting effects

6.4.1 Studies relevant to the assessment of potential endocrine disrupting effects in humans

6.4.1.1 In vitro studies

A. Receptor competitive binding assays

Blair *et al* (2000) assessed the relative binding affinity of 4-chloro-3-methylphenol in an oestrogen receptor (ER) competitive binding assay using uterine cytosol preparations from adult Sprague Dawley rats. Cytosol preparations were incubated with various concentrations of 4-chloro-3-methylphenol and low relative binding affinity in comparison to 17β-oestradiol was observed. On the basis of the data 4-chloro-3-methylphenol was classified as a weak binder to the oestrogen receptor.

B. Recombinant yeast assays

Nishihara *et al* (2000) used recombinant yeast cells transfected with the human oestrogen receptor (ERα) to test oestrogen-type responses of 4-chloro-3-methylphenol. The REC_{10} values (concentrations of substance resulting in 10% of the activity of 10^{-4} mM 17β-oestradiol) measured for 4-chloro-3-methylphenol was 5 x 10^{-2} mM.

Miller *et al* (2001) used yeast cells transfected with human ERα to investigate the relative potency of 4-chloro-3-methylphenol. This value was calculated by dividing the concentration of the test chemical required to produce a half-maximal response by the concentration of 17β-oestradiol required to produce the same response. The relative potency of 4-chloro-3-methylphenol was 3 x 10^6 times lower than that for 17β-oestradiol.

C. Mammalian cell growth assays

Körner *et al* (1996) investigated the effects of 4-chloro-3-methylphenol in a simplified modified E-Screen proliferation assay using a concentration series of 10^{-1} to 10^{-3} mM. In the study, the relative proliferation effect (RPE) and oestradiol proliferation equivalent (EE) were measured. 17β-oestradiol was used as a positive control at concentrations from 10^{-5} to 10^{-10} mM.

The RPE[1], the relative efficacy, compares the maximal proliferation induced by 17β-oestradiol with that induced by a test compound and full agonists (RPE = 100%) can be distinguished from partial agonists (RPE < 100%). The EE, the relative potency, is the quotient of the minimal concentration of 17β-oestradiol and the test compound required for maximal proliferation. The RPE for 4-chloro-3-methylphenol was 56% indicating a partial agonist, while the EE was 3 x 10^6 indicating a weakly positive response with effects only being evident at concentrations markedly higher than those for 17β-oestradiol.

The dose-response curve for relative proliferation compared to the control was U shaped with values increasing from 10^{-3} mM up to a maximum at ~8 x 10^{-2} mM. At higher concentrations relative proliferation values declined back to background levels at 5 x 10^{-1} mM. It is probable that the effects observed at these relatively high exposure concentrations were due to cytotoxic effects , which are consistent with the biocidal activity of 4-chloro-3-methylphenol.

Cotreatment of 4-chloro-3-methylphenol with 5 x 10^{-3} mM tamoxifen (an anti-oestrogen) completely antagonised the proliferation effect.

In a repeat of the earlier study Körner *et al* (1998) reported that the relative potency of 4-chloro-3-methylphenol was 2 x 10^6, while the relative efficacy was 44%.

Summary of *in vitro* data

Table 6.3 summarises the available *in vitro* data for 4-chloro-3-methylphenol which primarily relates to *in vitro* assays assessing oestrogenic mechanisms of action in mammalian cells and tissues. The data indicates no or weak binding of 4-chloro-3-methylphenol to the human oestrogen receptor and weakly positive results in a modified E-Screen assay. No data is

[1] RPE = [(Proliferation effect of test compound)/(proliferation effect of 17β oestradiol)] x 100

where PE = (Maximum cell number with test compound)/Cell number of negative control)

available on the androgenic and anti-androgenc effects of 4-chloro-3-methylphenol and effects on thyroid function and hormone synthesis and secretion and steroidogenesis in mammalian cells and tissues.

Table 6.3 Summary of the *in vitro* data in isolated mammalian cells and tissues relating to different mechanisms of action of 4-chloro-3-methylphenol

Mechanism of endocrine disruption	Responses observed in *in vitro* systems
Oestrogenicity/anti-oestrogenicity	Data indicates no or weak binding of 4-chloro-3-methylphenol to the human oestrogen receptor weakly positive results in a modified E-Screen assay.
Androgenicity/anti-androgenicity	No data identified
Thyroid effects	No data identified
Effects on hormone synthesis or secretion	No data identified
Effects on steroidogenesis	No data identified

6.4.1.2 In vivo studies

Tables 6.4 and 6.5 summarise the information on endocrine mediated responses in laboratory mammals following oral and dermal exposure to 4-chloro-3-methylphenol.

A. Effects on endocrine glands and hormone sensitive tissues

Eiben *et al* (1988) exposed groups of male and female rats (Wistar) to 4-chloro-3-methyl in the diet at doses of 0, 150, 500 and 1500 ppm for 13 weeks. The groups contained 20 animals of each sex and the exposure regime resulted in doses of approximately 0, 10, 40 and 120 mg kg body weight^{-1} day^{-1} for males and 0, 20, 50 and 170 mg kg body weight^{-1} day^{-1} for females. No substance-related effects were observed in any dose group with respect to behaviour, food intake or mortality. In the study no histopathological effects were found at any test dose in tissues or organs potentially susceptible to endocrine disrupting substances including the adrenals, epididymis, mammary gland, ovaries, pituitary, prostate, seminal vesicles, testes, thyroid and uterus). The growth of the male animals treated with 150 ppm was not affected, but in the 500 and 1500 ppm doses the body weights of males were lower than those in the control group by an average of 5.9% (maximum 7.8%) and 5.4% (maximum 7.0%) respectively. No effects on growth of females was evident at any test dose. Clinical laboratory investigations of organ weight data, necropsies and histopathology produced no indications of test substance related effects. However, tubular hyperplasia occurred in the kidney of females at the highest dose (1500 ppm or 170 mg kg body weight^{-1} day^{-1}).

Leser (1991) exposed groups of male and female rats (Wistar) to 4-chloro-3-methyl (formulated in PEG 400 under occlusive conditions) via the dermal route at doses of 0, 20, 100 and 500 mg kg body weight^{-1} day^{-1} for 13 weeks. The test was carried out to OECD Test Guideline 411 (Sub-chronic Dermal Toxicity – 90 day Study) and GLP. The groups of animals, which comprised 10 males and 10 females, were exposed for 6 hours a day for 5 days a week. No indications of effects on behaviour, body weight development, food and

water intake or mortality were evident at any test dose. No histopathological effects were found at any test dose in tissues or organs potentially susceptible to endocrine disrupting substances including the adrenals, epididymis, mammary gland, ovaries, pituitary, prostate, seminal vesicles, testes, thyroid and uterus). As a result the NOEL for effects on endocrine organs or hormone sensitive tissues was established as 500 mg kg body weight^{-1} day^{-1}. No effects were established by clinical laboratory, organ weight data, gross pathological and histopathological investigations. Thus a sub-chronic systemic toxicity NOEL of 500 mg kg body weight^{-1} day^{-1} (the highest dose tested) was established.

B. Reproduction and fertility studies

No data has been located on the effects of 4-chloro-3-methylphenol on the reproduction and fertility of laboratory mammals.

C. Developmental and teratogenicity studies

Bartmann (1991) exposed groups of pregnant female rats (Wistar) (25 per dose) by oral gavage to doses of 0, 30, 100 and 300 mg kg body weight^{-1} day^{-1} from days 6 to 15 of gestation. On day 20 of gestation, the progeny were delivered by Caesarean section. The study was carried out to OECD Test Guideline 414 (Teratogenicity) and GLP. In the study the weight and external appearance of the placentas, the sex of the foetuses and the development of the skeletal system were not affected at doses up to and including 300 mg kg^{-1}. The gestation and resorption rate, the number and weight of the foetuses as well as the number and types of malformations were not influenced at doses up to and including 100 mg kg^{-1}. Due to the complete resorption in two dams of the 300 mg kg^{-1} group, gestation rate and number of foetuses were statistically decreased in this group. At the highest dose the number of spontaneous malformations was slightly increased, but was within the historical range for control animals. At 30 mg kg^{-1} no effects on the appearance, behaviour, food and water intake as well as body weight development of the adult females were observed. In contrast at 100 mg kg^{-1} laboured breathing was observed in some animals at this and highest dose reductions in food and water intake and body weight gain and increases in the discharge of urine were observed. All dams in the 300 mg kg^{-1} group exhibited marked clinical signs (piloerection, sunken flanks, bloody muzzle, laboured breathing, reduced motility, high-stepping gait, lateral and sternal recumbency, convulsions and gasping breathing). Mortality was also elevated in the 300 mg kg^{-1} group. Necropsy of the animals dying intercurrently or sacrificed in moribund conditions established intestines filled with gas, bloody vaginas, thoraxes filled with serous fluid, suppurative foci in the pulmonary tissue, reddened oesophagus as well as stomachs and spleens which were reduced in size.

Overall the results indicated that there was no substance-related teratogenicity observed even in the presence of severe maternal toxicity. The doses tolerated with no adverse effects (NOAELs) were 30 mg kg^{-1} for the dams (maternal toxicity), 100 mg kg for intra-uterine development and 300 mg kg^{-1} for teratogenicity.

D. Carcinogenecity and oncology studies

Leser (1993) investigated the long-term effects of 4-chloro-3-methylphenol in rats (Wistar) in a combined chronic toxicity and oncogenicity study carried out according to OECD Test Guideline 453 and to GLP. Groups of animals (60 males and 60 females per dose) were exposed in the diet to doses of 0, 400, 2000 and 10000 ppm. The resulting doses were estimated to be 0, 21, 103 and 559 mg kg body weight^{-1} day^{-1} for males and 0, 28, 134 and

Table 6.4 Summary of the data on potential endocrine mediated responses in laboratory mammals following oral exposure

Species	Life stage of the test organism at start of test	Exposure route and dose series	Description of endocrine disruption measurement parameter(s) and effect doses	Reference	Test Relevance	Study Validity
Rat (Wistar)	Adult males and females	Dietary exposure at 0, 150, 500 and 1500 ppm for 13 weeks (males = 0, 10, 40 and 120 mg kg body weight^{-1} day^{-1} and females = 0, 20, 50 and 170 mg kg body weight^{-1} day^{-1}	No significant histopathological effects (relative to the controls) on endocrine glands and hormone sensitive tissues (adrenals, epididymus, mammary glands, ovaries, parathyroid, pituitary, prostate, seminal vesicles, testes, thyoid, uterus and vagina) at any test dose *(NOEL for effects on endocrine glands and hormone sensitive tissues = 120 mg kg body weight^{-1} day^{-1})*	Eiben *et al* (1988)	Medium	Valid
	Adult males and females	Dietary exposure at 0, 400, 2000 and 10000 ppm over a 24 month period (males = 0, 21, 103 and 559 mg kg body weight^{-1} day^{-1} and females = 0, 28, 134 and 774 mg kg body weight^{-1} day^{-1})	No significant histopathological effects (relative to the controls) on endocrine glands and hormone sensitive tissues (adrenals, epididymus, mammary glands, ovaries,parathyroid, pituitary, prostate, seminal vesicles, testes, thyoid, uterus and vagina) at any test dose *(NOAEL for effects on endocrine glands and endocrine sensitive tissues = 559 mg kg body weight^{-1} day^{-1})*	Leser (1993)	High	Valid
	Pregnant females	Oral gavage at 0, 30, 100 and 300 mg kg body weight^{-1} day^{-1} on gestation days 6 to 15	Significant effects (relative to the controls) on embryo and foetal development at 300 mg kg body weight^{-1} day^{-1} *(NOAEL for foetotoxicity and developmental toxcity = 100 mg kg body weight^{-1} day^{-1})*	Bartmann (1991)	High	Valid

Table 6.5 Summary of the data on potential endocrine mediated responses in laboratory mammals following dermal exposure

Species	Life stage of the test organism at start of test	Exposure route and dose series	Description of endocrine disruption measurement parameter(s) and effect doses	Reference	Test Relevance	Study Validity
Rat (Wistar)	Adult males and females	Dermal exposure at 0, 20, 100 and 500 mg kg body weight^{-1} day^{-1} for 13 weeks	No significant histopathological effects (relative to the controls) on endocrine glands and hormone sensitive tissues (adrenals, epididymus, mammary glands, ovaries, parathyroid, pituitary, prostate, seminal vesicles, testes, thyroid, uterus and vagina) at any test dose *(NOEL for effects on endocrine glands and hormone sensitive tissues = 500 mg kg body weight^{-1} day^{-1})*	Leser (1991)	Medium	Valid

774 mg kg body weight^{-1} day^{-1} for females. In the study 10 rats per group were used for a 12 month assessment whereas 50 rats per group were used for a 24 month assessment.

No histopathological effects were found at any test dose in tissues or organs potentially susceptible to endocrine disrupting substances including the adrenals, epididymis, mammary gland, ovaries, pituitary, prostate, seminal vesicles, testes, thyroid and uterus). As a result the NOEL for effects on endocrine organs or hormone sensitive tissues was established as 559 mg kg body weight^{-1} day^{-1} for males and 774 mg kg body weight^{-1} day^{-1} for females.

Clinical observations on animals at doses up to and including 2000 ppm produced no indications of treatment-related effects. Food intake and mortalities were not signficantly affected at any test dose and body weights and water intake were unaffected up to doses of 2000 ppm. At 10000 ppm an increased number of females in poor general condition and a reduction of growth of both sexes (maximum of 8% in males and 20% in females) were observed. Clinical laboratory investigations, opthalmological examinations, necropsis and histopathological investigations produced no evidence of damage to the organs in females up to and including 10000 ppm and males up to and including 2000 ppm. Histopathological investigations revealed damage to the kidney in males of the highest dose group (10000 ppm) which consisted of papillary necroses, cortical dilations and fibroses. There were no indications of carcinogenic effects at any dose as there were no dose dependent increases in tumour incidences which were outside the range of historical control data. On the basis of the results a systemic chronic toxicity NOAEL of 103 mg kg body weight^{-1} day^{-1} was established (specifically 103 mg kg body weight^{-1} day^{-1} for males and 134 mg kg body weight^{-1} day^{-1} for females).

E. General conclusions on potential endocrine mediated responses in laboratory mammals in in vivo studies

A series of definitive oral and dermal exposure studies (see Table 6.6) carried out to OECD Test Guidelines and GLP have assessed whether 4-chloro-3-methylphenol affects developmental endpoints which may be endocrine mediated. Exposure of pregnant rats to 4-chloro-3-methylphenol during the period of organogenesis induced embryo or foetotoxicity or malformations at the highest dose tested (300 mg kg body weight^{-1} day^{-1}), but no teratogenic effects. Therefore, the lowest NOAEL recorded from the studies on laboratory mammals is a value of 100 mg kg body weight^{-1} day^{-1} for foetotoxic effects (on intra-uterine development) (Bartmann 1991). However in the study, toxic effects on the dams were evident at the lowest exposure dose (30 mg kg body weight^{-1} day^{-1}). No data has been located on the effects of 4-chloro-3-methylphenol on reproductive endpoints, including that of definitive significance from a multi-generation reproduction study, which may be endocrine mediated. The available studies provide no consideration of changes in endocrine function.

The lowest doses tested in the oral and dermal exposure studies with mammals were in the region of 10 mg kg body weight^{-1} day^{-1} and no effects which may be endocrine mediated were evident at these doses.

6.4.1.3 Human studies

No information on endocrine mediated responses of workers and consumers following exposure to 4-chloro-3-methylphenol have been identified.

Table 6.6 Summary of the potential endocrine mediated responses reported in *in vivo* studies in laboratory mammals

Type of study	Species and exposure route used	Dose series used	NOEL (mg kg body weight^{-1} day^{-1})		Reference
			Potential endocrine mediated responses	Systemic toxicity	
Sub-chronic oral toxicity (OECD 408)	Rat (Wistar) - dietary	0, 10, 40 and 120 mg kg body weight^{-1} day^{-1} (males) 0, 20, 50 and 170 mg kg body weight^{-1} day^{-1} (females)	120 (Histopathology - males) 170 (Histopathology - females))	No data given in study	Eiben *et al* (1988)
Sub-chronic dermal toxicity (OECD 411)	Rat (Wistar) - dermal	0, 20, 100 and 500 mg kg body weight^{-1} day^{-1}	500 (Histopathology)	500	Leser (1991)
Reproduction – One generation (OECD 415)	No data	-	-	-	-
Reproduction – Two generation (OECD 416)	No data	-	-	-	-
Development/ Teratogenicity (OECD 414)	Rat (Wistar) – oral gavage	0, 30, 100 and 300 mg kg body weight^{-1} day^{-1}	100 (NOAEL - Foetotoxicity) 300 (NOAEL - Teratogenicity)	30 (NOAEL – Maternal toxicity)	Bartmann (1991)
Combined chronic toxicity/ Oncogenicity (OECD 453)	Rat (Wistar)	0, 21, 103 and 559 mg kg body weight^{-1} day^{-1} (males) 0, 28, 134 and 774 mg kg body weight^{-1} day^{-1} (females)	559 (Oncogenicity - males) 774 (Oncogenicity - females)	103 (NOAEL – males) 134 (NOAEL – females)	Leser (1993)

6.4.2 Studies relevant to the assessment of potential endocrine disrupting effects in wildlife

6.4.2.1 In vitro studies

No data has been located on the conduct of *in vitro* studies using cells and tissues from wildlife species.

6.4.2.2 In vivo studies

A. Studies on aquatic organisms

The only data that has been located on the potential endocrine disrupting effects of 4-chloro-3-methylphenol on aquatic species is for the invertebrate *Daphnia magna*.

Fish

No data has been located on the potential endocrine disrupting effects of 4-chloro-3-methylphenol on fish.

Invertebrates

Kuhn *et al* (1989) investigated the effects of 4-chloro-3-methylphenol on the reproduction of the water flea *Daphnia magna*. In the 21 day study the concentration range used was 0.04, 0.08, 0.16, 0.32, 0.64, 1.25, 2.5 and 5.0 mg l^{-1}. The No Observed Effect Concentration for effects on juvenile production was 1.25 mg l^{-1}, but there is no information of the mechanism of action for the effects. The effect observed in the *Daphnia magna* reproduction test is probably not caused by direct oestrogenic effects. Other studies have shown an absence of reproductive impairment at 0.39 mg l^{-1} when animals are exposed to the synthetic steroid 17α-ethinyestradiol (Schweinfurth *et al* 1996).

B. Studies on terrestrial organisms

No data has been located on the potential endocrine disrupting effects of 4-chloro-3-methylphenol on terrestrial organisms. However, given that 4-chloro-3-methylphenol does not strongly sorb to organic carbon and is degraded in soil the absence of data on terrestrial organisms does not represent a key area of uncertainty with regard to the potential endocrine effects of the substance. It should also be recognised that there are currently no internationally agreed methods specifically developed to assess potential endocrine disrupting effects in terrestrial organisms.

C. Studies on aerial organisms

No data has been located on the potential endocrine disrupting effects of 4-chloro-3-methylphenol on aerial organisms. However, given that 4-chloro-3-methylphenol is not volatile and is rapidly degraded in air the absence of data on aerial organisms does not represent a key area of uncertainty with regard to the potential endocrine effects of the substance. It should also be recognised that there are currently no internationally agreed methods specifically developed to assess potential endocrine disrupting effects in aerial organisms.

D. General conclusions on potential endocrine mediated responses in in vivo studies in wildlife species

The data that has been located assessing the potential endocrine disrupting effects of 4-chloro-3-methylphenol on wildlife is limited to one study on the effects on the reproduction of the water flea (*Daphnia magna*) (see Table 6.7). The no observed effect concentration for reproductive impairment was 1.25 mg l^{-1}, but there is no information on the mechanism of action. There is an absence of data for wildlife species, particularly reproduction and development in fish, which represents an area of uncertainty.

Table 6.7 Summary of the studies assessing potential endocrine mediated responses in wildlife

Environmental compartment	Taxonomic group	Type of study	Species and exposure route used	Concentration series used	Lowest reported NOEC	Reference
Aquatic	Amphibians	No data	-	-	-	-
	Fish	No data	-	-	-	-
	Invertebrates	Reproduction (OECD TG 211)	*Daphnia magna* – aqueous exposure	0, 0.04, 0.08, 0.16, 0.32, 0.64, 1.25, 2.5, 5.0	1.25 mg l^{-1} (a)	Kuhn *et al* (1989)
Terrestrial	Birds	No data	-	-	-	-
	Invertebrates	No data	-	-	-	-
Aerial	Invertebrates	No data	-	-	-	-

a – No information on the mechanism of action is available

6.5 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity

The general toxicity data in this section has largely been obtained from the IUCLID data set for chlorocresol and has been taken as accurate. Individual source documents have not been checked unless they are considered to be key studies which have a major influence on the outcome of the review. All information taken from IUCLID as been referenced as being from that source and individual references have not been given in the references.

6.5.1 Studies relevant to the assessment of general toxicity in humans

Table 6.8 summarises the general toxicity data from acute and repeat dose studies with 4-chloro-3-methylphenol.

6.5.1.1 Acute studies

A. Oral Exposure

In oral toxicity tests values ranging from 400 to 5129 mg kg body weight^{-1} have been recorded in studies with rats (see Table 6.8). Mobay (1981) reported oral LD_{50} values of 5129 and 3636 mg kg body weight^{-1} for male and female rats respectively. Female rats were exposed to 5 test concentrations ranging from 1500 to 5762 mg kg body weight^{-1} and males were exposed to 5 test concentrations ranging from 200 to 7683 mg kg body weight^{-1}. Symptoms of toxicity were observed in all treated rats, and included ataxia, wheezing, muscle fasciculations, tremors, convulsions, salivation, diarrhea, lacrimation, bloody urine and urine stain, pilo-erection, decreased activity and immobilisation. Dead rats exhibited fluid in the stomach and/or intestines at gross necropsy. Bayer (1982) exposed male and female rats to 6 doses ranging from 500 to 2000 mg kg body weight^{-1} and reported oral LD_{50} values of 1610 mg kg body weight^{-1} for male rats and 1360 mg kg body weight^{-1} for female rats. Leser (1978) conducted an oral toxicity test with male Wistar rats and reported an LD_{50} value of 1830 mg kg body weight^{-1} . While none of the studies meet all the conditions of a GLP guideline investigation certain of them meet the scientific principals for assessment of an oral LD_{50} in rats and can be considered valid.

B. Dermal Exposure

Two studies with rats and one with rabbits have investigated the acute toxicity following dermal exposure (see Table 6.8). Bayer (1970) recorded a 7 day LD_{50} value for rats of >500 mg kg body weight^{-1} and over a 24 hour exposure period an LD_{50} value of >2000 mg kg body weight^{-1} (Bayer 1988). Hazleton (1979) recorded a 14 day LD_{50} value of >5000 mg kg body weight^{-1} for rabbits. The skin on one half of 60 (30 male and 30 female) New Zealand White rabbits was abraded with minor incisions and 250, 500, 1000 and 5000 mg kg body weight^{-1} was applicated for 24 hours. No deaths occurred during the 14 day observation period but edema, moderate to severe erythema, dermal thickening, epidermal scaling, epidermal and dermal necrosis were observed.

C. Inhalation Studies

Two studies reported by Bayer (1981) investigated the acute toxicity in male and female rats following inhalation exposure (see Table 6.8). The reported 4 hour LC_{50} values were >0.58 mg l^{-1} and >0.70 mg l^{-1}.

D. Other routes of exposure

Two studies investigated the acute toxicity in rats, and one in mice following sub-cutaneous injection (Table 6.8). Wien (1939) exposed ten rats to 300, 400 and 500 mg kg body weight^{-1} and reported an LD_{50} value of 400 mg kg body weight^{-1}. The kidneys from some animals at high doses showed some damage to the tubules. In a single dose toxicity study Robenek *et al* (1980) reported a 60 hour LD_{50} value of 400 mg kg body weight^{-1} for rats. Thirty minutes following administration a common uneasiness and a ruffled-up coat were noticed and after one hour apathetic motions, liver slightly enlarged, intercellular spaces enlarged, increase in lysosomes and mitochondria and rough endoplasmic reticulum showed excessive loss of ribosomes. Wien (1939) reported an LD_{50} value of 360 mg kg body weight^{-1} for mice. Five exposure concentrations were used ranging from 100 – 500 mg kg body weight^{-1}. Toxic symptoms appeared within five minutes including severe muscular tremors, stage of narcosis, respiration irregular and feeble, death resulted from respiratory failure (within 0.5 to 3 hours). Kidneys from some animals on high doses showed some damage to the tubules.

One study investigated the acute toxicity in mice following intra-venous injection (Table 6.8). Wien (1939) reported an LD_{50} value of 70 mg kg body weight^{-1}. Animals (10 or 20 per group) were exposed to 5 test concentrations ranging from 30 to 120 mg kg body weight^{-1}. Kidneys from some animals on high doses showed some damage to the tubules.

6.5.1.2 Repeat dose studies

A number of repeat dose studies are listed in IUCLID (2000) with varying degrees of information on the findings being reported. Table 6.8 summarises these studies but this section focuses on the results with the lowest NOELs (or NOAELs) and/or information on mechanisms of action which are relevant to this review.

A. Oral Exposure

In an oral toxicity study, rats were exposed continuously for 28 days at doses of 0, 189, 385 and 790 mg kg body weight^{-1} (males) and 0, 216, 443 and 920 mg kg body weight^{-1} (females) (Bayer 1989). The haematological and clinical chemical parameters were found to be within the normal range in all the exposure concentrations with no pathological anatomical effects. In the male group, body weight gain was reduced in rats at the highest dose. The no observable effect level (NOAEL) was reported as 385 mg kg body weight^{-1} (males) and 920 mg kg body weight^{-1} (females).

In an oral toxicity study male and female rats were exposed continuously for 3 months at concentrations of 0, 11, 37 and 110 mg kg body weight^{-} (Bayer 1981, 1988). The clinical chemical parameters were found to be within the normal range at all the exposure concentrations and no histopathological changes were observed. In the male group slightly reduced body weight gain at 37 and 110 mg kg body weight day^{-1} was observed. The no observable effect level (NOAEL) was reported as 11 mg kg body weight^{-1} (males) and 110 mg kg body weight^{-1} (females).

An oral toxicity GLP guideline study conducted according to OECD Guideline 453, exposed male and female rats daily in their diets for 105 weeks at concentrations of 0, 400, 2000 and 10000 ppm (Bayer 1993). At 10000 ppm both males and females had reduced weight gain. The females were in poor condition and males had an increase in water intake and the kidneys showed papillo necrosis, cortical dilation and fibrosis. The no observable effect level (NOAEL) was reported as 2000 ppm.

Madsen (1986) treated male and female Wistar rats by oral gavage for 28 days at doses of 0, 50, 200 and 400 mg kg body weight day^{-1}. Animals in the highest dose group exhibited a significant decrease in weight gain during the last week of dosing. No other clinical signs of adverse effects were observed. The haematological and clinical chemical parameters were found to be within the normal range in all exposure groups. The no observable effect level (NOAEL) was reported as 200 mg kg body weight^{-1}.

B. Dermal exposure

In a dermal toxicity GLP study carried out according to OECD Guideline 411 ("Subchronic Dermal Toxicity: 90-day Study") male and female Wistar rats were exposed 6 hours a day, 5 days a week at 0, 20, 100 and 500 mg kg body weight^{-1} for a period of 90 days (Bayer 1991). Preventol CMK was tolerated without damage by all animals in a dermally applied dose up to and including 500 mg kg body weight^{-1}. The no observable effect level (NOAEL) was reported as 500 mg kg body weight^{-1}.

In a dermal toxicity study male rats were exposed 6 hours a day, 5 days a week at 0, 40, 200 and 1000 mg kg body weight^{-1} for a period of 28 days (Bayer 1993). At the highest exposure concentration erythema, odema, scars, significant reduced weight gain, reduced food consumption, increased water intake and histopathological skin changes were observed and one moribund animal was killed on day 16.

Hazleton (1980) conducted a GLP Guideline study where male and female New Zealand White rabbits were dermally exposed 6 hours a day, 5 days a week at 0, 10, 40 and 160 mg kg body weight^{-1} for a period of 21 days. The dermal irritation findings showed slight to well-defined erythema, skin thickening and epidermal scaling in all treated groups. Pronounced frequency of blanching, raw areas, necrosis, and brown scab-like areas in the mid and high-dose groups. Microscopic evaluation revealed compound-related findings, epidermal and dermal necrosis with destruction of adnexal structures in the mid and high-dose rabbits.

C. Inhalation studies

No data has been located on the repeat dose toxicity of 4-chloro-3-methylphenol to laboratory mammals following inhalation exposure.

D. Other routes of exposure

Wien (1939) conducted a sub-cutaneous toxicity study where rats were exposed once a day for 14 days to 0 and 80 mg kg body weight day^{-1}. Observations showed no effect on the growth of young rats, no effect on urine proteins and casts, normal red and white blood cell counts and mild inflammatory reaction with some leucocytic infiltration at injection sites. The author also conducted a sub-cutaneous toxicity study exposing rabbits (application as 0.25% solution) once a day for 28 days to 12.5 mg animal day^{-1}. No effect on blood cell counts and urine protein and no degenerative changes in the liver and the kidney were observed.

Swift (1950) conducted a sub-cutaneous toxicity study where rabbits were exposed (application as 0.4% solution) once a day for 28 days to 0 and 20 mg animal day^{-1}. No abnormalities in the urine, body weight, red and white cell counts or general health of the animals and no sign of necrosis at the site of injection were observed.

Table 6.8 Summary of the general mammalian toxicity data (Information from IUCLID 2000)

Test type	Test species	Exposure period	Test dose series used	Endpoint	Effect dose	Reference	Study validity
Acute Oral Toxicity	Rat	Not relevant	No data	Median lethal dose (LD_{50})	>500 mg kg body weight^{-1}	Dieke (1947)[1]	Use with care[2]
	Rat (Wistar) - males	Not relevant	1000 - 5000 mg kg body weight^{-1}	Median lethal dose (LD_{50})	1830 mg kg body weight^{-1}	Loeser (1978), Bayer (1992)[1]	Valid[2]
	Rat (Wistar)	Not relevant	No data	Median lethal dose (LD_{50})	400 mg kg body weight^{-1}	Meiss *et al* (1980, 1981)[1]	Use with care[2]
	Rat (Wistar) - males	Not relevant	No data	Median lethal dose (LD_{50})	400 mg kg body weight^{-1}	Robenek *et al* (1980)[1]	Use with care[2]
	Rat (Sprague-Dawley) – males and females	Not relevant	Male: 2000 – 7683 mg kg body weight^{-1} Females: 1500 – 5762 mg kg body weight^{-1}	Median lethal dose (LD_{50})	5129 mg kg body weight^{-1} (males) 3636 mg kg body weight^{-1} (females)	Mobay (1981)[1]	Valid[2]
	Rat (Wistar) – males and females	Not relevant	500 – 2000 mg kg body weight^{-1}	Median lethal dose (LD_{50})	1610 mg kg body weight^{-1} (males) 1360 mg kg body weight^{-1} (females)	Bayer (1982)[1]	Valid[2]
	Rat (Wistar)	Not relevant	No data	Median lethal dose (LD_{50})	<2000 mg kg body weight^{-1}	Bayer (1988)[1]	Use with care[2]
Acute Dermal Toxicity	Rat - males	7 days	No data	Median lethal dose (LD_{50})	>500 mg kg body weight^{-1}	Bayer (1970)[1]	Use with care[2]
	Rat (Wistar) – males and females	24 hours	No data	Median lethal dose (LD_{50})	>2000 mg kg body weight^{-1}	Bayer (1988)[1]	Use with care[2]
	Rabbit (New Zealand White) – males and females	14 days	250, 500, 1000 and 5000 mg kg body weight^{-1}	Median lethal dose (LD_{50})	>5000 mg kg body weight^{-1}	Hazleton (1979)[1]	Use with care[2]

Table 6.8 Continued

Test type	Test species	Exposure period	Test dose series used	Endpoint	Effect dose	Reference	Study validity
Acute Inhalation Toxicity	Rat (Wistar) – males and females	4 hours	No data	Median lethal dose (LC_{50})	>0.70 mg l^{-1}	Bayer (1981) [1]	Use with care [2]
		4 hours	No data	Median lethal dose (LC_{50})	>0.58 mg l^{-1}	Bayer (1981) [1]	Use with care [2]
Acute Toxicity (Sub-cutaneous injection)	Rat	No data	300, 400 and 500 mg kg body $weight^{-1}$	Median lethal dose (LD_{50})	400 mg kg body $weight^{-1}$	Wien (1939) [1]	Use with care [2]
	Rat (Wistar) - males	60 hours	400 mg kg body $weight^{-1}$	Median lethal dose (LD_{50})	400 mg kg body $weight^{-1}$	Robenek *et al* (1980)	Use with care [2]
	Mouse	No data	100 – 500 mg kg^{-1} body $weight^{-1}$	Median lethal dose (LD_{50})	360 mg kg body $weight^{-1}$	Wien (1939) [1]	Use with care
Acute Toxicity (Intra-venous injection)	Mouse	No data	30 - 120 mg kg body $weight^{-1}$	Median lethal dose (LD_{50})	70 mg kg body $weight^{-1}$	Wien (1939) [1]	Use with care [2]
Repeat Dose Toxicity (Oral)	Rats (Wistar) – males and females	3 months	Daily in diet at: Male: 11, 37 and 110 mg kg body $weight^{-1}$ Female: 0, 11, 37, and 110 mg kg body $weight^{-1}$	NOAEL NOAEL	11 mg kg body $weight^{-1}$ day^{-1} 110 mg kg body $weight^{-1}$ day^{-1}	Bayer (1981, 1988) [1]	Valid [2]
		28 days	Daily in diet at: Male: 0, 189, 385 and 790 mg kg body $weight^{-1}$ Female: 0, 216, 443 and 920 mg kg body $weight^{-1}$	NOAEL NOAEL	385 mg kg body $weight^{-1}$ day^{-1} 920 mg kg body $weight^{-1}$ day^{-1}	Bayer (1989) [1]	Valid [2]
		105 weeks	Daily in diet at 0, 400, 2000 and 10000 ppm	NOAEL	2000 ppm	Bayer (1993) [1]	Valid [2]

Table 6.8 Continued

Test type	Test species	Exposure period	Test dose series used	Endpoint	Effect dose	Reference	Study validity
Repeat Dose Toxicity (Oral)	Rat (Wistar) – males and females	28 days	Oral gavage at 0, 50, 200 and 400 mg kg body weight day^{-1}	NOAEL	200 mg kg body $weight^{-1}$ day^{-1}	Madsen (1986) [1]	Valid[2]
Repeat Dose Toxicity (Dermal)	Rat (Wistar) – males and females	90 days	Exposure 6 hours a day, 5 days a week at 0, 20, 100 and 500 mg kg body $weight^{-1}$	NOAEL	500 mg kg body $weight^{-1}$	Bayer (1991) [1]	Valid[2]
	Rat (Wistar) - males	28 days	Exposure 6 hours a day, 5 days a week at 0, 40, 200 and 1000 mg kg body $weight^{-1}$	NOAEL	1000	Bayer (1993) [1]	Valid[2]
	Rabbit (New Zealand White) – males and females	21 days	Exposure 6 hours a day, 5 days a week at 0, 10, 40 and 160 mg kg body $weight^{-1}$	NOAEL	No data	Hazleton (1980) [1]	Valid[2]
Repeat Dose Toxicity (Sub-cutaneous injection)	Rat	14 days	Injection once per day at 80 mg kg body weight day^{-1}	NOAEL	No data	Wien (1939) [1]	Use with care[2]
	Rabbit	28 days	Injection once per day at 12.5 mg $animal^{-1}$ day^{-1}	NOAEL	No data	Wien (1939) [1]	Use with care[2]
		28 days	Injection once per day at 20 mg $animal^{-1}$ day^{-1}	NOAEL	No data	Swift (1950) [1]	Use with care[2]

[1] – Cited in IUCLID (2000), [2] – Assessment based on data in IUCLID (2000)

6.5.1.3 Comparison of data from studies of potential endocrine disrupting effects and/or general toxicity in mammals

A series of oral and dermal exposure studies (see Table 6.4 and 6.5) have provided no evidence that 4-chloro-3-methylphenol affects developmental endpoints even in the presence of (high dose) toxicity of parental animals. Exposure of 4-chloro-3-methylphenol to pregnant rats during the period of organogenesis induced embryo or foetotoxicity or malformations at the highest dose tested (300 mg kg body weight^{-1} day^{-1}). Therefore, the lowest NOEL recorded from the studies on laboratory mammals is a value of 100 mg kg body weight^{-1} day^{-1} for foetotoxic effects (on intra-uterine development) (Bartmann 1991). However in the study, toxic effects on the dams were evident at the lowest exposure dose (30 mg kg body weight^{-1} day^{-1}). However, the absence of data on reproductive effects of 4-chloro-3-methylphenol could mean subtle effects on organisms post-natally may not have been detected.

In acute and repeat-dose studies the general systemic toxicity data for laboratory mammals indicates that the threshold in rats for an absence of effects which are not directly endocrine mediated occurs at a dose of 11 mg kg body weight^{-1} day^{-1} (in males)(Leser 1989). The NOEAL reported in the study related to reduced body weight gain in a 3 month oral exposure study. As a result it appears that on the basis of the available data endocrine mediated responses are not the mechanism responsible for the most toxic effects observed in laboratory mammals.

6.5.2 Studies relevant to the assessment of general toxicity in wildlife

6.5.2.1 Studies on aquatic organisms

Table 6.9 summarises the general toxicity data for aquatic organisms exposed to 4-chloro-3-methylphenol.

A. Fish

Acute toxicity

Data are available for two salmonid species, *Oncorhynchus mykiss* (rainbow trout) and *Salmo trutta* (brown trout), both of which indicate moderate to high sensitivity to 4-chloro-3-methylphenol. A 96-hour NOEC of 0.37 mg l^{-1} and an 96-hour LC_{50} of 0.92 mg l^{-1} are reported for the rainbow trout (Gagliano and Bowers 1993). These data appear to be reliable with water quality monitoring data available, analytical monitoring performed and the test being conducted in accordance with the FIFRA Guideline 72-1 Acute Toxicity Test for freshwater fish. In addition, LC_{50} values ranging from 1.3 to 50 mg l^{-1} have been reported for the brown trout (Hattula *et al* 1981, Kirk and Lester 1989), though these data are of lower reliability.

Acute (24 to 96 hour) LC_{50} values for non-salmonid fish range from 1 to 35 mg l^{-1} (Ruebelt *et al* 1982, Benoit-Guyod *et al.* 1984a,b, Holcombe *et al* 1984, Devillers *et al* 1985, MITI 1992, Bayer plc unpublished data cited in Dixon *et al* 1997, and Ramos *et al* 1998). LC_0 values range from 0.5 to 2 mg l^{-1} (Ruebelt *et al.* 1982 and Bayer plc unpublished data cited in Dixon *et al* 1997). Sub-lethal effect data on *Pimephales promelas* (fathead minnow) after a 96 hour exposure ranged from 2.7 mg l^{-1} for fish swimming at the water surface to 22.1 mg l^{-1} for hyperactivity with fish darting and rolling (Holcombe *et al* 1984).

Chronic toxicity

No chronic data appear to have been published for salmonid fish species. However, some limited data relating to the chronic toxicity of 4-chloro-3-methylphenol are available for non-salmonid fish species. A 14 day NOEC of 1 mg l^{-1} and LOEC of 3.2 mg l^{-1} have been reported for *Brachydanio rerio* (zebra fish) (Bayer plc 1991) in a study using a German Environmental Protection Office procedure and with measured exposure concentrations and water quality parameters reported.

B. Invertebrates

Toxicity data for invertebrates have only been located for the crustaceans *Daphnia magna* (or *pulex*) and the gastropod *Lymnaea stagnalis* (pond snail).

Acute (24 to 48 hour immobilisation) values reported for *Daphnia magna* range from 1.5 to 10 mg l^{-1} (Devillers *et al* 1985, Gersich and Mayer 1986, Kuhn *et al* 1989, Ramos *et al* 1998). The lowest reported acute EC_{50} (immobilisation) was 2 mg l^{-1} for the 48 hour immobilisation of *Daphnia magna* which were < 24 hours old (Gersich and Mayes, 1986). Procedures used in this test were based on the guidelines recommended by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) and the ASTM Subcommittee on Safety to Aquatic Organisms (1980). However, concentrations reported were nominal and not measured. In acute tests, a 24 hour EC_0 (immobilisation) of 1.9 mg l^{-1} has been reported for *Daphnia magna* (Kuhn *et al* 1989). The test was conducted in accordance with recommendations made by the German Federal Environment Agency. Exposure concentrations were measured, although the method of analysis is not given in the reference, and water quality parameters were monitored.

A chronic (21 day) NOEC for survival of 1.25 mg l^{-1} has been reported for *Daphnia magna* by Kuhn *et al* (1989), with the test being performed under reliable test conditions. A 21 day EC_{50} for reproduction of 2.5 mg l^{-1} was obtained by the same author (Kuhn *et al* 1988) in a test conducted according to the method proposed by the German Federal Environment Agency and therefore likely to be of a similar reliable standard.

Only one study was located for non-crustacean invertebrate involving an acute semi-static test on the pond snail (*Lymnaea stagnalis*) (Ramos *et al* 1998). The test reported a 96 hour LC_{50} of 14.0 mg l^{-1}, indicating lower sensitivity to 4-chloro-3-methylphenol than *Daphnia*.

6.5.2.2 Studies on terrestrial organisms

The only information located on the toxicity of 4-chloro-3-methylphenol to terrestrial organisms is for the quail or Northern bobwhite (*Colinus virginianus*) by oral administration and the slug (*Deroceras reticulatum*) by injection.

In the study with the slug the toxicity of 4-chloro-3-methylphenol was investigated by injection of the dissolved substance into the intestine of the animal (Briggs *et al* 1987). The resulting LD_0 and LD_{100} values were 20 and 200 μg per animal, although other experimental details are limited.

The original studies for the quail could not be obtained, however, summary information was available (Office of Pesticide Programs 1995). In the first study, 16 week old birds were exposed orally for 14 days with a resulting 14 day LD_{50} of 15409 mg kg^{-1} organism reported. In

Table 6.9 Summary of general toxicity data for aquatic organisms (Information from IUCLID 2000)

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study validity
Acute Fish Toxicity	*Brown trout* (Salmo trutta)	24h	No data	LC_{50}	1.3 mg l^{-1}	Hattula *et al* (1981)[1]	Valid[2]
		24h	No data	LC_{50}	50 mg l^{-1}	Kirk and Lester (1989)[1]	Use with care[2]
	Fathead minnow *(Pimephales promelas)* 2-3 months old	24h	No data	LC_{50}	13.3 mg l^{-1}	Holcombe *et al* (1984)[1]	Valid[2]
		48h	"	LC_{50}	11.4 mg l^{-1}		
		72h	"	LC_{50}	9.2 mg l^{-1}		
		96h	"	LC_{50}	7.6 mg l^{-1}		
	Fathead minnow *(Pimephales promelas)* 30 days old	96h	No data	LC_{50}	4.0 – 7.4 mg l^{-1}	Gieger *et al* (1985)[1]	Valid[2]
	Fathead minnow *(Pimephales promelas)* 30-35 days old	96h	No data	LC_{50}	5.5 mg l^{-1}	Schultz *et al* (1986)[1]	Valid[2]
	Fathead minnow *(Pimephales promelas)* 30-35 days old	96h	No data	LC_{50}	4.1 mg l^{-1}	Broderius *et al* (1995)[1]	Valid[2]
	Fathead minnow *(Pimephales promelas)* 30-35 days old	24h	No data	LC_{50}	7.3 mg l^{-1}	Ramos *et al* (1998)[1]	Valid[2]
		48h	"	LC_{50}	7.3 mg l^{-1}		
		72h	"	LC_{50}	7.3 mg l^{-1}		
		96h	"	LC_{50}	6.7 mg l^{-1}		
	Golden orfe *(Leuciscus idus melanotus)*	48h	No data	LC_{0}	0.5 mg l^{-1}	Bayer AG (unpublished data)[1]	Valid[2]
		48h	"	LC_{50}	1.2 mg l^{-1}		
		48h	"	LC_{100}	2.0 mg l^{-1}		
		48h	No data	LC_{0}	2.0 mg l^{-1}	Ruebelt *et al* (1982)[1]	Valid[2]
		48h	"	LC_{50}	2.4 mg l^{-1}		
		48h	"	LC_{100}	3.0 mg l^{-1}		
	Guppy *(Poecilia reticulata)*	24h	No data	LC_{50}	2.2 mg l^{-1}	Benoit-Guyod *et al* (1984a,b)[1]	Use with care[2]

Table 6.9 Continued

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study validity
Acute Fish Toxicity	Japanese medaka *(Oryzias latipes)*	48h	No data	LC_{50}	4.6 mg l^{-1}	CITI (1992) [1]	Valid[2]
	Rainbow trout (Oncoryhnchus mykiss)	96h	No data	NOEC	0.37 mg l^{-1}	Gagliano and Bowers (1993) [1]	Valid[2]
		96h	No data	LC_{50}	0.92 mg l^{-1}		
	Zebrafish *(Danio rerio)*	24h	No data	LC_{50}	1.0 – 3.5 mg l^{-1}	Devillers *et al* (1985) [1]	Use with care[2]
Chronic Fish Toxicity	*Zebrafish (Danio rerio)*	14 days	No data	NOEC	1.0 mg l^{-1}	Bayer AG (1991) [1]	Valid[2]
		14 days	No data	LOEC	3.2 mg l^{-1}		
Acute Invertebrate Toxicity	Water flea *(Daphnia magna)* <72h old	24h	No data	EC_{50}	3.5 - 10 mg l^{-1}	Devillers *et al* (1985) [1]	Use with care[2]
		48h	No data	EC_{50}	2.0 mg l^{-1}	Gersich and Mayes (1986) [1]	Valid[2]
		24h	No data	EC_0	1.9 mg l^{-1}	Kuhn *et al* (1989) [1]	Valid[2]
		24h	No data	EC_{50}	4.4 mg l^{-1}		
		24h	No data	EC_{50}	2.8 mg l^{-1}	Ramos *et al* (1998) [1]	Valid[2]
		48h	No data	EC_{50}	1.5 mg l^{-1}		
	Water flea *(Daphnia pulex)* <24h old	96h	No data	EC_{50}	3.1 mg l^{-1}	Trabalka and Burch (1978) [1]	Use with care
	Pond snail *(Lymnaea stagnalis) 2-3 months old*	24h	No data	LC_{50}	>22.8 mg l^{-1}	Ramos *et al* (1998) [1]	Valid[2]
		48h	No data	LC_{50}	16.2 mg l^{-1}		
		72h	No data	LC_{50}	14.0 mg l^{-1}		
		96h	No data	LC_{50}	14.0 mg l^{-1}		
Chronic Invertebrate Toxicity	Water flea *(Daphnia magna) <24h old*	21 days	No data	NOEC	1.25 mg l^{-1}	Kuhn *et al* (1989) [1]	Valid[2]

[1] – Cited in IUCLID (2000), [2] – Assessment based on data in IUCLID (2000)

the second study, organisms were younger (10 days) and exposure, again orally, was over a shorter period (8 days). A resulting 8 day LC_{50} of >3180 ppm was reported.

6.5.2.3 Studies on aerial organisms

No general toxicity data for aerial organisms following exposure to 4-chloro-3-methylphenol has been located.

6.5.2.4 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity in wildlife

Comparison of the limited data on potential endocrine mediated responses in the aquatic invertebrate *Daphnia magna* with the acute and chronic data for this species indicates that mortalities were evident at only slightly higher concentrations than the threshold concentration for effects on reproduction. However, no comparisons could be made for fish due to the absence of data on potential endocrine mediated responses in this taxonomic group, which represents an area of uncertainty.

6.6 Current classification of 4-chloro-3-methylphenol against European Commission and national regulations

Table 6.10 summarises the current classification of the substance against Council Directives in order to assess the regulations to which 4-chloro-3-methylphenol is subject.

4-chloro-3-methylphenol is listed as an evaluation and approved preservative in Annex V1A under Directive 76/768/EEC and notification has been filed under Directive 98/8/EEC.

Table 6.10 Current classification of 4-chloro-3-methylphenol against Council Directives

Directive	Status (listed or not)
67/548/EEC - Classification, packaging and labelling of dangerous substances	Classified: Xn, N R phrases: 21/22-41-43-50
76/768/EEC - Approximation of laws relating to cosmetic products	Listed as an evaluated and approved preservative in Annex VIA

Under Directive 67/548/EEC the R phrases indicate that the substance is very toxic to aquatic organisms (R50).

In the United Kingdom statutory environmental quality standards of 40 μg l^{-1} in freshwaters and saltwaters (as annual averages) have been established.

6.7 Exposure data

6.7.1 Worker exposure

Data on concentrations of 4-chloro-3-methylphenol to which workers are potentially exposed during the production and use of the substance has been sought from the relevant CEFIC Sector Group. Table 6.11 summarises the suggested addition doses of the disinfectants/

preservatives Preventol CMK (>99.8% 4-chloro-3-methylphenol) and Preventol CMK-Na (>71% sodium salt of 4-chloro-3-methylphenol) for different applications.

Table 6.10 Suggested addition doses of Preventol CMK and Preventol CMK-Na for different applications

Application	Use	Suggested addition doses (%)	
		Preventol CMK	Preventol CMK-Na
Disinfection and cleaning agents	Disinfection		
Disinfection concentrates		5 - 15	8 – 24
Ready to use disinfectant concentrates		0.075 - 0.15	0.12 – 0.24
Metal working fluids	Preservation		
Concentrates for metal working fluids		2 - 4	1.6 - 4.8
Ready to use metal working fluid dilutions		0.15 - 0.3	0.24 - 0.48
Leather	Preservation		
Chrome leather		0.1 - 0.2	0.16 - 0.32
Leather pigments (casein-based)		0.2 - 0.4	0.32 - 0.64
Construction materials			
Concrete additives		0.15 - 0.3	0.24 - 0.48
Glues and adhesives	Preservation		
Casein solutions		0.2 - 0.3	0.32 - 0.48
Dextrin and cellulose glues		0.05 - 0.1	0.08 - 0.16
Starch glues (dry)		0.1 – 0.15	0.16 - 0.24
Paper coatings	Preservation		
Filler suspensions, coating compounds		0.05 - 0.1	0.08 - 0.16
Casein-based preparations		0.1 - 0.15	0.16 - 0.24
Textile auxillaries	Preservation of use dilutions		
Print thickeners (powder)		1.0 - 2.0	1.6 - 3.2

The highest potential exposure levels of 4-chloro-3-methylphenol occur when handling the concentrates for disinfection and cleaning agents. However, given that appropriate safety equipment will be worn at this time the risk to workers should be minimised. The materials containing 4-chloro-3-methylphenol which are used for preservation in other areas have lower levels of the substance (typically <1% vol/vol or ~ 10 mg l^{-1})

6.7.2 Consumer exposure

4- chloro-3-methylphenol is used in medicinal hand and skin disinfection products including products which are used to disinfect skin prior to injections. An example of this an alcohol based gel for hand and skin disinfection which contains 22 mg of 4-chloro-3-methylphenol per 100 ml of gel as well as 9 mg of clorofen, 70% volume % 2-propanol and small amounts of viscosity regulator and perfume. This product is designed to be effective against bacteria, including mycobacteria, fungi, fungal spores, Hepatitis B and HIV.

Exposure to 4-chloro-3-methylphenol during hygienic hand disinfection involves rubbing 2 x 3 ml of undiluted product into the hands for one minute Over an area of 400 cm^2 to a depth of 0.015 cm this would involve exposure to 1.32 mg of 4-chloro-3-methylphenol per event[2] (see

[2] Exposure per event (mg) = 0.22 (Weight fraction of substance) x 450 (Surface area of exposed skin) x 0.015 (Thickness of product layer)

Table 2.11). Based on a scenario of 5 events per day a 60 kg female would be exposed to 0.11 mg kg body weight day^{-1} and a 70 kg male to 0.094 mg kg body weight day^{-1}. These calculations assume that there is 100% adsorption through the skin. For children the exposure levels could be higher given the smaller body weights.

The data on potentially endocrine mediated responses indicates that the lowest NOEL recorded from the studies on laboratory mammals is a value of 100 mg kg body $weight^{-1}$ day^{-1} for foetotoxic effects (on intra-uterine development) (Bartmann 1991). The use of a margin of safety (MOS)[3] approach assuming the effects on laboratory mammals were endocrine mediated would result in values of 909-1064. On the basis that an MOS of 100 should be required for the risk to be acceptable then 4-chloro-3-methylphenol does not apparently present a risk to consumers in terms of endocrine disrupting effects. However, it needs to be recognised that the actual margin of safety may be smaller given the uncertainty resulting from the absence of data for reproduction effects of 4-chloro-3-methylphenol (which may be endocrine mediated).

For skin disinfection the area to be treated is wiped with the product and allowed to dry. This exposure regime will usually involve the use of smaller amount of product applied to smaller surface areas of the skin than is the case for hand disinfection. As a result the margins of safety will be higher than those for exposure through use of products to disinfect the hands.

4-chloro-3-methylphenol is also used as a preservative in many pharmaceutical creams and lotions, but especially steroid creams. It is not used in cosmetic products because it may interfere with the perfumes present in these products. The risk to consumers from 4-chloro-3-methylphenol in pharmaceutical creams and lotions is minimal given the small weight fractions of the material typically present (for example 0.1% or 1 mg of 4-chloro-3-methylphenol in each 1 g tube of cortico-steroid based eye preparation) and the amount of cream or lotion recommended for use at one time.

6.7.3 Environmental monitoring data

6.7.3.1 Aquatic environment

Treatment plant discharges and sewage sludges

Körner *et al* (1998) investigated the presence of various endocrine disrupting chemicals in the influent and effluent of a municipal sewage plant in southern Germany in March and June 1998. 4-chloro-3-methylphenol was detected at mean concentrations of 0.13 and 0.5 µg l^{-1} in the influent for March and June respectively. No 4-chloro-3-methylphenol was detected in the effluents and the authors of the study estimated a removal efficiency of >94% at this treatment works.

Schnaak *et al* (1997) detected the presence of 4-chloro-3-methylphenol in sewage sludges from Brandenburg, Germany, in samples taken in summer and winter. The summer median concentration of 4-chloro-3-methylphenol was just above 10 µg kg dry $matter^{-1}$, with the maximum just above 100 µg kg dry $matter^{-1}$: in winter the median was between 1 and 10 µg kg dry $matter^{-1}$, but a higher maximum of just below 1000 µg kg dry $matter^{-1}$ was recorded.

[3] Margin of safety (MOS) = (Lowest NOEL for endocrine mediated responses)/Exposure dose

Bolz *et al* (2001) detected 4-chloro-3-methylphenol, in the range 14-40 µg kg dry matter^{-1}, in the sewage sludge of a municipal sewage treatment works in the state Baden-Württemberg, South West Germany during 1998 and 1999.

Surface waters and sediments

In the United Kingdom Environmental Quality Standards (EQSs) for 4-chloro-3-methylphenol has been established for fresh and saline waters.

Dixon *et al* (1997) summarised data provided by the United Kingdom Environment Agency for 1995 describing the environmental concentrations of 4-chloro-3-methylphenol in fresh, ground and marine waters in the Midlands and North West regions of England. The average concentrations for freshwater was 0.060 µg l^{-1} and for marine and estuarine waters was 0.3 µg l^{-1}, all concentrations in groundwater were <LOD. It should be noted that these annual average concentrations may be artificially low due to the treatment of <LOD results as zero, particularly when the LOD is high. In the mid 1990s 4-chloro-3-methylphenol concentrations in surface waters ranged from 0.5 to 6.9 µg l^{-1} in the Midland region and from 0.7 to 6.6 µg l^{-1} in the North West regions. Only one result reported for marine waters was greater that the LOD, this was a concentration of 0.6 µg l^{-1} for an estuary in the North West region and was towards the lower end of the concentration range reported for freshwaters in the two regions.

In surface water screening of rivers, canals and lakes in Berlin, Schmidt-Bäumler *et al* (1999), found 4-chloro-3-methylphenol to be present in 22 of the 30 water samples taken, with concentrations in the range 0.05 to 0.14 µg l^{-1}. No correlation was found between the presence of this chemical and the input of sewage effluents.

If the United Kingdom EQS of 40 µg l^{-1} (see Section 6.6) were applied across Europe all the available monitoring data would comply with the standard.

Bolz *et al* (2001) analysed surface water and sediments from different stream and rivers in the state Baden-Württemberg, South West Germany during 1998 and 1999. 4-chloro-3-methylphenol was found in the sediment of 5 of the 7 sites tested, with concentrations ranging from 1 to 15 µg kg dry matter^{-1}.

6.7.3.2 Terrestrial environment

Bayer AG (the relevant CEFIC Sector Group for 4-chloro-3-methylphenol) have indicated that the substance and substance related products are not directly applied or released to terrestrial environments. Furthermore, given that 4-chloro-3-methylphenol is not considered to strongly sorb to organic carbon and soil biodegradation is rapid (see Table 6.1) the potential for terrestrial organisms to be exposed to the substance is limited.

6.7.3.3 Aerial environment

No information on aerial environmental concentrations of 4-chloro-3-methylphenol has been obtained. However, given that 4-chloro-3-methylphenol is not considered to be volatile the potential for aerial organisms to be exposed to the substance should be limited.

6.7.3.4 Comparison of environmental monitoring data and exposure concentrations causing endocrine mediated responses

The limited data on the concentrations of 4-methyl-3-chlorophenol in European surface waters (see Section 6.7.3.1) indicates that typical levels are in the range 0.05 – 6.9 μg l^{-1}, though most values are probably at the lower end of the range. The only data on potentially endocrine mediated responses in aquatic organisms is a 21 day NOEC for reproduction in *Daphnia magna* of 1300 μg l^{-1}, though there is no information on the mechanism of action for the effects. The use of a margin of safety (MOS)[4] approach assuming the effects on *Daphnia magna* reproduction were endocrine mediated would result in values of 188 – 26000. On the basis that an MOS of 100 should be required for the risk to be acceptable then 4-chloro-3-methylphenol does not apparently present a risk to aquatic organisms in terms of endocrine disrupting effects. However, there is considerable uncertainty associated with the MOS due to the absence of data for key taxa such as fish.

6.8 Overall conclusions on 4-chloro-3-methylphenol

The following conclusions have been drawn from the review of the data on 4-chloro-3-methylphenol.

6.8.1 Data from studies assessing potential endocrine disrupting effects

6.8.1.1 Human related studies

- *In vivo* sub-chronic studies in rats using oral exposure (up to doses of 559 mg kg body weight^{-1} day^{-1} in males and 774 mg kg body weight^{-1} day^{-1} in females) and dermal exposure (up to doses of 500 mg kg body weight^{-1} day^{-1} in males and females) resulted in no histopathological effects on endocrine glands and endocrine sensitive tissues.

- *In vivo* exposure of pregnant rats to 4-chloro-3-methylphenol during the period of organogenesis induced embryo or foetotoxicity or malformations at the highest dose tested (300 mg kg body weight^{-1} day^{-1}). Therefore, the lowest NOEL recorded from the studies on laboratory mammals is a value of 100 mg kg body weight^{-1} day^{-1} for foetotoxic effects (intra-uterine development) (Bartmann 1991). However in the study, toxic effects on the dams were evident at the lowest exposure dose (30 mg kg body weight^{-1} day^{-1}).

- No data was available on the potential effects of 4-chloro-3-methylphenol on the reproduction and fertility of laboratory mammals.

- *In vitro* screening studies using mammalian cells and tissues have shown weak oestrogen type activity in oestrogen receptor competitive binding assays, recombinant yeast assays and cell culture assays. The potency of 4-chloro-3-methylphenol is approximately 10^6 times lower than that of the endogenous vertebrate oestrogen 17β-oestradiol. No information was available on the androgenic and anti-androgenic effects of 4-chloro-3-methylphenol and effects on thyroid function and hormone synthesis and secretion or steroidogenesis in mammalian cells and tissues.

6.8.1.2 Wildlife studies

[4] Margin of safety (MOS) = (Lowest NOEC for endocrine mediated responses)/Environmental concentration

- The data on potential endocrine disrupting effects in wildlife was limited to a reproduction study in the water flea *Daphnia magna* which showed a NOEL of 1.25 mg l^{-1}, though no information is available on the mechanism of action for the effects. No data was available on the effects of 4-chloro-3-methylphenol on the reproduction and development of fish.

- No data on potential endocrine mediated responses in terrestrial or aerial species could be located, though these are probably not key areas of uncertainty given that the physico-chemical properties of 4-chloro-3-methylphenol indicate that the substance should not partition into the terrestrial and aerial compartments.

- No *in vitro* data was available from assays using cells and tissues from wildlife species.

6.8.2 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity

6.8.2.1 Human related studies

- In acute and repeat-dose studies the general systemic toxicity data for laboratory mammals indicates that the threshold in rats for an absence of effects which are not endocrine mediated occurs at a dose of 11 mg kg body $weight^{-1}$ day^{-1} (in males)(Leser 1989). The NOEAL reported in the study related to reduced body weight gain in a 3 month oral exposure study. As a result it appears that on the basis of the available data endocrine mediated responses are not the mechanism responsible for the most toxic effects observed in laboratory mammals. The absence of data on reproductive effects of 4-chloro-3-methylphenol (which may be endocrine mediated) represents an area of uncertainty. The available studies provide no consideration of changes in endocrine function.

6.8.2.2 Wildlife studies

- In wildlife studies data was only available for aquatic species (invertebrates and fish) and not for terrestrial or aerial species. The lowest NOECs for survival in invertebrates and fish were 1.25 mg l^{-1} (21 day value for the water flea *Daphnia magna*) and 1 mg l^{-1} (14 day value for the zebrafish *Danio rerio*). These values were similar to the NOEC for effects on reproduction in *Daphnia magna,* though there is no information on the mechanism of action for these effects.

6.8.3 Exposure data

6.8.3.1 Workers

- The data on concentrations of 4-chloro-3-methylphenol to which workers are exposed indicates that the risk should be minimal if proper safe handling procedures are adopted.

6.8.3.2 Consumers

- Information on the use of 4-chloro-3-methylphenol in hand and skin disinfectants to which consumers could be exposed indicate that the derived margin of safety (MOS) for potential endocrine mediated effects exceeded the threshold of 100 for acceptable risk. However,

there was uncertainty associated with the MOS due to the absence of data on reproductive effects which may be endocrine mediated.

- The risk to consumers from 4-chloro-3-methylphenol in pharmaceutical creams and lotions is minimal given the small weight fractions of the material typically present (for example 0.1% or 1 mg of 4-chloro-3-methylphenol in each 1 g of cortico-steroid based eye preparation).

6.8.3.3 Environment

- The environmental monitoring data for aquatic systems indicated that typical European surface water concentrations were in the range 0.05 to 6.9 $\mu g\ l^{-1}$.

- The derived margin of safety for potential endocrine mediated effects in aquatic organisms exceeded the threshold of 100 for acceptable risk but there was considerable uncertainty given the limited data.

- No data has been located on concentrations of 4-chloro-3-methylphenol in terrestrial or aerial environments.

6.9 Summary of the weight of evidence for endocrine disrupting effects in humans and wildlife and associated uncertainties

The summary of the weight of evidence for endocrine disrupting effects of 4-chloro-3-methylphenol in humans and wildlife along with associated uncertainties are given in Table 6.12.

Table 6.12 Summary of the weight of evidence conclusion and uncertainties associated with the assessment of the endocrine disrupting effects of 4-chloro-3-methylphenol

	Target group	
	Humans	Wildlife
Weight of evidence	The available data from *in vivo* studies in laboratory mammals (using oral or dermal exposure routes) indicates that 4-chloro-3-methylphenol does not cause adverse effects on reproductive and developmental endpoints (which may be endocrine mediated) at exposure levels where general systemic toxic effects are observed. The lowest NOEL in the *in vivo* studies was 100 mg kg body weight $^{-1}$ day^{-1} for foetotoxic effects (on intra-uterine development) At higher exposure doses where adverse effects on development (foetotoxicity) were evident no information on changes in endocrine function was available. The available data indicate that 4-chloro-3-methylphenol in hand and skin disinfectants and as a preservative in pharmaceuticals does not present a risk to consumers.	The available aquatic effects data shows that the threshold exposure concentration of 4-chloro-3-methylphenol above which reproduction of the invertebrate *Daphnia magna* is reduced (NOEC = 1.25 mg l^{-1}) is slightly lower than the threshold level for general toxic effects (i.e. lethality). However there is no information on the mechanism of action for the effects on reproduction observed in *Daphnia magna*. The available exposure data indicate that 4-chloro-3-methylphenol does not represent a risk to aquatic organisms.
Uncertainties	There are uncertainties with regard to the evaluation of potential adverse effects of 4-chloro-3-methylphenol on reproductive and developmental endpoints since data is not available from a definitive multi-generation study. Mechanistic uncertainties exist because the available studies provide no direct measurement of changes in endocrine function (for example changes in hormone levels). Limited exposure data have been located for workers and consumers.	There are uncertainties with regard to potential adverse effects of 4-chloro-3-methylphenol on reproduction and development in wildlife due to the absence of data for a wider range of aquatic taxa, particularly fish. The absence of data for terrestrial and aerial organisms is not a major uncertainty since the physico-chemical properties of 4-chloro-3-methylphenol indicate that the substance should not partition into the terrestrial and aerial compartments. No environmental exposure data for 4-chloro-3-methylphenol in the terrestrial and aerial compartments has been located.

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7. REVIEW OF DATA FOR 2,4-DICHLOROPHENOL

Table of Contents

Notes:

This section contains information collected and collated from a range of sources including published papers, reports of studies conducted by industrial companies or sector groups and data compilations such as IUCLID (2002). The data from IUCLID has been taken as accurate and individual source documents have not been checked unless they are considered to be key studies which have a major influence on the outcome of the review. All information taken from IUCLID has been referenced as being from that source and individual references have not been given in the references.

This review has been carried out in accordance with the evaluation framework described in Section 2. In the review the International Programme for Chemical Safety (IPCS) definition of an endocrine disrupter has been adopted, namely that it is "*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*".

In the context of the review it is recognised that there are various laboratory-based *in vivo* and *in vitro* methods utilising a range of (eco)toxicological endpoints that are claimed by different sources to be relevant to the assessment of endocrine disruption in humans and wildlife. However, since this field is still in an early stage of development there is uncertainty regarding the significance of many of the current findings.

From the numerous recent reviews of potential test methods (such as the Detailed Review Paper prepared by OECD in 1997) there is a clear consensus in terms of the hierarchy of the relevance of test methods. In this hierarchy longer-term *in vivo* studies considering effects on reproduction and/or development (and including mechanistic information) are of greater relevance than short-term *in vivo* screening tests which are of greater relevance than *in vitro* assays. The greater relevance of chronic *in vivo* tests or those assessing effects during critical windows of sensitivity is also evidenced by the fact that these are the key (eco)toxicological methods being developed in the OECD Endocrine Disruption Testing and Assessment (EDTA) Programme. This hierarchy approach to data relevance has been adopted in the review along with a weight of evidence consideration of the available data.

The review has been carried out to address three key questions:

1. Does the available data indicate there is evidence that a chemical causes endocrine disrupting effects in target groups of humans and/or wildlife?

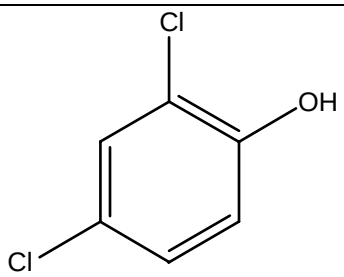
2. Do endocrine disrupting effects of the chemical in target groups of humans and/or wildlife occur at lower concentrations than those causing effects on general systemic toxicological endpoints?

3. Are particular target groups of workers, consumers or organisms in the environment likely to be exposed to concentrations of chemicals which exceed effects thresholds due to current emission patterns.

It should be recognised that this review is not designed to be a full Risk Assessment of a substance under the Existing Substances Regulation 793/93.

7.1 Physico-chemical data for 2,4-dichlorophenol

7.1.1 Summary details on the substance

CAS Number	120-83-2
EINECS Number	204-429-6
IUPAC Name	2,4-dichlorophenol
Other names	4-Hydroxy-1,3-dichlorobenzene
Molecular weight	163.0
Chemical formula	$C_6H_4Cl_2O$
Chemical structure	

7.1.2 Physico-chemical properties and environmental fate information (from IUCLID 2002)

The data on the physico-chemical properties of 2,4-Dichlorophenol and its environmental fate (see Table 7.1) indicate that the substance was not biodegradable in a ready biodegradability test (0% loss in 28 days), but was inherently biodegradable in a modified Zahn-Wellens test (98% loss in 120 hours). In the aquatic environment photolysis is the main route of degradation while hydrolysis is not expected to be an important fate process.

Volatilisation is unlikely to represent a major removal process from the aquatic environment even though the Henry's Law Constant of 3.12×10^{-1} Pa-m^3 mol^{-1} (3.16×10^{-6} atm-m^3 mol^{-1}) for 2,4-dichlorophenol being slightly lower than a value range of 1 - 100 Pa-m^3 mol^{-1} which is considered to indicate volatility.

The low organic carbon water partition coefficient (log Koc) of 2.54 indicates low to moderate adsorption, but pH affects the affinity of 2,4-dichlorophenol to bind to organic matter in soils. In soil 2,4-dichlorophenol undergoes rapid biodegradation.

Table 7.1 Physico-chemical properties and environmental fate information (from IUCLID 2002)

Physico-chemical property	Value (and comments)
Physical state at ambient temperature	Solid
Water solubility	4.5 g l^{-1} at 20°C
Octanol-water partition coefficient (log Kow)	3.21 – 3.25 at 20°C
Organic carbon water partition coefficient (Koc)	2.54
Henry's Law Constant	3.16 x 10^{-6} atm-m^3 mol^{-1}
Type of degradation	
Aquatic - abiotic	Photolysis is the main route of degradation while hydrolysis is not expected to be an important fate process.
Aquatic - biotic	2,4-dichlorophenol was not biodegradable in a ready biodegradability test (0% loss in 28 days), but was inherently biodegradable in a modified Zahn-Wellens test (98% loss in 120 hours).
Terrestrial	2,4-dichlorophenol undergoes rapid biodegradation in soils.
Atmospheric	No data

A Mackay Level 1 fugacity model has shown that for a discharge of 1000 tonnes of 2,4-dichlorophenol 57.8% of the substance will partition into the soil (Table 7.2), with 38.4% partitioning into the water. Amounts present in other compartments are minimal.

Table 7.2 Summary of the results of a Mackay Level 1 fugacity model

Compartment	Volumes of different compartments	% of substance present in different compartments
Water	2 x 10^{11}	38.4
Suspended sediment	10^6	0.04
Bottom sediment	10^8	1.28
Fish	2 x 10^5	0.0033
Air	10^{14}	2.42
Aerosol	2000	2.21 x 10^{-5}
Soil	9 x 10^9	57.8

7.2 Production and Uses

7.2.1 Production Patterns

2,4-Dichlorophenol is produced by the stepwise chlorination of phenol or a lower chlorinated phenol at elevated temperatures and pressure. Information from the relevant CEFIC Sector Group has indicated that 18500 tonnes of 2,4 Dichlorophenol was produced in the EU in 1997, a reduction of 16% on the 22000 tonnes produced in 1983.

7.2.2 Use Patterns

The principal uses of 2,4-dichlorophenol is as an intermediates for the production of the pesticide 2,4-Dichlorophenoxyacetic acid (2,4-D).

Other minor uses are as intermediates for the production of Sesone, nitrofen, meacide, genite-EM-923, as a raw material for polyester films and also for use in mothproofing agents and in miticides.

7.3 Toxicokinetics, metabolism and bioaccumulation

7.3.1 Toxicokinetics and metabolism

The metabolism and distribution of 2,4-dichlorophenol in mammals has been investigated in both *in vitro* and *in vivo* studies.

In a metabolism study in isolated rat liver, 2,4-dichlorophenol was reported to be conjugated into its glucuronide or metabolised into dicloromethoxyphenols (Somani and Khalique 1984). In an *in vitro* study on the human P450 3A4-mediated metabolism of 2,4-dichlorophenol, 2-chloro-1,4-hydroxyquinone, 2-chloro-1,4-benzoquinone and 1,2,4-hydroxybenzene are detected by thin-layer chromatography (Mehmood *et al* 1997).

Somani and Khalique (1982) administered 2,4-dichlorophenol to male Sprague-Dawley (SD) rats (250-300g) by a single intravenous injection at 10 mg kg^{-1} and found the test substance was rapidly transferred into a glucuronide conjugate or other conjugates (no description about the name of the conjugated substances). The half-lives of 2,4-dichlorophenol and its metabolites (no description about the name of the conjugated substances) in the brain, liver, kidney and plasma are 4-30 minutes. Within 10-15 minutes after administration, 2,4-dichlorophenol and its conjugates (no description about the name of the conjugated substances) were detected in the brain, liver, kidney and plasma and 2,4-dichlorophenol alone in the adipose tissues. At 1 hour after administration, 76% of the total administered dose was detected in the kidney, with maximal concentration in the renal tissues of 17.7 mg kg^{-1} (kidney weight).

In rabbits, 2,4-dichlorophenol is excreted mainly as its glucuronide conjugate but some fraction (16% or less) of the administered dose is reported to be converted into its sulphate conjugate (HSDB 2001). In calves, it is reported that the entire amount of 2,4-dichlorophenol (20 g per calf) administered is excreted within 24 hours after administration (HSDB 2001).

IPCS (1989) found that 2,4-dichlorophenol is relatively rapidly absorbed from the digestive tract, skin and respiratory organs.

7.3.2 Bioaccumulation

There is no evidence of bioaccumulation in laboratory mammals which is consistent with the octanol water partition coefficient (log Kow) of 3.21 – 3.25.

Bioaccumulation of 2,4-dichlorophenol has been investigated in two fish species; the carp (*Cyprinus carpio*) and the goldfish (*Carassius auratus*) with low BCF values being reported. In a study performed according to OECD Guideline 305C carp were exposed to 0.03 mg l^{-1} 2,4-dichlorophenol for 56 days at 25 ^{o}C in a flow-through system. The test water was analysed

twice a week and test fish were analysed every 14 days. A bioconcentration factor of 7.1 to 69 was calculated (IUCLID 2002).

Kobayashi *et al* (1979) investigated bioaccumulation of goldfish exposed to 7.8 mg l^{-1} 2,4-dichlorophenol over a 25 hour period at 20 ^{o}C. The water was renewed at 8 hour intervals and a BCF of 34 was determined.

7.4 Studies relevant to the assessment of potential endocrine disrupting effects

7.4.1 Studies relevant to the assessment of the potential endocrine disrupting effects in humans

7.4.1.1 In vitro studies

A. Receptor competitive binding assays

In competitive receptor binding assays, 2,4-dichlorophenol did not bind to human and calf oestrogen receptors (ER) up to a concentration of 5 x 10^{-2} mM (8.2 mg l^{-1}) (Kramer and Giesy 1999, CERI 2001a). In the reporter gene assay using cultured recombinant cells, 2,4-dichlorophenol does not induce ERE- (Oestrogen Response Element) – dependent gene transcription activation within a concentration range of 10^{-8}-10^{-2} mM (CERI 2001a).

B. Recombinant yeast assays

Tran *et al* (1996) investigated the effects of 2,4-dichlorophenol on the human progesterone receptor – progesterone response element using a reporter gene assay using yeast cells transfected with the human progesterone receptor genes. Not all of the experimental details in the study were reported but 2,4-dichlorophenol had no agonist or antagonist activities after a 12 hour exposure period at a 2,4-dichlorophenol concentrations of 10^{-3} mM (0.16 mg l^{-1}). The simultaneous exposure of yeast cells to 10^{-5} mM of progesterone and 10^{-3} mM of 2,4-dichlorophenol had no effect on progesterone activity.

Nishihara *et al* (2000) used a yeast two-hybrid assay (transfected with the human ER ligand domain genes) to assess the effects of 2,4-dichlorophenol on oestrogenic activity. The assay was based on the ligand-dependent interaction of two proteins, a hormone receptor (ERα) and a cofactor (TIF2). Not all of the experimental details in the study were reported but 2,4-dichlorophenol did not induce gene transcription activation. The REC_{10} (the concentration corresponding to 10% of the activity) reported for the assay was 4 x 10^{-2} mM.

C. Mammalian cell growth assays

Jobling *et al* (1995) investigated the effects of 2,4 dichlorophenol on cell growth of the human breast cancer cell line ZR-75. The study indicated that exposure to 10^{-2} mM (1.6 mg l^{-1}) 2,4-dichlorophenol did not enhance breast cancer cell growth.

In an *in vitro* cell proliferation assay with MCF-7 cells (human breast tumour cells), a weak concentration-dependent proliferation of the tumour cells was reported in the range 10^{-1} to 10^{-6} mM (0.000163 – 16.3 mg l^{-1}) after 6 days exposure (Jones *et al* 1998).

Körner *et al* (1998) assessed the effects of 2,4-dichlorophenol on oestrogenic activity via the E-Screen (which utilises the MCF-7 cell line). The study indicated that 2,4-dichlorophenol did not show any oestrogenic activity up to exposure concentrations of 0.1 mM (16.3 mg l^{-1}).

Summary of *in vitro* data

Table 7.3 summarises the available *in vitro* data for 2,4-dichlorophenol which only relates to *in vitro* assays assessing oestrogenic mechanisms of action in mammalian cells and tissues. The data indicates an absence of induction of oestrogen-sensitive gene products and no or weak binding of 2,4-dichlorophenol to the human oestrogen receptor. Exposure of human mammary tumour cells to 2,4-dichlorophenol results in a weak induction of cell proliferation. No data has been identified on the androgenic and anti-androgenic effects of 2,4-dichlorophenol and effects on thyroid function and hormone synthesis and secretion and steroidogenesis in mammalian cells and tissues.

Seyler *et al* (1984) investigated the effects of 2,4-dichlorophenol on *in vitro* fertilisation. Using the oocytes from superovulated female CB6F1 mice aged 6-8 weeks and sperm from male CD-1 mice with proven fertility, *in vitro* fertilisation was performed in medium containing 2,4-dichlorophenol at concentrations of 0, 0.1, 0.3 and 1.0 mM (0, 16.3, 48.9 and 163 mg l^{-1}). In the groups treated with 2,4-dichlorophenol there was no effect on sperm motility and sperm penetration rate into the oocytes. In another *in vitro* fertilisation test, male CD-1 mice were treated with 2,4-dichlorophenol in drinking water at doses of 0, 50, 150 and 500 mg kg^{-1} day^{-1} for 90 days and the sperm from these males was collected and fertilised in medium with the oocytes from untreated and superovulated females. In this experiment, 2,4-dichlorophenol is reported to have no effect on the sperm motility or fertilisation rate.

Table 7.3 Summary of the *in vitro* data in isolated mammalian cells and tissues relating to different mechanisms of action of 2,4-dichlorophenol

Mechanism of endocrine disruption	Responses observed in *in vitro* systems
Oestrogenicity/anti-oestrogenicity	Data indicates an absence of induction of oestrogen-sensitive gene products and no or weak binding of 2,4-dichlorophenol to the human oestrogen receptor. Exposure of human mammary tumour cells to 2,4-dichlorophenol results in no or weak induction of cell proliferation.
Androgenicity/anti-androgenicity	No data identified
Thyroid effects	No data identified
Effects on hormone synthesis or secretion	No data identified
Effects on steroidogenesis	No data identified

7.4.1.2 In vivo studies

Tables 7.4. and 7.5 summarise the information on potential endocrine mediated responses in laboratory mammals following oral exposure (Table 7.4) and sub-cutaneous injection (Table 7.5) to 2,4-dichlorophenol.

A. Effects on endocrine glands and hormone sensitive tissues

In a Uterotrophic assay, ovariectomised 8-week-old Wistar Hannover rats were treated orally with 2,4-DCP alone at doses of 0, 100, 200 and 400 mg kg^{-1} day^{-1} (to detect the oestrogenic effect) or in combination with 17α-ethinylestradiol subcutaneously at 0.5 μg kg^{-1} day^{-1} (to detect the anti-oestrogenic effect) for 3 days, but no treatment-related abnormal changes were observed in the uterine weight in either group (CERI 2001b) indicating that 2,4-dichlorophenol did not cause oestrogenic or anti-oestrogenic effects at the doses tested.

In a Hershberger assay[1] (carried out in accordance with the OECD Draft Guidelines) castrated 8-week-old Wistar Hannover rats were treated orally with 2,4-dichlorophenol alone at doses of 0, 50, 100 and 200 mg kg^{-1} day^{-1} or in combination with testosterone propionate sub-cutaneously at 0.4 mg kg^{-1} day^{-1} for 10 days. However, no treatment-related abnormal changes were observed in the weights of any male accessory reproductive organs in either group (CERI 2001b), indicating that 2,4-dichlorophenol did not cause androgenic or anti-androgenic effects at the doses tested.

B. Reproduction and fertility studies

In a one generation study in SD rats (Exon *et al* 1984, Exon and Koller 1985), 2,4-dichlorophenol (purity 99%) was administered in drinking water to 3-week-old females (10 females per group) at concentrations of 0, 3, 30, and 300 ppm (corresponding to 0, 0.5, 5 and 50 mg kg^{-1} day^{-1}). Administration was continued during mating with untreated males at the age of 13 weeks through gestation and lactation periods until weaning. The offspring were weaned at the age of three weeks and treated with 2,4-dichlorophenol in drinking water until the age of 12 weeks. The parameters of reproduction recorded included percent conception, litter size, number of still-born, birth and weaning weight and survival to weaning. The study was performed in a way which was consistent with OECD Test Guideline 415 (except that each dose group contained less than 50 animals of each sex) but was not carried out to GLP.

Exposure of pregnant female rats to levels of 3 to 300 ppm 2,4 dichlorophenol had no significant effects on reproduction parameters such as conception, litter size and weight, number of still-born pups, or survival to weaning. Although there was a dose-dependent trend toward an increase in body weight at weaning age, this effect was probably more related to a non-significant smaller litter size and was not evident, after 6 weeks of age. The number of still-born pups also tended to be higher, but not significantly, in 2,4-dichlorophenol litters.

It was postulated that chlorinated phenols, or some metabolite of these chemicals, are able to cross the placenta and are toxic to the developing foetus. The toxic effects of substances such as 2,4-dichlorophenol could be related to their effects on oxidative phosphorylation at a time during organogenesis of foetal tissues when maximum production of cellular energy is required.

[1] The Hershberger assay is designed to screen for androgenic and anti-androgenic effects

In the offspring in the 300 ppm group, the spleen and liver weights increased and, haematologically, increases in the red blood cells and haemoglobin were observed. In this study, the liver and spleen showed no histopathological changes despite the increases in their weights and hyperplasia of these organs is suspected to be responsible for their increased weight. Prolonged exposure to 2,4-dichlorophenol was also shown to alter major immune function in rats, although the mechanism of immune dysfunction was not apparent. Antibody levels to Keyhole Limpet Haemocyanin (KLH) in the serum of rats exposed to 2,4-dichlorophenol were consistently greater than the controls and were significantly elevated in the 300 ppm group. The Delayed Type Hypersensitivity (DTH) responses in the 30 and 300 ppm exposed rats were significantly depressed compared to the controls.

Borzelleca *et al* (1985) conducted a one generation reproduction study in CD-1 mice in which animals were exposed to 0, 50, 150 and 500 mg kg body weight^{-1} day^{-1} via drinking water. An absolute control (de-ionised water) and a vehicle control (10% Emulphor) were used. The 2,4-DCP was administered in water containing 21% Emulphor (which was intended to enhance the solubility and palatability of the substance). Administration occurred in males and females for 90 days prior to mating then during mating and the gestation period. After 90 days 10 males and 10 females per treatment were mated. The test was terminated 18 days after mating when all females were sacrificed and the following parameters were measured or calculated: total implants, total resorptions, total number of live pups, weight of individual pups and fertility index. Pups were not examined for histopathological parameters. The study was carried out in a manner consistent to OECD Test Guideline 415 but was not carried out to GLP.

No significant effects were observed on any reproductive parameters at any test dose. The only effect observed was an increase in the resorption rate at 150 mg kg body weight^{-1} day^{-1} but this change was not statistically significant.

C. Developmental and teratogenicity studies

NTIS (1968) reported on a study in which 2,4-dichlorophenol was administered sub-cutaneously at 74 mg kg^{-1} (dissolved in DMSO) to pregnant female C57BL/6 and AKR mice (6 dams per group) on gestation days 6-14 and 6-15 respectively. The dams were caesarean sectioned on gestation days 18 (C57BL/6) and 19 (AKR). Foetal mortality increased in the C57BL/6 mice and in the AKR mice. The 2,4-dichlorophenol-related toxicities included a decrease in the relative liver weight in dams, a decrease in the foetal body weight, excessive extension of four limbs in 4 of 40 foetuses (untreated control group: 6 of 251 foetuses, DMSO group: 1 of 229 foetuses), cystic kidney (1 foetus), short limb (2 foetuses) and thenar dysplasia (1 foetus).

In a teratogenicity study which was carried out to a procedure consistent with OECD Test Guideline 414 but not to GLP, 2,4-dichlorophenol (purity 99.2%) was administered by oral gavage to female F344 rats (34 females) at doses of 0, 200, 375 and 750 mg kg^{-1} day^{-1} (in corn oil) on gestation days 6-15. Dams were caesarian-sectioned on gestation day 20. In the 200 mg kg^{-1} day^{-1} or higher groups, a suppression of the body weight gain and soiling of the external genitalia were observed in dams. In the 750 mg kg^{-1} day^{-1} group, the maternal toxicities included alopecia, abnormal respiratory sound, adhesion of a blood-like substance around the eye, nostrils and mouth and death (4 of 34 dams) and delayed ossification of the sternebrae and vertebral arches were observed in foetuses. It was concluded that 2,4-dichlorophenol has no teratogenic potential but caused delayed foetal development secondary to the maternal toxicities at 750 mg kg^{-1} day^{-1} (Rodwell *et al* 1989).

D. Carcingenicity and oncogenicity studies

In a study of Sutter mice (8-12 weeks of age) by topical application, 25 µl of 0.3% DMBA (dimethylbenz anthracene) (DMBA: 75µg) was topically applied to the back skin of the mice for 1 week, followed by the topical application of 25 µl of 20% 2,4-dichlorophenol (corresponding to 5 mg per mouse) to the back skin twice weekly for 15-24 weeks. The development of papilloma was observed at the application site in 13 of 27 (48%) and 12 of 16 (75%) mice at weeks 15 and 24 of application respectively (control group at week 24 of application: 3 of 27 mice or 11%). At week 24 of application, a skin cancer was confirmed at the application site in one mouse (Boutwell and Bosch 1959).

2,4-dichlorophenol was administered in drinking water to female SD rats from 3 weeks of age at the concentrations of 0, 3, 30 and 300 ppm. After mating at the age of 13 weeks, the females received EU (ethyl urea), which is a precursor of ENU (ethyl nitrosurea), as an initiator and nitrogen dioxide in drinking water together with 2,4-dichlorophenol at concentrations of 0.15% and 1ppm respectively, on gestation days 14 through 21. Then the weanlings were either treated or not treated with 2,4-dichlorophenol in drinking water. In a third experiment, dams were treated with the initiator alone in drinking water on gestation days 14-21 and the pups with 2,4-dichlorophenol in drinking water at concentrations of 0, 3, 30 and 300 ppm. To evaluate the tumour-promoting action of 2,4-DCP, the results of these experiments were compared with the values in the control group which received the initiator alone. 2,4-dichlorophenol exhibited no tumour-promoting action on the ENU-initiated cells (Exon and Koller 1985).

In a 2-year carcinogenicity study in F344/N rats 2,4-dichlorophenol (purity $\geq$99%) was administered in the diet to males at dietary concentrations of 0, 5000 and 10000 ppm (corresponding to 0, 210 and 440 mg kg^{-1} day^{-1}) and to females at 0, 2500 and 5000 ppm (corresponding to 0, 120 and 250 mg kg^{-1} day^{-1}). The incidence of mononuclear cell leukaemia decreased in treated males (control group: 62%, 5000 ppm group: 34%, 10000 ppm group: 34%). However, since these values were almost similar to the background incidence in untreated males (36.3%), it was concluded that these decreases are unrelated to 2,4-dichlorophenol exposure (NTP 1989).

In another 2-year carcinogenicity study 2,4-dichlorophenol (purity $\geq$99%) was administered in diet to 8-week-old B6C3F1 mice (50 mice per sex per group) at dietary concentrations of 0, 5000, and 10000 ppm (corresponding to 0, 800 and 1300 mg kg^{-1} day^{-1} in males and to 0, 430 and 820 mg kg^{-1} day^{-1} in females). The body weight gain was suppressed in females in the 10000 ppm group and the incidence of multinuclear hepatocytes increased markedly in males in a dose-dependent manner (control group:11 of 50, 5000 ppm group: 33 of 49, 10000 ppm group: 42 of 48). In females in the treated groups, the incidence of malignant lymphoma decreased (control group: 12 of 50, 5000 ppm group: 6 of 50, 10000 ppm group: 4 of 50). However, it was concluded that this change was not related to 2,4-dichlorophenol since these incidences are within the range of the background data for this strain of mice. One male (1 of 50) in the 10000 ppm group had squamous cell carcinoma of the forestomach which is rare in this strain of mice (control group: 8%). However, since the 2,4-dichlorophenol did not promote hyperplasia of the forestomach, it was concluded that the results showed no evidence of carcinogenicity (NTP 1989).

Table 7.4 Summary of the data on potential endocrine mediated responses in laboratory mammals following oral exposure

Species	Life stage of the test organism at start of test	Exposure route and dose series	Description of endocrine disruption measurement parameter(s) and effect doses	Reference	Test Relevance	Study validity
Rat (Sprague-Dawley)	3 week old females	Daily in drinking water at 0, 3, 30 and 300 ppm (0, 0.5, 5 and 50 mg kg body weight^{-1} day^{-1}) for 16 weeks	No significant effects (relative to the controls) on reproductive parameters at any test dose *(NOEL for effects on reproduction = 300ppm (50 mg kg body weight^{-1} day^{-1})*	Exon *et al* (1984), Exon and Koller (1985)	Medium	Valid
Rat (F344)	Pregnant females	Oral gavage at 0, 200, 375 and 750 mg kg body weight^{-1} day^{-1} on gestation days 6 to 15	No significant effects (relative to the controls) on embryo development at any test dose but delayed foetal development at highest test dose secondary to maternal toxicity *(NOEL for teratogenicity = 750 mg kg body weight^{-1} day^{-1})*	Rodwell *et al* (1989)	Medium	Valid
Rats (Wistar-Hannover)	Ovariectomised females (operation at 6 weeks of age)	Oral at doses of 0, 100, 200 and 400 mg kg body weight^{-1} for 3 consecutive days	No significant effects (relative to the controls) in the weights or histopathology of the uterus at any test dose *(NOEL for effects on hormone sensitive tissues = 400 mg kg body weight^{-1})*	CERI (2001b)	Medium	Valid
Rats (Wistar-Hannover	Castrated males (operation at 6 weeks of age)	Oral at doses of 0, 50, 100 and 200 mg kg^{-1} body weight^{-1} for 10 consecutive days	No significant effects (relative to the controls) in the weights or histopathology of male reproductive organs at any test dose *(NOEL for effects on hormone sensitive tissues = 200 mg kg body weight^{-1})*	CERI (2001b)	Medium	Valid
Mice (CD-1)	Males and females	Daily in drinking water at 0, 50 , 150 and 500 mg kg body weight^{-1} day^{-1}	No significant effects (relative to the controls) on reproductive parameters at any test dose *(NOEL for effects on reproduction = 500 mg kg body weight^{-1} day^{-1})*	Borzelleca *et al* (1985)	Medium	Valid

Table 7.5 Summary of the data on potential endocrine mediated responses in laboratory mammals following sub-cutaneous injection

Species	Life stage of the test organism at start of test	Exposure route and dose series	Description of endocrine disruption measurement parameter(s) and effect doses	Reference	Test Relevance	Study validity
Mice (C57BL/6)	Pregnant females	Exposure by sub-cutaneous injection at 74 mg kg^{-1} (dissolved in DMSO) on gestation days 6 to 14	No significant effects (relative to the controls) on embryo and foetal development at any test dose *(NOEL for embryo and foetal development = 74 mg kg^{-1} body $weight^{-1}$)*	NTIS (1968)	Medium	Use with care
Mice (AKR)	Pregnant females	Exposure by sub-cutaneous injection at 74 mg kg^{-1} (dissolved in DMSO) on gestation days 6 to 15	No significant effects (relative to the controls) on embryo and foetal development at any test dose *(NOEL for embryo and foetal development = 74 mg kg^{-1} body $weight^{-1}$)*	NTIS (1968)	Medium	Use with care

E. General conclusions on the potential endocrine mediated responses in laboratory mammals in in vivo studies

A series of oral and sub-cutaneous exposure studies (see Table 7.6) have been conducted on 2,4-dichlorophenol. One generation reproduction studies with 2,4-dichlorophenol (which were consistent with OECD Test Guideline 415 but were not carried out to GLP) did not result in effects on reproductive parameters even at the highest test doses of 300 ppm (50 mg kg body weight^{-1} day^{-1}) for rats (Exon *et al* 1984, Exon and Koller 1985) or 500 mg kg body weight^{-1} day^{-1} for mice (Borzelleca *et al* 1985). However, a multi-generation reproductive toxicity study of definitive significance has not yet been performed and the effects of this compound on the reproductive performance and development of subsequent generations of animals remains unknown. A multi-generation reproduction study is currently being conducted in Japan by MITI which will address the uncertainties resulting from the available data.

In the studies considering development and teratogenicity, 2,4-dichlorophenol has been reported to have toxic effects on foetuses secondary to the maternal toxicities, for example decreases in the litter size and increases in the organ weights.

The results of the *in vivo* studies screening including the Uterotrophic assay and rodent Hershberger assay indicated that 2,4-dichlorophenol did not cause sex hormone receptor-mediated endocrine disrupting effects at the dose tested.

Table 7.6 Summary of the potential endocrine mediated responses reported in *in vivo* studies with laboratory mammals

Type of study	Species and exposure route used	Dose series used	NOEL (mg kg body weight^{-1} day^{-1})		Reference
			Potential endocrine mediated responses	Systemic toxicity	
Sub-chronic oral toxicity (OECD 408)	-	-	-	-	-
Reproduction – One generation (OECD 415)	Rat (Sprague-Dawley)	0, 3, 30 and 300 ppm in drinking water (0, 0.5, 5 and 50 mg kg body weight^{-1} day^{-1}	50 (300ppm) (Reproduction)	No data given in study	Exon *et al* (1984), Exon and Koller (1985)
	Mice (CD-1)	0, 50, 150 and 500 mg kg body weight^{-1} day^{-1} in drinking water	50 (300ppm) (Reproduction)	No data given in study	Borzelleca *et al* (1985)
Reproduction – Two generation (OECD 416)	No data	-	-	-	-
Development/ Teratogenicity (OECD 414)	Rat (F344)	0, 200, 375 and 750 mg kg body weight^{-1} day^{-1}	750 (Embryo and foetal development)	No data given in study	Rodwell *et al* (1989)
	Mice (C57BL/6)	0, 74 mg kg body weight^{-1} day^{-1}	74 (Embryo and foetal development)	No data given in study	NTIS (1968)
	Mice (AKR)	0, 74 mg kg body weight^{-1} day^{-1}	74 (Embryo and foetal development)	No data given in study	

Studies in rats and mice (NTP 1989) showed no evidence of carcinogenic activity of 2,4-dichlorophenol. These data are consistent with the conclusions of IARC that although polychlorophenols and their salts are classified in group 2B there is evidence suggesting a lack of carcinogenicity of 2,4-DCP in experimental animals (IARC 1999).

7.4.1.3 Human studies

The acute symptoms of 2,4-dichlorophenol gastrointestinal toxicities include burning sensation in the mouth, white necrosis of the oral and upper gastrointestinal mucosae, abdominal pain, vomiting and diarrhoea and those of the neurological toxicities include pallor, sweating, debilitation, headache, vertigo, tinnitus and urinary incontinence. The toxicities of the cardiovascular system include slight arrhythmia, hypertension, cyanosis and marked body temperature changes and those of the respiratory system include rales, frothing in the nose and mouth and pulmonary oedema. Brown urine and renal failure are the acute symptoms of renal toxicities and, as the haematological toxicities, methemoglobinemia, haemolytic Heinz body anaemia and hyperbilirubinemia have been reported, 2,4-dichlorophenol may cause death due to respiratory, circulatory or heart failure (HSDB 2001).

In 1998 a male worker who was sprayed with hot pressurised steam containing 2,4-dichlorophenol (amount and purity are unknown) fell unconscious and died 1 hour later. The skin contamination involved his forearm, knee, thigh and face and 2,4-dichlorophenol was detected in blood and urine at concentrations of 13.1 and 6.2 mg l^{-1}, respectively (US EPA 2000). When a worker was splattered with an almost 100% pure molten 2,4-dichlorophenol over his right thigh through right arm (less than 10% of the body surface) an epileptic seizure occurred within 20 minutes and the worker subsequently died. The blood, urine, bile and gastric 2,4-dichlorophenol concentrations were 24.3, 5.3, 18.7 and 1.2 mg l^{-1} respectively (Kintz *et al* 1992). Accidental skin exposure to 2,4-DCP has also been linked with the deaths of two other workers since 1980 (US EPA 2000).

These cases indicate that molten or hot 2,4-dichlorophenol is immediately absorbed through the skin in amounts which are lethal for humans unless the skin areas are immediately decontaminated by washing with water. The skin exposure to molten 2,4-dichlorophenol in particular was shown to be fatal even if it involves as little as 1% of the body surface area. The US EPA together with OSHA issued a warning of CANPR (Chemical Advisory and Notice of Potential Risk) for 2,4-dichlorophenol (US EPA 2000).

7.4.2 Studies relevant to the assessment of potential endocrine disrupting effects in wildlife

7.4.2.1 In vitro studies

Jobling *et al* (1995) used an initial screening assay to investigate the direct binding of 2,4-dichlorophenol to the rainbow trout oestrogen receptor. The assay used a cytosoloic liver extract and cells were exposed to a saturated concentration of 5 nM 17β-oestradiol alone and with 2,4-dichlorophenol concentrations up to 1 mM (163 mg l^{-1}). Reduced binding of tritiated 17β-oestradiol was reported, although it was not evident whether this inhibitiory effects was due to direct competition.

7.4.2.2 *In vivo* studies

A. Studies on aquatic organisms

A series of 14 - 21 day water flea (*Daphnia magna)* reproduction studies have been conducted to investigate the effects of 2,4-dichlorophenol on juvenile production (Gersich and Milazzo 1988, Shigeoka *et al* 1988, Kuhn *et al* 1989).

Gersich and Milazzo (1988) conducted a 21-day life cycle toxicity study with *Daphnia magna* according to OECD Guideline 211 under static renewal conditions with test medium replacement every Monday, Wednesday and Friday. Exposure levels ranged from 0.38 to 6.0 mg l^{-1} based on measured concentrations and each test concentration and the controls had four replicates resulting in twenty daphnids being exposed to each concentration. After 21 days exposure at 0.74 mg l^{-1} no effects (relative to the controls) were evident while at 1.48 mg l^{-1} 15 % mortality and a 57 % and 41% reduction in young per adult and brood size per adult was observed. At 2.96 mg l^{-1} 95 % of the daphnids died and no offspring were produced while at an exposure concentration of 5.94 mg l^{-1} all the daphnids died. Statistical analysis after a 21 day exposure period indicated that reproduction of *Daphnia magna* was significantly different from the controls in the mean measured concentration of 1.48 mg l^{-1} (Lowest Observed Effect Concentration), while no effects (relative to the controls) were evident at 0.74 mg l^{-1} (No Observed Effect Concentration).

Shigeoka *et al* (1988) reported a MATC for the total number of *Daphnia magna* offspring produced of 0.55 mg l^{-1} after 14 days exposure. Kuhn *et al* (1989) reported a NOEC for *Daphnia magna* reproduction of 0.21 mg l^{-1} after 21 days exposure.

In all the studies reported there is no information on the mechanism of action. The effects observed in the *Daphnia magna* reproduction tests are probably not caused by direct oestrogenic effects since other studies have shown an absence of reproductive impairment at 0.39 mg l^{-1} when animals are exposed to the synthetic steroid 17α-ethinylestradiol (Schweinfurth *et al* 1986).

B. Studies on terrestrial organisms

A 34 day chronic toxicity test with 10 – 12 day old springtails (*Folsomia candida)* was conducted in artificial soil (pH 5.9 – 6.4, humidity: 40 – 50 % of the maximal water capacity and organic content of soil 1.21%) according to ISO Guideline 11267. After 34 days exposure an EC_{10} value of 3.8 mg kg dw^{-1}, an EC_{50} value of 7.1 mg kg soil dw^{-1} and an EC_{90} value of 13.3 mg kg dry $weight^{-1}$ were reported for reproduction rate (Rombke *et al* 1995). However, there is no information on the mechanism of action.

C. Studies on aerial organisms

No data has been located on the potential endocrine disrupting effects of 2,4-dichlorophenol on aerial organisms. However, given that 2,4-dichlorophenol is not volatile (see Section 7.1) the absence of data on aerial organisms does not represent a key area of uncertainty with regard to the potential endocrine effects of the substance. It should also be recognised that there are currently no internationally agreed methods specifically developed to assess endocrine disrupting effects in aerial organisms.

D. General conclusions on potential endocrine mediated responses in in vivo studies with wildlife species

The data that has been located on the potential endocrine disrupting effects of 2,4-dichlorophenol on wildlife (see Table 7.7) is limited to studies on effects on the reproduction on the water flea *Daphnia magna* and a study on the reproduction of terrestrial springtails (*Folsomia candida)* (see Table 7.7). There is uncertainty due to an absence of data for wildlife species particularly in relation to reproduction and development in fish.

7.5 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity

The general toxicity data in this section has largely been obtained from the IUCLID data set for 2,4-dichlorophenol (IUCLID 2002) and has been taken as accurate. Individual source documents have not been checked unless they are considered to be key studies which have a major influence on the outcome of the review. All information taken from IUCLID as been referenced as being from that source and individual references have not been given in the references.

7.5.1 Studies relevant to the assessment of general toxicity in humans

Table 7.8 summarises the general toxicity data from acute and repeat does studies with 2,4-dichlorophenol.

7.5.1.1 Acute studies

A. Oral exposure

2,4-dichlorophenol is of moderate acute oral toxicity to mammals with median lethal dose (LD_{50}) values in rats ranging from 580 to 4500 mg kg body weight^{-1} day^{-1} (Deichmann 1943, Kobayashi *et al* 1972, Vernot *et al* 1977) and in mice ranging from 1276 to 1630 mg kg body weight^{-1} day^{-1} (Kobayashi *et al* 1972, Borzelleca *et al* 1985).

B. Dermal exposure

The acute dermal toxicity of 2,4-dichlorophenol as LD_{50} values ranges from 780 mg kg body weight^{-1} day^{-1} for rats (Rhone Poulenc 1992) to 3100 mg kg body weight^{-1} day^{-1} (Allen *et al* 1979).

C. Inhalation exposure

The only acute inhalation toxicity study for 2,4-dichlorophenol resulted in a 4 hour LC_{50} value of 0.97 mg l^{-1} in Sprague-Dawley rats (Rhone Poluenc 1980).

D. Other routes of exposure

In studies by Farquharson *et al* (1958) and Biagi *et al* (1975) single intra-peritoneal injection of 2,4-dichlorophenol in male albino rats and mice resulted in a LD_{50} values of 430 and 163 mg kg body weight^{-1} day^{-1} respectively. Deichmann (1943) reported an LD_{50} value of 1730 mg kg body weight^{-1} day^{-1} in rats following sub-cutaneous injection. However, all these data are questionable due to issues of experimental design.

Table 7.7 Summary of the potential endocrine mediated responses in wildlife

Environmental compartment	Taxonomic group	Type of study	Species and exposure route used	Concentration series used	Lowest reported NOEC	Reference
Aquatic	Amphibians	No data	-	-	-	-
	Fish	No data	-	-	-	-
	Invertebrates	Reproduction (OECD TG 211)	Water flea (*Daphnia magna*) –aqueous exposure	No data	0.21 mg l^{-1} (a)	Kuhn *et al* (1989)
Terrestrial	Birds	No data	-	-	-	-
	Invertebrates	Reproduction (ISO 11267)	Springtails (*Folsomia candida*) – soil exposure	No data	3.8 mg kg dry $weight^{-1}$ (EC_{10}) (a)	Rombke *et al* (1995)
Aerial	Invertebrates	No data	-	-	-	-

a – No information is available on the mechanism of action

7.5.1.2 Repeat dose studies

A. Oral exposure

Kobayashi *et al* (1972) carried out a six-month dosed-feed study of 2,4-DCP in ICR mice (7 males per group) using the dietary concentrations of 0, 0.02, 0.05, 0.1 and 0.2% (corresponding to 18, 45, 100 and 230 mg kg^{-1} day^{-1}). In the study the relative liver weight decreased in the 230 mg kg^{-1} day^{-1} group, with hepatocellular swelling in one, small round cell infiltration in the interstitium in two and thinning of the adrenal cortex in two males. The data resulted in a No Observed Adverse Effect Level (NOAEL) of 100 mg kg^{-1} day^{-1} for 2,4-dichlorophenol.

In a two-year dosed-feed study of 2,4-dichlorophenol (purity 99% or more) in F344 rats using the dietary concentrations of 0, 5000 and 10000 ppm (corresponding to 0, 210 and 400 mg kg^{-1} day^{-1}) for females, 2,4-dichlorophenol had no effect on the survival rate at any dose levels but caused a suppression of the body weight gain in both sexes in the high dose group. In males in the 2,4-dichlorophenol groups, the incidence of diffuse degeneration of the respiratory epithelium tended to increase (control group: 35 of 45, 5000 ppm group: 38 of 48, 10000 ppm group:42 of 46) (NTP 1989).

In a 13-week dosed-feed study of 2,4-DCP (purity $\geq$99%) in F344 rats (10 rats per sex per group) using the dietary concentrations of 0, 2500, 5000, 10000, 20000 and 40000 ppm, atrophy of the bone marrow and marked decreases in the erythrocytes and myelocytes were observed in 6 of 10 females in the 10000 ppm group and all rats in the 20000 ppm or higher groups. In addition a suppression of the body weight gain, hunchback posture, rough hair coat and a decrease in the food consumption in the 40000 ppm group was observed (NTP 1989). The NOAEL was estimated to be 10000 ppm (corresponding to 1000 mg kg^{-1} day^{-1}) for males and 5000 ppm (corresponding to 500 mg kg^{-1} day^{-1}) for females under the conditions tested.

In a 4-week dosed-feed study of 2,4-dichlorophenol in F344 rats (5 rats per sex per group) using the dietary concentrations of 0, 200, 1000, 5000 and 20000 ppm (corresponding to 0, 20, 101, 493 and 1,782 mg kg^{-1} day^{-1}) (OECD TG407), a suppression of the body weight gain, an increase in γ-GTP activity and a prolongation of the clotting time were observed in both sexes in the 20,000 ppm group (BUA 1996).

A 13-week dosed-feed study of 2,4-dichlorophenol (purity $\geq$99%) in B6C3F1 mice (10 mice per sex per group) was carried out using dietary concentrations of 0, 2500, 5000, 10000, 20,000 and 40000 ppm, 2,4-dichlorophenol caused rough hair coat in both sexes and appearance of multinuclear hepatocytes in males at 10000 ppm or above, suppression of the body weight gain and a decrease in the food consumption at 20000 ppm with cellular necrosis in all males and death of all mice within 3 weeks and epithelial necrosis of the urinary tubules at 40000 ppm (NTP 1989).

When 2,4-DCP was administered in the diet to B6C3F1 mice (50 mice per sex per group) at the dietary concentrations of 0, 5000, and 10000 ppm (males; 0, 800 and 1,300 mg kg^{-1} day^{-1}, females 0, 430 and 820 mg kg^{-1} day^{-1}) for two years, the body weight gain was suppressed in the 10000 ppm group and the incidence of multinuclear hepatocytes increased dose-dependently in males (control group: 11 of 50, 5000 ppm group: 33 of 49, 10000 ppm group: 42 of 48) (NTP 1989).

Table 7.8 Summary of general mammalian toxicity data (Information from IUCLID 2002)

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect dose	Reference	Study validity
Acute Oral Toxicity	Rat	Not relevant	No data	Median lethal dose (LD_{50})	580 mg kg^{-1} body $weight^{-1}$	Deichmann (1943)[1]	Invalid[2]
	Rat	Not relevant	0, 2000, 2250 and 3000 mg kg^{-1}	Median lethal dose (LD_{50})	3670 mg kg^{-1} body $weight^{-1}$ (males) 4500 mg kg^{-1} body $weight^{-1}$ (females)	Kobayashi *et al* (1972) [1]	Use with care[2]
	Rat	Not relevant	0, 100, 1000 and 10000 mg kg^{-1}	Median lethal dose (LD_{50})	2830 mg kg^{-1} body $weight^{-1}$	Vernot *et al* (1977) [1]	Valid[2]
	Mouse (Males and Females)	Not relevant	0, 667, 1000, 1500 and 2250 mg kg^{-1}	Median lethal dose (LD_{50})	1630 mg kg^{-1} body $weight^{-1}$ (males) 1630 mg kg^{-1} body $weight^{-1}$ (females)	Kobayashi *et al* (1972) [1]	Use with care[2]
	Mouse (CS1 Males and Females, six weeks old)	Not relevant	10 ml kg^{-1}	Median lethal dose (LD_{50})	1276 mg kg^{-1} body $weight^{-1}$ (males) 1352 mg kg^{-1} body $weight^{-1}$ (females)	Borzelleca *et al* (1985) [1]	Valid[2]
Acute Dermal Toxicity	Rat (Males and Females)	2 weeks	0, 200, 300, 1400 and 2000 mg kg^{-1} (Males) 0, 200 and 2000 mg kg^{-1} (Females)	Median lethal dose (LD_{50})	780 mg kg^{-1} body $weight^{-1}$	Rhone Poulenc (1992) [1]	Valid[2]
	Mouse	Not relevant	No data	Median lethal dose (LD_{50})	3100 mg kg^{-1} body $weight^{-1}$	Allen *et al* (1979) [1]	Use with care[2]

Table 7.8 Continued

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect dose	Reference	Study validity
Acute Inhalation Toxicity	Rat (Sprague-Dawley strain)	4 hours	0, 0.77, 0.84, 0.97, 1.07 and 1.13 mg l^{-1}	LC_{50}	0.97 mg l^{-1}	Rhone Poulenc (1980) [1]	Valid[2]
Acute Toxicity (Intra-peritoneal injection)	Rat (Male Albino)	No data	10 ml kg^{-1}	Median lethal dose (LD_{50})	430 mg kg^{-1} body $weight^{-1}$	Farquharson *et al* (1958) [1]	Invalid[2]
	Mouse	No data	No data	Median lethal dose (LD_{50})	163 mg kg^{-1} body $weight^{-1}$	Biagi *et al* (1975) [1]	Invalid[2]
Acute Toxicity (Sub-cutaneous injection)	Rat	No data	No data	Median lethal dose (LD_{50})	1730 mg kg^{-1} body $weight^{-1}$	Deichmann (1943) [1]	Invalid[2]
Repeated Dose Toxicity (Oral)	Rat (Males and females)	90 days	0, 2500, 5000, 10000, 20000 and 40000 ppm	NOAEL (female) LOAEL (female)	5000ppm (400 mg kg^{-1}) 10000 ppm (800 mg kg^{-1})	NTP (1989) [1]	Valid[2]
	Mouse (ICR)	6 months	0, 0.02, 0.05, 0.1 and 0.2% (0, 18, 45, 100 and 230 mg kg^{-1})	NOAEL LOAEL	1000 mg kg^{-1} 230 mg kg^{-1}	Kobayashi *et al* (1972) [1]	Valid[2]
	Mouse (CD-1 Males and Females)	90 days	0, 200, 600 and 200 mg l^{-1}	NOAEL (males)	>2000 mg l^{-1} (>383 mg kg^{-1})	Borzelleca *et al* (1985) [1]	Valid[2]
	Mouse (B6C3F1 Males and Females)	90 days	0, 2500, 5000, 10000, 20000 and 40000 ppm	NOAEL	<2500 ppm (<750 mg kg^{-1})	NTP (1989) [1]	Valid[2]

[1] – Cited in IUCLID (2002), [2] – Assessment made on basis of data in IUCLID (2002)

B. Dermal exposure

No data has been located on the repeat dose toxicity of 2,4-dichloropehnol to laboratory mammals following dermal exposure.

C. Inhalation exposure

No data has been located on the repeat dose toxicity of 2,4-dichlorophenol to laboratory mammals following inhalation exposure.

D. Other routes of exposure

No data has been located on the repeat dose toxicity of 2,4-dichlorophenol to laboratory mammals following exposure by sub-cutaneous or intra-peritoneal injection.

7.5.1.3 Comparison of data from studies assessing potential endocrine disrupting effects and general toxicity in mammals

The lowest NO(A)EL identified from the review of data on potentially endocrine mediated responses in laboratory mammals was 50 mg kg body weight^{-1} day^{-1} based on an absence of effects on reproductive parameters in Sprague-Dawley rats even at the highest test dose of 300 ppm (50 mg kg body weight^{-1} day^{-1}) (Exon *et al* 1984, Exon and Koller 1985). In developmental/teratogenicity studies (NTIS 1968, Rodwell *et al* 1989) no endocrine mediated responses have been identified even at the highest doses tested (>74 mg kg body weight^{-1} day^{-1}).

In acute and repeat-dose studies the general systemic toxicity data for laboratory mammals indicates that the threshold in rats for an absence of effects which are not directly endocrine mediated occurs at a doses of >100 mg kg body weight^{-1} day^{-1}. As a result it appears that on the basis of the available data that foetuses are markedly more sensitive to 2,4-dichlorophenol than juveniles or adults. However, the mechanism responsible has not been elucidated.

7.5.2 Studies relevant to the assessment of general toxicity in wildlife

7.5.2.1 Studies on aquatic organisms

A. Fish

Table 7.9 summarises all of the toxicity test results found for aquatic organisms exposed to 2,4-dichlorophenol.

Acute toxicity

Data for acute tests with salmonids is limited with Hattula *et al* (1981) reporting a 24 hour LC_{50} of 1.7 mg l^{-1} for brown trout (*Salmo trutta*). Kaiser *et al* (1995) reported a 1 hour LOEC of 0.03 mg l^{-1} for rainbow trout (*Oncorhynchus mykiss*) in a flow-through test. However, the test was not performed according to a standardised method, no information concerning test substance purity was given and the test period was extremely short.

An acute 96-hour LC_{50} value of 7.75 mg l^{-1} has been reported for 30 day old fathead minnow (*Pimephales promelas*) in a flow-through test. The study was carried out according to OECD Guideline 203 “Fish, Acute Toxicity Test” and the test concentrations were measured.

However, while the chemical source is known its purity was not determined (Geiger *et al* 1977).

Phipps *et al* (1981) also reported on a flow-through study with 30 to 35 day old fathead minnow (*Pimephales promelas*) in which an acute 96-hour LC_{50} value of 8.2-8.3 mg l^{-1} and a 192-hour LC_{50} value of 6.5 mg l^{-1} were reported. Five test concentrations and a control all in duplicate, were used and water was cycled in the flow-through studies at a rate sufficient to obtain a renewal of 10 tank volumes a day. Concentrations were measured and the study was carried out according to the US EPA method, but the test substance origin and purity was not specified.

Broderius *et al* (1995) reported an acute 96-hour LC_{50} value of 11.6 mg l^{-1} for 26 to 34 day old fathead minnow (*Pimephales promelas*) in a flow-through test. Exposures were conducted at four or five toxicant concentrations and a control in duplicate and toxicant concentrations were measured daily, but test substance purity was not controlled.

In semi-static tests with the guppy (*Poecilia reticulata*) Konemann and Musch (1981) reported a 24 hour LC_{50} values of 5.9 mg l^{-1} at pH 7.8, 4.2 mg l^{-1} at pH 7.3 and 3.3 mg l^{-1} at pH 6.1. However, test concentrations were not determined during the exposure period and there was no control. Bernot-Guyod *et al* (1984) reported a 24 hour LC_{50} value of 6.8 mg l^{-1} for male 2-3 month old guppy (*Lebistes reticulatus*) in a static test. No analytical confirmation of exposure concentrations was carried out and the test substance was of reagent grade. In addition, no control information is provided.

A series of acute studies were carried out by Kischino and Kobayashi (1995, 1996a,b) using goldfish (*Carracius auratus*) but no confirmation of test concentrations was conducted. A 2.5 hour LC_{50} value of 8.0 mg l^{-1}, 5 hour LC_{50} values of 5-7 mg l^{-1}, 7.8 mg l^{-}, 7–10 mg $l^{-1}$and >100 mg l^{-1} and a 25 hour LC_{50} value of 7.8 mg l^{-1} were reported. However, no control animals were used in the 2.5 hour study, and no information on test purity was available.

In static tests with the bluegill sunfish (*Lepomis macrochirus*) Buccafuso *et al* (1981) reported a 24 hour LC_{50} value of 4.7 mg l^{-1} and a 96 hour LC_{50} value of 2.0 mg l^{-1}. However, the dissolved oxygen concentration after 96 hours dropped to 0.3 mg l^{-1} invalidating the 96 hour value.

An acute 48-hour LC_{50} value of 8.6 mg l^{-1} has been reported for Japanese medaka (*Oryzias latipes)* in a semi-static test. The study was carried out according a Japanese Industrial Standard: JIS K 0102-1986-71 but the test concentrations were not measured (IUCLID 2002). Shigeoka *et al* (1988a) reported a 96 hour LC_{50} of 6.3 mg l^{-1} for Japanese medaka (*Oryzias latipes)*.

An acute 96-hour LC_{50} value of 6.0 mg l^{-1} has been reported for flounder (*Platichthys flesus*) in a semi-static test carried out according to the principles of OECD Guideline 203 “Fish, Acute Toxicity Test (Smith *et al* 1994). However, while no mortality was observed in the control vessels the loading rate of 33.6 g fish l^{-1} greatly exceeded the OECD recommended loading rate of 1 g fish l^{-1}, and flounder are not listed in the OECD recommended species for acute toxicity tests. A further study conducted using the same conditions obtained an LC_{50} result of 6.8 mg l^{-1}.

Smith *et al* (1994) also reported an acute 96-hour LC_{50} value of 5.13 mg l^{-1} for sole (*Solea solea)* in a semi-static test carried out according to the principles of OECD Guideline 203 “Fish, Acute Toxicity Test. Again no mortality was observed in the control vessels but the

loading rate of 27 g fish l^{-1} greatly exceeded the OECD recommended loading rate of 1 g l^{-1}. In addition sole are not listed in the OECD recommended species for acute toxicity tests.

Chronic toxicity

Hodson *et al* (1991) carried out an 85 day flow-through early life stage test with rainbow trout (*Oncorhynchus mykiss*) embryos. The study reported a NOEC (mortality) of 0.1 mg l^{-1}, a LOEC (mortality) of 0.18 mg l^{-1} and a MATC (mortality) of 0.13 mg l^{-1}. The NOEC, LOEC and MATC values for growth and development of the fish were 0.18, 0.32 and 0.24 mg l^{-1} respectively.

Mayes *et al* (1988) reported a 7 day MATC (survival) value of 3.48 mg l^{-1}, and 73% mortality value at 4.85 mg $l^{-1}$for fathead minnow (*Pimephales promelas*) fry (<24 h old at the start of the test). Shigeoka *et al* (1998b) reported a 40 day MATC (mortality) value of 0.32 - 0.63 mg l^{-1} for juvenile Japanese medaka (*Oryzias latipes*).

Holcombe *et al* (1982) carried out an early life-stage test with fathead minnow (*Pimephales promelas*) over a 28 day exposure period. The test was carried out according to OECD Guideline Draft "Early Life Stage Test (ELS Test)" and the toxicant concentrations were measured in all tanks once a week. The reported results were 0.29 mg l^{-1} for NOEC (mortality) and 0.46 mg l^{-1} for LOEC (mortality). The LOEC for inhibition of hatching was >1.24 mg l^{-1} while the NOEC and LOECs for the growth of larval and juvenile stages were 0.77 and 1.24 mg l^{-1}.

B. Invertebrates

Table 7.9 summarises all of the toxicity test results found for 2-4-dichlorophenol to aquatic invertebrates.

Acute toxicity

Only limited toxicity data are available for freshwater invertebrates, mostly for the water flea *Daphnia magna.*

LeBlanc (1980) reported a 24 hour EC_{50} value for *Daphnia magna* of >10 mg l^{-1} and a 48 hour LC_{50} value of 2.6 mg l^{-1} in a test performed according to US-EPA method but the test substance concentrations were not measured. In a further study a 24 hour EC_{50} value of 6.95 mg l^{-1} was reported (LeBlanc *et al* 1988), but no experimental details of this study were provided. Devillers and Chambon (1986) reported a 24 hour EC_{50} value of 2.68 mg l^{-1} for *Daphnia magna* in static tests.

Shigeoka *et al* (1988a) reported 24 hour EC_{50} values of 6.0, 6.6 and 7.0 mg l^{-1} for *Daphnia magna*, *Daphnia pulex* and *Daphnia carcinata* respectively in static tests. Steinburg *et al* (1992) reported a 24 hour EC_{50} value of 2.84 mg l^{-1} for *Daphnia magna* in a static tests. A similar 24 hour LC_{50} value of 2.7 mg l^{-1} was also reported by Beirat (1989).

Zhao *et al* (1998) reported a 24 hour EC_{50} value of 3.25 mg l^{-1} for *Daphnia magna* in a static test. The study was not performed according to standardised guidelines and the test substance concentrations were not measured during the exposure period.

Kuhn *et al* (1989) reported on a study carried out in accordance with procedure DIN 38412 where the purity of the test substance and test substance concentrations were not measured

during the exposure period. In the study after 24 hours an EC_0 value of 1 mg l^{-1}, an EC_{50} of 2.5 mg l^{-1} and an EC_{100} of 5.1 mg l^{-1} were reported whilst after 48 hours an EC_0 value of 0.7 mg l^{-1}, an EC_{50} of 1.4 mg l^{-1} and an EC_{100} of 3.6 mg l^{-1} were reported. Twenty animals were exposed to each concentration and fewer than 10 % daphnids in the control solutions were immobile.

An acute 24 hour EC_{50} value of 2.68 mg l^{-1} to *Daphnia magna* was reported where the test was performed according to the French standard method (NF T90301). The daphnids were <72 hours old (instead of <24 hours old in the OECD Guidelines), 7 concentrations were tested (0.1 – 100 mg l^{-1}) and 20 daphnids were exposed to each concentration. The test substance purity was not controlled and no analytical monitoring was carried out, but the test was carried out in the dark to avoid photodegradation processes (IUCLID 2002). A similar 24 hour EC_{50} value of 2.9 mg l^{-1} was reported for *Daphnia magna* at pH 7.8 (pH 7.8 ± 1), and in the same study EC_{50} values of 2.14 mg l^{-1} at pH 6 (pH 6 ± 1) and 3.4 mg l^{-1} at pH 9 (pH 9 ± 1) were also calculated. The study was conducted in a manner consistent with standardised guidelines but test substance concentrations were not measured (IUCLID 2002).

Rao *et al* (1981) reported a 96 hour EC_{50} value of 2.16-2.55 mg l^{-1} for grass *shrimp* (*Palaemonetes pugio*) (20 ± 1°C, pH 7.6 – 7.7). The average adult length was 25 mm and moult stage was determined. To prevent cannibalism, 20 shrimps were maintained individually in glass jars exposed to each of the five test concentrations and the control media (seawater and ethanol containing seawater). While this test species is not recommended by the OECD Guidelines it is often used as an acute toxicity test to aquatic invertebrates. However, test substance concentrations were not measured during the exposure period.

Smith *et al* (1994) investigated the effects of 2-4-dichlorophenol to 6 day old copepods (*Tisbe battagliai*) and reported a 24 hour EC_{50} value of 16 mg l^{-1} (confidence limits 14.2 – 18.6 mg l^{-1}). A static system was carried out and five concentrations were tested (pH 8 ± 0.1). No test concentrations were determined during exposure but the method used was well described.

Chronic toxicity

Shigeoka *et al* (1988a) reported a MATC for immobility of *Daphnia magna* adults of 1.7 mg l^{-1} in a 14 day study where reproduction was also measured (see Section 7.4.2).

Gersich and Milazzo (1988) conducted a 21-day life cycle toxicity study with *Daphnia magna* according to OECD Guideline 211 under static conditions with test concentrations renewals every Monday, Wednesday and Friday. Exposure levels ranged from 0.38 to 6.0 mg l^{-1} based on measured concentrations and each test concentration and the controls had four replicates resulting in twenty daphnids being exposed to each concentration. After 21 days exposure no effects (relative to the controls) were evident at 0.74 mg l^{-1} while at 1.48 mg l^{-1} 15 % mortality and a 57 % and 41% reduction in young per adult and brood size per adult was observed. At 2.96 mg l^{-1} 95 % of the daphnids died and no offspring were produced while at an exposure concentration of 5.94 mg l^{-1} all the daphnids died.

Tessier *et al* (2000) carried out a 20 day net-spinning behaviour study with *Hydropsyche slossonae* under a dynamic flow system using nominal exposure concentrations (temperature 15 ± 1°C). A LOEC (based on numbers of net spun) was observed at 0.0035 mg l^{-1}. However, the test was not performed according to a standardised protocol though the test conditions are well described.

Table 7.9 Summary of general toxicity data for aquatic organisms (Information from IUCLID 2002)

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study validity
Acute Fish Toxicity	Bluegill sunfish (*Lepomis macrochirus*)	24h	No data	LC_{50}	4.7 mg l^{-1}	Buccafusco *et al (*1981)[1]	Use with care[2]
	Brown trout (*Salmo trutta*)	24h	No data	LC_{50}	1.7 mg l^{-1}	Hattula *et al* (1981) [1]	Use with care[2]
	Fathead minnow *(Pimephales promelas*)	96h	No data	LC_{50}	7.75 mg l^{-1}	Geiger *et al* (1977) [1]	Valid[2]
		96h	No data	LC_{50}	8.2 – 8.3 mg l^{-1}	Phipps *et al* (1981) [1]	Valid[2]
		96h	No data	LC_{50}	11.6 mg l^{-1}	Broderius *et al* (1995) [1]	Valid[2]
	Flounder (*Platichthys flesus*	96h	No data	LC_{50}	6.0 mg l^{-1}	Smith *et al* (1994) [1]	Use with care[2]
	Goldfish *(Carracius auratus*)	2.5h	No data	LC_{50}	8.0 mg l^{-1}	Kischino and Kobayashi (1995, 1996a,b) [1]	Use with care[2]
		5h	No data	LC_{50}	5 - 7, 7 - 10 and > 100 mg l^{-1}		
		5h	No data	LC_{50}	7.8 mg l^{-1}		
		25h	No data	LC_{50}	7.8 mg l^{-1}		
	Guppy (*Lebistes reticulatus*)	24h	No data	LC_{50}	6.8 mg l^{-1}	Benot-Guyod *et al* (1984) [1]	Use with care[2]
	Guppy *(Poecilia reticulata*)	24h	No data	LC_{50}	3.3 - 5.9 mg l^{-1}	Konemann and Musch (1981) [1]	Invalid[2]

Table 7.9 Continued

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study validity
Acute Fish Toxicity	Japanese medaka *(Oryzias latipes)*	96h	No data	LC_{50}	6.3 mg l^{-1}	Shigeoka *et al* (1988a) [1]	Use with care [2]
	Sole (*Solea solea*)	96h	No data	LC_{50}	5.13 mg l^{-1}	Smith *et al* (1994) [1]	Use with care [2]
Chronic Fish Toxicity	Fathead minnow *(Pimephales promelas)*	28 days	0, 0.29, 0.46, 0.77 and 1.24 mg l^{-1}	NOEC (Mortality) LOEC (Mortality)	0.29 mg l^{-1} 0.46 mg l^{-1}	Holcombe *et al* (1982) [1]	Valid [2]
		7 days	No data	MATC (Mortality)	3.48 mg l^{-1}	Mayes *et al* (1988) [1]	Use with care [2]
	Japanese Medaka (*Oryzias latipes*)	40 days	No data	MATC (Mortality)	0.32 – 0.63 mg l^{-1}	Shigeoka et al (1988b) [1]	Use with care [2]
	Rainbow trout (*Oncorhynchus mykiss)*	85 days	0, 0.1, 0.18 and 0.32 mg l^{-1}	NOEC (Mortality) LOEC (Mortality) MATC (Mortality) NOEC (Growth) LOEC (Growth) MATC (Growth)	0.1 mg l^{-1} 0.18 mg l^{-1} 0.13 mg l^{-1} 0.18 mg l^{-1} 0.32 mg l^{-1} 0.24 mg l^{-1}	Hodson *et al* (1991) [1]	Valid [2]
Acute Invertebrate Toxicity	Water flea *(Daphnia carcinata)*	24h	No data	EC_{50} (Immobility)	7.0 mg l^{-1}	Shigeoka *et al* (1988a) [1]	Use with care [2]
	Water flea *(Daphnia magna)*	48h	No data	LC_{50}	2.6 mg l^{-1}	LeBlanc (1980) [1]	Use with care [2]
		24h	No data	EC_{50}	2.68 mg	Devillers and Chambon (1986) [1]	Use with care [2]

Table 7.9 Continued

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study validity
Acute Invertebrate Toxicity	Water flea *(Daphnia magna)*	24h	No data	EC_{50} (immobility)	6.0 mg l^{-1}	Shigeoka *et al* (1988a) [1]	Use with care [2]
		24h	No data	EC_{50}	2.7 mg l^{-1}	Beirat (1989) [1]	Use with care [2]
		48h	No data	EC_0 (Immobility)	0.7 mg l^{-1}	Kuhn *et al* (1989) [1]	Use with care [2]
		48h	No data	EC_{50} (Immobility)	1.4 mg l^{-1}		
		48h	No data	EC_{100} (Immobility)	3.6 mg l^{-1}		
		24h	No data	LC_{50}	6.95 mg l^{-1}	LeBlanc *et al* (1988) [1]	Use with care [2]
		24h	No data	EC_{50} (Immobility)	2.84 mg l^{-1}	Steinburg *et al* (1992) [1]	Use with care [2]
	Water flea *(Daphnia pulex)*	24h	No data	EC_{50} (Immobility)	6.6 mg l^{-1}	Shigeoka *et al* (1998a) [1]	Use with care [2]
	Copepod *(Tisbe battagliai)*	24h	No data	EC_{50}	16 mg l^{-1}	Smith *et al* (1994) [1]	Use with care [2]
	Grass shrimp *(Palaemonetes pugio)*	96h	No data	EC_{50} (lethality)	2.16 – 2.55 mg l^{-1}	Rao *et al* (1981) [1]	Use with care [2]
Chronic Invertebrate Toxicity	Water flea *(Daphnia magna)*	21 days	No data	EC_{95} EC_{100}	2.96 mg l^{-1} 5.94 mg l^{-1}	Gersich and Milazzo (1988) [1]	Valid [2]
		14 days	No data	MATC (Immobility)	1.7 mg l^{-1}	Shigeoka *et al* (1998a) [1]	Use with care [2]
	Hydropsyche slossonae (larvae)	20 days	No data	LOEC (net spinning behaviour)	0.0035 mg l^{-1}	Tessier *et al* (2000) [1]	Use with care [2]

[1] - Cited in IUCLID (2002), [2] – Assessment made on basis of data in IUCLID (2002)

No chronic toxicity data was identified for saltwater species.

7.5.2.2 Studies on terrestrial organisms

Table 7.10 summarises all of the toxicity test results found for 2-4-dichlorophenol to terrestrial organisms.

Acute toxicity

A 24 hour acute toxicity test to Springtails (*Folsomia candida)* was conducted in artificial soil (pH 5.9 – 6.4, humidity: 40 – 50 % of the maximal water capacity and organic content of soil 1.21%) where the exposure concentrations were measured (Rombke *et al* 1995). A 24 hour LC_0 value of 10 mg kg dry weight^{-1}, an LC_{50} of 55 mg kg dry weight^{-1} and an LC_{100} of 100 mg kg dry weight^{-1} were reported.

A 34 day chronic toxicity test with 10 – 12 day old springtails (*Folsomia candida)* was conducted in artificial soil (pH 5.9 – 6.4, humidity: 40 – 50 % of the maximal water capacity and organic content of soil 1.21%) according to ISO Guideline 11267. After 34 days exposure an LC_{10} value of 0.7 mg kg dw^{-1}, an LC_{50} value of 3.5 mg kg soil dw^{-1} and an LC_{90} value of 18.7 mg kg dry weight^{-1} were reported for adult mortality (Rombke *et al* 1995).

A 14 day acute toxicity test to 3 month old Earthworms (*Eisenia fetida*) was conducted in artificial soil according to OECD Guide-line 207 “Earthworm, Acute Toxicity Test”. The test concentration range was 250 – 600 mg kg soil dw^{-1} and ten animals were exposed at each test concentration (temperature 20 ± 2 °C). At 192 mg kg^{-1} dry weight^{-1} no mortality was observed and at 333 mg kg dry weight^{-1} 90% mortality was observed. A 14 day NOEC value of 192 mg kg dry weight^{-1}, an LC_{50} of 253 mg kg^{-1} dry weight^{-1} and an LC_{100} of 577 mg kg dry weight^{-1} were reported (Rombke *et al* 1995).

A 14 day acute toxicity test to 4 – 12 week old Ground Beetle (*Poecilus cupreus*) was conducted in artificial soil (temperature 20-24 °C and humidity: 70 % of the maximal water capacity) where the feeding rate of the beetles was assessed. At a concentration of 5 mg kg^{-1}, the feeding rate of the beetles was reduced by 41% in comparison with the control (Rombke *et al* 1995).

Chronic toxicity

A 34 day chronic toxicity test to 10 – 12 day old Springtails (*Folsomia candida)* was conducted in artificial soil (pH 5.9 – 6.4, humidity: 40 – 50 % of the maximal water capacity and organic content of soil 1.21%) according to ISO Guideline 11267. After 34 days exposure an EC_{10} value of 0.7 mg kg dry weight^{-1}, an EC_{50} value of 3.5 mg kg dry weight^{-1} and an EC_{90} value of 18.7 mg kg dry weight^{-1} were reported for adult mortality (Rombke *et al* 1995).

7.5.2.3 Studies on aerial organisms

No general toxicity data for aerial organisms following exposure to 2,4-dichlorophenol has been located.

Table 7.10 Summary of general toxicity data for terrestrial organisms (Information from IUCLID 2002)

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study validity
Acute Invertebrate Toxicity	Earthworm *(Eisenia fetida)*	2 days	No data	LC_{50}	0.00021 mg kg^{-1}	Callahan *et al* (1994)[1]	Invalid[2]
		14 days	250 – 600 mg kg^{-1}	NOEC LC_{50} LC_{100}	192 mg kg^{-1} soil dry $weight^{-1}$ 253 mg kg^{-1} soil dry $weight^{-1}$ 577 mg kg^{-1} soil dry $weight^{-1}$	Rombke *et al* (1995) [1]	Valid[2]
	Ground beetle *Poecilus cupreus* (carabidan)	14 days	No data	Feeding rate of Calliphora (41 % reduction)	5 mg kg^{-1}	Rombke et al (1995) [1]	Valid[2]
	Springtail *(Folsomia candida)*	24 hours	No data	LC_0 LC_{50} LC_{100}	10 mg kg^{-1} soil dry $weight^{-1}$ 55 mg kg^{-1} soil dry $weight^{-1}$ 100 mg kg^{-1} soil dry $weight^{-1}$	Rombke *et al* (1995) [1]	Valid[2]
Chronic Invertebrate Toxicity	Springtail *(Folsomia candida)*	34 days	No data	Adult mortality LC_{10} LC_{50} LC_{90}	 0.7 mg kg^{-1} soil dry $weight^{-1}$ 3.5 mg kg^{-1} soil dry $weight^{-1}$ 18.7 mg kg^{-1} soil dry $weight^{-1}$	Rombke *et al* (1995) [1]	Valid[2]

[1] – Cited in IUCLID (2002), [2] – Assessment made on basis of data in IUCLID (2002)

7.5.2.4 Comparison of data from studies assessing potential endocrine disrupting effects an/or general toxicity in wildlife

Comparison of the limited data on potential endocrine mediated responses in the aquatic invertebrate *Daphnia magna* with the acute and chronic data for this species indicates that the threshold exposure concentration for mortalities (1.48 – 1.7 mg l^{-1}) was higher than the threshold concentration for effects on reproduction (0.21 mg l^{-1}). However, no comparisons could be made for fish due to the absence of data on endocrine mediated responses in this taxonomic group, which represents an area of uncertainty.

Data for the terrestrial springtail (*Folsomia candida*) indicated that the threshold exposure concentration for mortalities after 34 days exposure to 2,4-dichlorophenol (LC_{10} value of 0.7 mg kg^{-1} dry $weight^{-1}$) was lower than the threshold for reproduction (EC_{10} value of 3.8 mg kg^{-1} dry $weight^{-1}$).

7.6 Current classification of the substance against European Commission and national regulations

Table 7.11 summarises the current classification of the substance against Council Directives in order to assess the regulations to which 2,4-dichlorophenol is subject.

Table 7.11 Current classification of 2,4-dichlorophenol against Council Directives

Directive	Status (listed or not)
67/548/EEC - Classification, packaging and labelling of dangerous substances	Classified: C, N R phrases: 21/22-34-51/53

The World Health Organisation (WHO) derived a Tolerable Daily Intake (TDI) for 2,4-dichlorophenol in humans at 0.2 mg kg^{-1} day^{-1} by dividing the NOEL of 100 mg kg^{-1} day^{-1} at which no abnormal changes in the liver weight was observed in the 6-month dietary toxicity study in mice (Kobayashi *et al* 1972) by 500, a coefficient for uncertainties such as study period (EHC 1989).

Based on the decreased delayed hypersensitivity response data from the Exon and Koller (1985) study (see Section 7.4.2.1), the US EPA set the NOEL and NOAEL for 2,4-dichlorophenol at 3 ppm (corresponding to 0.5 mg kg^{-1} day^{-1} and 30 ppm (corresponding to 5 mg kg^{-1} day^{-1}) respectively (IRIS, 1988). However, it was recognised that these endpoints are not commonly used in the derivation of human health risk evaluations and confidence in the study was rated low.

There are water quality standards in both the Netherlands and the United Kingdom for the protection of the aquatic environment from 2,4-dichlorophenol. In the Netherlands the target value is 0.2 µg l^{-1} (based on total concentration) while the maximum permissible concentration (total) is 15 µg l^{-1}. In the United Kingdom the statutory environmental quality standards (as annual averages) for freshwaters and saltwaters are 20 µg l^{-1}.

7.7 Exposure data

7.7.1 Worker exposure data

No measured exposure data has been located for 2,4-dichlorophenol but since it is produced as an intermediate in closed systems the potential exposure of workers to the substance is limited providing appropriate safety procedures are followed.

7.7.2 Consumer exposure data

No information on concentrations of 2,4-dichlorophenol to which consumers are potentially exposed during the use of products has been obtained. However, the advisory on 2,4-dichlorophenol from the United States Environmental Protection Agency (US EPA) Office of Pollution Prevention and Toxics (OPPT) and US Department of Labour Occupational Safety and Health Administration (OSHA) stated that "*The substance, which is a solid at room temperature, is a high production volume chemical feedstock used to make herbicides and some other chemical products. It is not believed to be used outside of the chemical industry. The focus of concern is occupational and no risks are expected for consumers or community members.*".

7.7.3 Environmental exposure data

Limited measured environmental (aquatic, terrestrial or aerial) exposure data has been obtained based on searches of the COMMPS database and literature sources. Environmental contamination results from the use of 2,4-dichlorophenol in the production of methyl compounds used as mothproofing agents, antiseptics, miticides and seed disinfectants.

7.7.3.1 Aquatic environment

Data from the period 1996 – 1997 on surface water concentrations have been located for Denmark, France and the United Kingdom. In Denmark 4 samples taken were all below the limit of detection of 0.02 μg l^{-1}, while in France 11 samples taken were all below a limit of detection of 50 μg l^{-1}. In the United Kingdom 1077 samples were taken in 1996 with 201 (18.7%) being above the limit of detection of 0.05 μg l^{-1}. The highest value recorded was 100 μg l^{-1} in a sample from the River Doe Lea.

7.7.3.2 Terrestrial environment

Contamination of the terrestrial environment by 2,4-dichlorophenol results from the breakdown of the widely used herbicide 2,4-dichlorophenoxyacetic acid (Clark *et al* 1975).

7.7.3.3 Aerial environment

No information has been obtained on aerial concentrations of 2,4-dichlorophenol.

7.7.3.4 Comparison of environmental monitoring data and exposure concentrations causing endocrine mediated responses

The limited data on the concentrations of 2,4-dichlorophenol in European surface waters (see Section 7.7.3.1) indicates that typical levels are in the range 0.05 – 100 μg l^{-1}, though most values are probably at the lower end of the range. The only data on potentially endocrine mediated responses in aquatic organisms is a 21 day NOEC for reproduction in *Daphnia*

magna of 210 μg l^{-1}, though there is no information on the mechanism of action for the effects. The use of a margin of safety (MOS)[2] approach assuming the effects on *Daphnia magna* reproduction were endocrine mediated would result in values of 2.1 - 4200. On the basis that an MOS of 100 should be required for the risk to be acceptable then 2,4-dichlorophenol may present a risk to aquatic organisms in terms of potential endocrine disrupting effects if environmental concentrations exceed 2.1 μg l^{-1}. However, there is considerable uncertainty associated with the MOS due to the absence of data for key taxa such as fish.

7.8 Overall Conclusions on 2,4-dichlorophenol

The following conclusions have been drawn from a review of the data for 2,4-dichlorophenol:

7.8.1 Data from studies assessing potential endocrine disrupting effects

7.8.1.1 Human related studies

- A series of oral and sub-cutaneous exposure studies been conducted on 2,4-dichlorophenol. In a one generation reproduction study there was an absence of effects on reproductive parameters in Sprague-Dawley rats even at the highest test dose of 300 ppm (50 mg kg body weight^{-1} day^{-1})(Exon *et al* 1984, Exon and Koller 1985). However, multi-generation reproductive toxicity studies have not yet been performed and the effects of this compound on the reproductive performance and development of subsequent generations of animals remains unknown. A multi-generation reproduction study is currently being conducted in Japan by MITI which will address the uncertainties resulting from the currently available data.

- In the studies considering development and teratogenicity, 2,4-dichlorophenol has been reported to have toxic effects on foetuses secondary to the maternal toxicities, for example decreases in the litter size and increases in the organ weights.

- The results of the *in vivo* studies including the Uterotrophic assay and rodent Hershberger assay did not indicate that 2,4-dichlorophenol did not cause sex hormone receptor-mediated endocrine disrupting effects at the dose tested.

- The available *in vitro* data for 2,4-dichlorophenol only relates to *in vitro* assays assessing oestrogenic mechanisms of action in mammalian cells and tissues. The data indicates an absence of induction of oestrogen-sensitive gene products and limited binding of 2,4-dichlorophenol to the human oestrogen receptor. Exposure of human mammary tumour cells to 2,4-dichlorophenol results in a weak induction of cell proliferation. No data has been identified on the androgenic and anti-androgenic effects of 2,4-dichlorophenol and effects on thyroid function and hormone synthesis and secretion and steroidogenesis in mammalian cells and tissues.

- In *in vitro* fertilisation tests in mice no effects on sperm motility and penetration rate into the ovum were found.

[2] Margin of safety (MOS) = (Lowest NOEC for endocrine mediated responses)/Environmental concentration

7.8.1.2 Wildlife studies

- The data that has been located on the potential endocrine disrupting effects of 2,4-dichlorophenol on wildlife is limited to studies on the effects on the reproduction on the water flea *Daphnia magna* and a study on the reproduction of springtails (*Folsomia candida)*. There is uncertainty due to an absence of data for wildlife species particularly in relation to reproduction and development in fish.

- In an *in vitro* screening test reduced binding of tritiated 17β-oestradiol to the rainbow trout oestrogen receptor was measured although it was not evident whether the inhibitory effects was due to direct action.

7.8.2 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity

7.8.2.1 Human related studies

- In acute and repeat-dose studies the general systemic toxicity data for laboratory mammals indicates that the threshold in rats for an absence of effects which are not directly endocrine mediated occurs at a doses of >100 mg kg body weight^{-1} day^{-1}. As a result it appears that on the basis of the available data that foetuses are markedly more sensitive to 2,4-dichlorophenol than juveniles or adults. The mechanism responsible has not been elucidated.

7.8.2.2 Wildlife studies

- Comparison of the limited data on potential endocrine mediated responses in the aquatic invertebrate *Daphnia magna* with the acute and chronic data for this species indicates that the threshold exposure concentration for mortalities (1.48 – 1.7 mg l^{-1}) was higher than the threshold concentration for effects on reproduction (0.21 mg l^{-1}). However, no comparisons could be made for fish due to the absence of data on endocrine mediated responses in this taxonomic group, which represents an area of uncertainty.

- Data for the terrestrial springtail (*Folsomia candida*) indicated that the threshold exposure concentration for mortalities after 34 days exposure to 2,4-dichlorophenol (LC_{10} value of 0.7 mg kg dry weight^{-1}) was lower than the threshold for reproduction (EC_{10} value of 3.8 mg kg dry weight^{-1}).

7.8.3 Exposure data

7.8.3.1 Workers

- No measured exposure data has been located for 2,4-dichlorophenol but since it is produced as an intermediate in closed systems the potential exposure of workers to the substance is limited providing appropriate safety procedures are followed.

7.8.3.2 Consumers

- No information on concentrations of 2,4-dichlorophenol to which consumers are potentially exposed during the use of products has been obtained. However, the advisory on 2,4-

dichlorophenol from the United States Environmental Protection Agency (US EPA) Office of Pollution Prevention and Toxics (OPPT) and US Department of Labour Occupational Safety and Health Administration (OSHA) stated that "*The focus of concern is occupational and no risks are expected for consumers or community members.*".

7.8.3.3 Environment

- The environmental monitoring data for aquatic systems indicated that typical European surface water concentrations were in the range 0.05 to 100 μg l^{-1}.
- The derived margin of safety (MOS) for potential endocrine mediated effects in aquatic organisms spanned the threshold of 100 for acceptable risk but there was considerable uncertainty given the limited data.
- No data has been located on concentrations of 2,4-dichlorophenol in the terrestrial and aerial compartments.

7.9 Summary of the weight of evidence for endocrine disrupting effects in humans and wildlife and associated uncertainties

The summary of the weight of evidence for endocrine disrupting effects of 2,4-Dichlorophenol in humans and wildlife along with associated uncertainties are given in Table 7.12.

Table 7.12 Summary of the weight of evidence conclusion and uncertainties associated with the assessment of the endocrine disrupting effects of 2,4-Dichlorophenol

	Target group	
	Humans	Wildlife
Weight of evidence	The available data from *in vivo* studies in laboratory mammals (using oral or dermal exposure routes) indicates that 2,4-dichlorophenol does not cause adverse effects on reproductive and developmental endpoints (which may be endocrine mediated) at exposure levels where general systemic toxic effects are observed. The lowest NOEL in the *in vivo* studies was 50 mg kg body weight $^{-1}$ day^{-1} for reproductive parameters. No measured exposure data has been located for 2,4-dichlorophenol but since it is produced as an intermediate in closed systems the potential exposure of workers to the substance is limited providing appropriate safety procedures are followed. No information on concentrations of 2,4-dichlorophenol to which consumers are potentially exposed during the use of products has been obtained. However, the advisory on 2,4-dichlorophenol from the United States Environmental Protection Agency (US EPA) Office of Pollution Prevention and Toxics (OPPT) and US Department of Labour Occupational Safety and Health Administration (OSHA) stated that "*The focus of concern is occupational and no risks are expected for consumers or community members.*"	The available effects data shows that the threshold exposure concentration of 2,4-dichlorophenol above which reproduction of the aquatic invertebrate *Daphnia magna* is reduced (NOEC = 0.21 mg l^{-1}) is only slightly lower than the threshold level for general toxic effects (i.e. lethality). However there is no information on the mechanism of action for the effects observed in this species. The available exposure data indicate that 2,4-dichlorophenol may represent a risk to aquatic organisms. In contrast, the threshold exposure concentration of 2,4-dichlorophenol above which reproduction of the terrestrial invertebrate *Folsomia candida* is reduced (NOEC = 3.8 mg kg dry weight^{-1}) is slightly higher than the threshold levels for general toxic effects (i.e. lethality).
Uncertainties	There are uncertainties with regard to the evaluation of potential adverse effects of 2,4-dichlorophenol on reproductive and developmental endpoints since data is not available from a definitive multi-generation study. These issues will be addressed in a study initiated by MITI in 2002. Mechanistic uncertainties exist because the available studies provide no direct measurement of changes in endocrine function (for example changes in hormone levels).	There are uncertainties with regard to potential adverse effects of 2,4-dichlorophenol on reproduction and development in wildlife due to the absence of data for a wider range of aquatic taxa, particularly fish. The absence of data on aerial organisms is not a major uncertainty since the physico-chemical properties of 2,4-dichlorophenol indicate that the substance should not partition into the aerial compartment. No environmental exposure data for 2,4-dichlorophenol in the terrestrial and aerial compartments has been located.

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8. REVIEW OF DATA FOR 4-NITROTOLUENE

Table of Contents

Notes:

This section contains information collected and collated from a range of sources including published papers, reports of studies conducted by industrial companies or sector groups and data compilations such as IUCLID (2000). The data from IUCLID has been taken as accurate and individual source documents have not been checked unless they are considered to be key studies which have a major influence on the outcome of the review. All information taken from IUCLID has been referenced as being from that source and individual references have not been given in the references.

This review has been carried out in accordance with the evaluation framework described in Section 2. In the review the International programme for Chemical safety (IPCS) definition of an endocrine disrupter has been adopted, namely that it is "*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*".

In the context of the review it is recognised that there are various laboratory-based *in vivo* and *in vitro* methods utilising a range of (eco)toxicological endpoints that are claimed by different sources to be relevant to the assessment of endocrine disruption in humans and wildlife. However, since this field is still in an early stage of development there is uncertainty regarding the significance of many of the current findings.

From the numerous recent reviews of potential test methods (such as the Detailed Review Paper prepared by OECD in 1997) there is a clear consensus in terms of the hierarchy of the relevance of test methods. In this hierarchy longer-term *in vivo* studies considering effects on reproduction and/or development (and including mechanistic information) are of greater relevance than short-term *in vivo* screening tests which are of greater relevance than *in vitro* assays. The greater relevance of chronic *in vivo* tests or those assessing effects during critical windows of sensitivity is also evidenced by the fact that these are the key (eco) toxicological methods being developed in the OECD Endocrine Disruption Testing and Assessment (EDTA) Programme. This hierarchy approach to data relevance has been adopted in the review along with a weight of evidence consideration of the available data.

The review has been carried out to address three key questions:

1. Does the available data indicate there is evidence that a chemical causes endocrine disrupting effects in target groups of humans and/or wildlife?

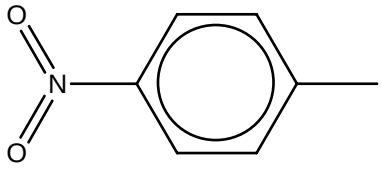
2. Do endocrine disrupting effects of the chemical in target groups of humans and/or wildlife occur at lower concentrations than those causing effect on general systemic toxicological endpoints?

3. Are particular target groups of workers, consumers or organisms in the environment likely to be exposed to concentrations of chemicals which exceed effects thresholds due to current emission patterns.

It should be recognised that this review is not designed to be a full Risk Assessment of a substance under the Existing Substances Regulation 793/93.

8.1 Physico-chemical data for 4-nitrotoluene

8.1.1 Summary details on the substance

CAS Number	99-99-0
EINECS Number	202-808-0
IUPAC Name	1-methyl-4-nitrobenzene
Other names	4-nitrotoluene, para-mononitrotoluene, 4-methylnitrobenzene, p-methylnitrobenzene, p-nitrophenylmethane
Molecular weight	137.1
Chemical formula	$C_7H_7NO_2$
Chemical structure	

8.1.2 Physico-chemical properties and environmental fate information (from IUCLID 2000)

The data on the physico-chemical properties of 4-nitrotoluene and its environmental fate (see Table 8.1) indicate that the substance is inherently biodegradable but does not tend to sorb to sludge in waste water treatment plants based on a low organic carbon water partition coefficient. In the aquatic environment 4-nitrotoluene is susceptible to photochemical degradation with a half-life of 5.9 hours (Simmons and Zepp 1986).

Volatilisation represents a major removal process from the aquatic environment based on the Henry's Law Constant of 2.38 Pa-m^3 mol^{-1} (2.41 x 10^{-5} atm-m^3 mol^{-1}) being similar to the value range of 1- 100 Pa-m^3 mol^{-1} which is considered to indicate volatility.

Table 8.1 Physico-chemical properties and environmental fate data (from IUCLID 2000)

Physico-chemical property	Value (and comments)
Physical state at ambient	Solid
Water solubility	262 to 345 mg l^{-1} at 20°C
Octanol-water partition coefficient (log Kow)	2.4
Organic carbon water partition coefficient (log Koc)	No data
Henry's Law Constant	2.38 Pa-m^3 mol^{-1} (2.41 x 10^{-5} atm m^3 mol^{-1})
Type of degradation	Information
Aquatic - abiotic	4-nitrotoluene is susceptible to photochemical degradation in water with a half-life of 5.9 hours
Aquatic - biotic	4-nitrotoluene was reported as inherently biodegradable in a closed bottle test and a Zahn-Wellens test
Terrestrial	No data
Atmospheric	No data

A Mackay Level 1 fugacity model has shown that for a discharge of 1000 tonnes of 4-nitrotoluene 53.2% of the substance will partition into the air (Table 8.2), with 38.1% partitioning into the water. Amounts present in other compartments are minimal.

Table 8.2 Summary of the results of a Mackay Level 1 fugacity model

Compartment	Volumes of different compartments	% of substance present in different compartments
Water	2×10^{11}	38.1
Suspended sediment	10^6	0.0059
Bottom sediment	10^8	0.19
Fish	2×10^5	4.8×10^{-4}
Air	10^{14}	53.2
Aerosol	2000	2.4×10^{-4}
Soil	9×10^9	8.48

8.2 Production and Uses

8.2.1 Production Patterns

4-Nitrotoluene is manufactured by the continuous nitration of toluene in a closed process with subsequent separation and purification. Information in IUCLID (2000) indicates an annual production in the EU of 50000 – 100000 tonnes.

8.2.2 Use Patterns

4-Nitrotoluene is a high production volume chemical which is only used as an intermediate in the synthesis of agricultural and rubber chemicals, pharmaceuticals and various dyes. Significant quantities of 4-nitrotoleune are used in the production of the stilbene derivative

4,4'-diaminostilbene-2,2'disulphonic acid (DAS) which is an intermediate in the manufacture of fluorescent whitening agents.

8.3 Toxicokinetics, metabolism and bioaccumulation

8.3.1 Toxicokinetics and metabolism

Following exposure of male rats to a single oral dose of 200 mg kg^{-1} of labelled 4-nitrotoluene excretion of radioactivity occurred mainly in the urine (76.7%) with the major metabolites being 4-nitrobenzoic acid, 4-acetamidobenzoic acid and 4-nitrohippuric acid. Radioactivity was also detected in the faeces (6.1%) but not in the expired air. The total proportion of labelled 4-nitrotoluene accounted for as radioactivity was 82.2% of the dose (Chism *et al* 1985, Rickert 1987). In a 2 year carcinogenicity study carried out as part of the National Toxicology Program (see Section 8.4.2.1) two urinary metabolites (4-nitrobenzoic acid and 4-acetamidobenzoic acid) were followed as biomarkers of exposure. The ratios of 4-nitrobenzoic acid to creatinine and 4- acetamidobenzoic acid to creatinine determined at 2 weeks and 3, 12 and 18 months were linearly related to exposure doses of 4-nitrotoluene in males and females (NTP 2001).

8.3.2 Bioaccumulation

In laboratory mammals there is no indication of bioaccumulation, which is consistent with the low octanol water partition coefficient for 4-nitrotoluene (log Kow = 2.4).

A 42 day Japanese study on carp (*Cyprinus carpio*) conducted to a guideline corresponding to OECD 305C (Bioaccumulation: Degree of Bioaccumulation in Fish) indicated that at 4-nitrotoluene exposure concentrations of 0.01 and 0.1 mg l^{-1} the resulting bioconcentration factors (BCFs) were 4.5 – 8 and 3.7 – 7.2 respectively (MITI 1992). These data confirm the hypothesis, based on the low log Kow, that 4-nitrotoluene does not bioaccumulate in aquatic organisms.

8.4 Studies relevant to the assessment of potential endocrine disrupting effects

8.4.1 Studies relevant to the assessment of potential endocrine disrupting effects in humans

8.4.1.1 In vitro studies

A. Receptor competitive binding assays

Kondo (2000) investigated the binding of 4-nitrotoluene to the human oestrogen receptor (ERα) and showed weak binding capacity at concentrations approximately 1.2 x 10^5 times higher than those of 17β-oestradiol and diethylstilbestrol.

B. Recombinant yeast assays

Nishihara *et al* (2000) investigated the oestrogen type responses of 4-nitrotoluene on recombinant yeast cells transfected with the human oestrogen receptor (ERα) though limited details on the experimental procedure are reported. The study found that concentrations up to 1 mM (137000 $\mu g\ l^{-1}$) did not induce any response in the assay.

C. Mammalian cell growth assays

Jobling *et al* (1985) investigated the effects of 4-nitrotoluene on the growth of human breast cancer ZR-75 cells. The cells were incubated with 10 μM (1370 μg l^{-1}) 4-nitrotoluene with or without 17β oestradiol (10 nM) for 10 days and cells were counted on days 0, 3, 6, 8 and 10. At the concentration tested 4-nitrotoluene did not significantly enhance breast cancer cell growth[1].

Summary of in vitro data

Table 8.3 summarises the available *in vitro* data for 4-nitrotoluene which relates to *in vitro* assays using mammalian cells and tissues assessing oestrogenic mechanisms of action. The data indicates no or weak binding of 4-nitrotoluene to the human oestrogen receptor and no substance-induced proliferation of human breast cancer cells. No data has been located on the androgenic and anti-androgenic effects of 4-nitrotoluene and effects on thyroid function and hormone synthesis and secretion and steroidogenesis in mammalian cells and tissues.

Table 8.3 Summary of the *in vitro* data in isolated mammalian cells and tissues relating to different mechanisms of action of 4-nitrotoluene

Mechanism of endocrine disruption	Responses observed in *in vitro* systems
Oestrogenecity/anti-oestrogenecity	Data indicates no or weak binding of 4-nitrotoluene to the oestrogen receptor and no substance-induced proliferation of human breast cancer cells.
Androgenicity/anti-androgenicity	No data identified
Thyroid effects	No data identified
Effects on hormone synthesis or secretion	No data identified
Effects on steroidogenesis	No data identified

8.4.1.2 In vivo studies

Table 8.4 summarises the information on endocrine mediated responses in laboratory mammals following oral exposure.

A. Effects on endocrine glands and hormone sensitive tissues

In a long-term study (Ciss 1980) rats received a single treatment of 400 mg kg body $weight^{-1}$ as a suspension in 1% methylcellulose once per day, 5 days per week for 24 weeks. Males showed reduced body weight gain and atrophy of testes in combination with necrosis of the seminiferous tubules. Females and offspring tolerated the single treatment dose without apparent effects.

[1] In a review of the *in vitro* methods available for assessing oestrogenic substances, Zacharewski (1997) has stated that cell proliferation observed in the "E-Screen" assay using human breast cancer MCF-7 cells can be due to other non-oestrogenic factors.

As part of the development of a screening system for reproductive toxicity in mice and rats by the United States National Toxicology Program, Morrisey *et al* (1988) reported data on the effects of a range of chemicals, including 4-nitrotoluene. The sperm morphology and vaginal cytology examinations (SMVCEs) were carried out at the end of 13 week exposure studies and included evaluations of:

- motility, concentration and head morphology of sperm from the cauda epididymus;
- male reproduction organ (cauda epididymis, epididymis and testis) weights;
- average oestrous cycle length and relative frequency of different oestrous stages in females.

In the 13 weeks study with 4-nitrotoluene groups of 10 Fischer 344/N rats per sex received doses of 0, 90, 180 and 360 mg kg body weight^{-1} of 4-nitrotoluene dissolved in corn oil by oral gavage. Groups of 10 B6C3F1 mice per sex received doses of 0, 40, 80 and 160 mg kg body weight^{-1} of 4-nitrotoluene dissolved in corn oil also by oral gavage. For the rats at the highest dose (360 mg kg body weight^{-1}), a decrease in terminal body weight and in absolute cauda epididymis, epididymis and testis weights and relative epididymus weight was evident in males but sperm parameters were not affected. In female rats no adverse effects (including oestrous cycle length) were reported. In the groups of mice no adverse effects on measured reproductive parameters (organ weight and histopathology and oestrous cycle length) were reported. In the mice no adverse effects on reproductive organ weights, sperm parameters or oestrous cycle were reported in males and females at any test dose

In another study (NTP TOX 23) conducted as part of the United States National Toxicology Program the effects of 4-nitrotoluene administered orally in the diet on male and female F344/N rats and B6C3F1 mice were investigated over a 14 day period (NTP 1992). Groups of rats (5 rats/sex/group) were exposed daily to doses ranging from 1250 to 20000 ppm (94 – 1500 mg kg body weight^{-1}). Groups of mice (5 rats/sex/group) were exposed daily to doses from 675 to 10000 ppm (101 to 1500 mg kg body weight^{-1}). There were no treatment-related effects on survival or clinical signs of toxicity in these studies. However, decreases in body weights and food consumption were noted, relative to controls, at 20000 ppm in rats. As a result 10000 ppm was selected as the highest dose for the subsequent longer-term (13 week) dietary exposure study for rats. In the mice, reduced body weight gain was evident at 10000 ppm.

Dunnick *et al* (1994) reported the data from this subsequent 13 week sub-chronic study (NTP TOX 23) on the effects of 4-nitrotoluene on male and female F344/N rats and B6C3F1 mice as part of the National Toxicology Programme (NTP 1992). The animals were placed on study at 6-8 weeks of age. Controls groups received NIH 07 feed while treatment groups received NIH 07 feed mixed with 4-nitrotoluene at doses ranging from 0, 625, 1250, 2500, 5000 and 10000 ppm. Feed was changed on a schedule of 2 days, 3 days and 2 days. The doses used equated to an approximate range for rats of 0, 42, 82, 165, 342 and 723 mg kg body weight^{-1} day^{-1} for males and 0, 44, 82, 164, 335 and 680 mg kg body weight^{-1} day^{-1} for females. For mice the estimated doses were 0, 131, 212, 439, 813 and 1491 mg kg body weight^{-1} day^{-1} for males and 0, 164, 320, 625, 1075 and 1634 mg kg body weight^{-1} day^{-1} for females. Body weights were recorded weekly and food consumption was measured at each feed replacement. In the studies with rats and mice 10 animals of each sex were used in the control group and treatment groups. Ten additional rats/sex/dose/level were included in the

studies for clinical chemistry and haematology evaluations at weeks 1 and 3 and core animals were used for these evaluations at study termination. A range of haematological parameters[2] and clinical chemistry measurements[3] were made. At the completion of dosing core animals were killed and a complete necropsy was performed. A wide range of tissues were examined in all animals in the controls and the high dose groups including the adrenal, epididymus, mammary glands, ovaries, parathyroid, prostate, seminal vesicles, testis, thyroid or uterus. The heart, liver, lungs, right kidney, thymus and right testis were weighed at necropsy. Tissues in which a treatment–related effect was identified were examined for all animals in the other dosed groups. Sperm morphology and vaginal cytology were also performed (according to the methods of Morrissey *et al* 1988).

In the *rats* decreased body weight gain was observed in the 5000 ppm (342 mg kg body weight^{-1} day^{-1}) and 10000 ppm (723 mg kg body weight^{-1} day^{-1}) groups with decreased food intake in the highest dose group. Reduced absolute testis weights and testicular degeneration was observed in males at the highest 4-nitrotoluene dose (10000 ppm or 723 mg kg body weight^{-1} day^{-1}). Testicular degeneration was characterised by decreased number of germinal cells and the presence of syncytial giant cells (degenerate spermatids) in seminiferous tubules. Epididymal sperm density and testicular spermatid head count were also reduced in high dose males (723 mg kg body weight^{-1} day^{-1}). The observed effects could result from direct cytotoxic action but could have an endocrine component, that is the loss of hormonal support due to effects on Leydig cells. At 5000 ppm (342 mg kg body weight^{-1}day^{-1}) there was a reduction in absolute testis weight without the other treatment related effects found in the testes at 10000 ppm. In female rats there was no discernable oestrous cycle in the highest dose group (10000 ppm or 680 mg kg body weight^{-1} day^{-1}) though this occurred without changes in the weight or gross pathology or histopathology of the uterus or ovaries. The vaginal oestrous cycle mirrors endogenous secretion of ovarian oestrogen and this lack of oestrous cycle is indicative of ovarian dysfunction.

Haematology showed typical effects secondary to methemoglobinemia in the highest dose group with minor effects in lower dose groups. Methemoglobin increased up to 8.1 % in males and 9.0 % in females. Hyaline droplet nephropathy and haematopoesis and pigmentation of the spleen occurred in the males in all dose groups with spleen congestion at the highest dose group. In the females there were effects on the kidney (karyomegaly and pigmentation) as well as the spleen (haematopoesis and pigmentation) in all dose groups and spleen congestion at the two highest doses. A systemic sub-chronic toxicity LOAEL of 42 mg kg body weight^{-1} day^{-1} for rats was derived in this study.

In the *mice* there was a decreased body weight gain in males in the 5000 ppm (813 mg kg body weight^{-1} day^{-1}) and 10000 ppm (1634 mg kg body weight^{-1} day^{-1}) groups. However, no histopathological changes were noted in organs and tissues potentially susceptible to endocrine disrupting substances (including the adrenals, epididymis, mammary gland, ovaries, parathyroid, pituitary, prostate, seminal vesicles, testis, thyroid and uterus). An increased relative liver weight in all dose groups in both sexes was also evident. A systemic

[2] Haematological parameters included erythrocyte count, mean corpuscular volume, haemoglobin concentration, packed cell volume, erythrocyte morphology, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, leukocyte count and differential reticulocyte count, platelet count and morphology, methemoglobin concentration and Heinz body formation

[3] Clinical chemistry measurements included serum levels of sorbitol dehydrogenase, alanine aminotransferase, alkaline phosphatase, total bile acids, creatine kinase, urea nitrogen, creatinine, total protein and albumin

sub-chronic toxicity LOAEL of 131 mg kg body weight^{-1} day^{-1} for mice was derived in this study.

Smith and Quinn (1992) investigated the oestrogenic effects of 4-nitrotoluene in immature female CD Sprague Dawley rats using the Uterotrophic screening assay. The study assessed the effects of single intra-peritoneal injections of 4-nitrotoluene dissolved in corn oil over a concentration range from 0.01 to 1000 mg kg body weight^{-1}. However, the exposures of 0.01, 0.1, 1.0, 10, 30, 100, 300 and 1000 mg kg body weight^{-1} were not carried out in a single study but were actually assessed in three separate experiments (0.01 to 10 mg kg body weight^{-1}, 30 to 100 mg kg body weight^{-1} and 300 to 1000 mg kg body weight^{-1}). The paper used all the data on change in uterine weight (after correction for body weight) by using the treatment data as a proportion of the corn oil only control. This showed that there was no dose-response evident and only the treatments of 30 and 100 mg kg body weight^{-1} caused increased uterine weights whereas treatments of 300 and 1000 mg kg body weight^{-1} caused no increase in uterine weight. However, there was marked variability in the level of control uterine weights evident in the different studies which raises issues as to whether the animals used in the separate experiments were of the same age.

In another Uterotrophic screening assay Kondo (2000) found that exposure of mice to 4-nitrotoluene did not result in any significant effects on uterus size and weights. However, only an English abstract is currently available and there are no further experimental details.

B. Reproduction and fertility studies

Ciss (1980) investigated the effects of 4-nitrotoluene on Wistar rats by exposing groups of male and females to 400 mg kg body weight^{-1} by oral gavage once per day, 5 days per week for 3 months. The rats were paired with exposed animals of the other sex and the treatment was continued for another 3 months. The males showed atrophy of the testes in combination with necrosis of the seminiferous tubules and an increase in spleen weight. The observed atrophy could result from direct cytotoxic effects or hormonal abnormalities. However, the presence of necrotic tissue indicates a direct toxic effect. No significant effects on reproduction or on the offspring were observed.

A study on reproductive toxicity (conducted to OECD Test Guideline 421) including additional investigations on reproductive organs is currently being conducted by Bayer AG and termination of the in life phase occurred on 11th March 2002. A draft report is expected by late 2002.

C. Developmental and teratogenicity studies

No data has been located on the effects of 4-nitrotoluene in developmental and teratogenicity studies.

D. Carcinogenecity and oncogenecity studies

In a two year carcinogenecity study (NTP TR-498) male and female F344/N rats and B6C3F1 mice were exposed to p-nitrotoluene (greater than 99% purity) in feed for 2 years (NTP 2001). Groups of 50 male and 50 female rats were fed diets containing 0, 1250, 2500, or 5000 ppm 4-nitrotoluene (equivalent to average daily doses of approximately 0, 55, 110 or 240 mg kg body weight^{-1} for males and 0, 60, 125 and 265 mg kg body weight^{-1} for females) for 105 to

106 weeks. Groups of 50 male and 50 female mice were also fed diets containing 0, 1250, 2500, or 5000 ppm (equivalent to average daily doses of approximately 0, 170, 345, and 690 mg kg body weight^{-1} for males and 0, 155, 315, or 660 mg kg body weight^{-1} for females) for 105 to 106 weeks. No interim kill was performed.

Survival of all exposed groups of *rats* was similar to that of the control groups and mortality generally was between 10 and 20 % in all groups. The exception was the 38% mortality recorded in male control rats. Mean body weights of 5000 ppm male rats (366 g) and 2500 and 5000 ppm female rats (262 and 210 g) were less than those of the controls (402 g in males and 294 g in females) during most of the study. Mean body weights of 1250 ppm females were less during the second year of the study. Food consumption by 5000 ppm females was less than that by the controls during year 2 of the study. In all male and female rats nasal and eye discharge were the only clinical signs of toxicity. However, haematological data or clinical chemistry data were not reported,

In male rats incidences of germinal epithelial atrophy of the testis in 5000 ppm (240 mg kg body weight^{-1} day^{-1}) males (30 of 50) were significantly increased relative to the controls (7 of 50), which could represent a direct cytotoxic effect or an endocrine mediated response. The incidence of interstitial cell adenoma of the testis in 5000 ppm males was significantly decreased relative to the controls (34 of 50 compared to 49 of 50).

In female rats the incidence of clitoral gland adenoma or carcinoma (combined) was significantly greater in 2500 ppm (125 mg kg body weight^{-1} day^{-1}) females (20 in 50) than that in the controls (8 in 50) and exceeded the historical control ranges. The incidence of clitoral gland neoplasms was not increased in 5000 ppm females (8 of 49), possibly because of the lower body weights in this group. These effects are probably not endocrine mediated. In 2500 and 5000 ppm females incidences of endometrial cystic hyperplasia of the uterus (which could be an oestrogenic mediated response) were significantly increased relative to controls**.** The incidences of mononuclear cell leukemia were significantly decreased in all exposed groups except 1250 ppm females. The incidence of mammary gland fibroadenoma in 5000 ppm females was significantly decreased with the lower incidence possibly being related to the lower body weights in this group. The incidences of subcutaneous fibroma and of subcutaneous fibroma or fibrosarcoma (combined) in 2500 ppm male rats were significantly increased and exceeded the historical control ranges. However, significant effects were not evident in males exposed to the highest dose.

Target organs for non-neoplastic lesions were the kidneys, livers and spleen in males and females. In both sexes the significant lesions in all exposed groups consisted of renal tubule hyaline droplets and renal tubule pigmentation which are signs of tissue degeneration. The incidences of several non-neoplastic kidney lesions were significantly increased in exposed groups of rats, and the severities of these lesions generally increased with increasing exposure concentration. In females mineralisation was evident in the 2500 and 5000 ppm groups while renal tubule hyperplasia was noted in the 5000 ppm group. In the spleen, incidences of haematopoietic cell proliferation and pigmentation were significantly increased in the 2500 and 5000 ppm groups. The significant adverse effects in the liver were basophilic foci and clear cell foci in males of the 2500 ppm and 5000 ppm-groups. Eosinophilic foci in the liver were observed in females of the 2500 and 5000 ppm groups and in males of highest dose group.

Survival of all exposed groups of male and female *mice* was similar to that of the control groups with mortality levels being between 10 and 20%. Mean body weights of 5000 ppm males and females were less than those of the control groups during most of the study. Mean

body weights of 2500 ppm males were less than those of the controls after week 92. Food consumption by all exposed groups of mice was similar to that by the control groups. No clinical signs of toxicity were observed in male and female mice.

In the study no effects on reproductive organs were reported at any test dose. There were also no neoplastic lesions in males and females which were attributed to the treatment. The alveolar/bronchiolar adenomas or carcinomas found in males at the highest dose group (19 of 50) were significantly increased when compared to the concurrent control (8 of 50), but were within the historical control range. Non-neoplastic lesions, attributed to the treatment, were observed in the lungs of females and males including alveolar epithelial bronchiolization and alveolar epithelial hyperplasia (the latter only in males) in all treatment groups.

On the basis of the study data the NTP stated that there was equivocal evidence of carcinogenic activity of 4-nitrotoluene in male rats (based on increased incidences of sub-cutaneous skin neoplasms) and mice (based on increased incidences of alveolar/bronchiolar neoplams). There was some evidence of carcinogenic activity in female rats (based on increased incidences of clitoral gland neoplasms) but no evidence of carcinogenic activity in female mice. The observed significant effects on male reproductive organs in rats occurred at systemically toxic doses characterised by reduced body weights and toxicity to the spleen triggered by the haematoxic effect of 4-nitrotoluene and could be considered to be secondary to erythrocyte damage caused by methemoglobinemia. However, this could not be substantiated due to a lack of haemaotology data. A lowest observed adverse effect level (LOAEL) for systemic effects of 55 mg kg body weight^{-1} day^{-1} in rats and 155 mg kg body weight^{-1} day^{-1} for mice was observed.

Table 8.4 Summary of the data on potential endocrine mediated responses in laboratory mammals following oral exposure

Species	Life stage of test organism	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Rats (Wistar)	Males and females	Oral (gavage) exposure to 0 and 400 mg kg body weight^{-1} for 3 months	No significant effects (relative to the controls) on reproduction or on the offspring	Ciss (1980)	Medium	Use with care
Rats (F344/N)	Males	Oral (gavage) exposure to 0, 90, 180 and 360 mg kg body weight^{-1} for 13 weeks	Significant decrease (relative to the controls) in absolute cauda epididymus, epididymus and testis weights and relative epididymus weight at 360 kg body weight^{-1} after 13 weeks No significant effect (relative to the controls) on sperm parameters (motility, concentration and head abnormalities) at any test dose after 13 weeks *(NOEL for effects on endocrine glands and hormone sensitive tissues = 180 mg kg body weight^{-1})*	Morrisey *et al* (1988)	Medium	Valid
	Females	"	No significant effects (relative to the controls) on average oestrous cycle length and relative frequency of different oestrous stages at any test dose after 13 weeks *(NOEL for effects on endocrine glands and hormone sensitive tissues = 360 mg kg body weight^{-1})*			
Mice (B6C3F1)	Males	Oral (gavage) exposure to 0, 40, 80 and 160 mg kg body weight^{-1} for 13 weeks	No significant effects (relative to the controls) in absolute or relative cauda epididymus, epididymus and testis weights at any test dose after 13 weeks No significant effect (relative to controls) on sperm parameters (motility, concentration and head abnormalities) at any test dose after 13 weeks *(NOEL for effects on endocrine glands and hormone sensitive tissues = 160 mg kg body weight^{-1})*	Morrisey *et al* (1988)	Medium	Valid
	Females	"	No significant effects (relative to controls) on average oestrous cycle length and relative frequency of different oestrous stages at any test dose after 13 weeks *(NOEL for effects on endocrine glands and hormone sensitive tissues = 160 mg kg body weight^{-1})*			

Table 8.4 Continued

Species	Life stage of test organism	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Rats (F344/N)	Males	Dietary exposure to 0, 625, 1250, 2500, 5000 and 10000 ppm (0, 42, 82, 165, 342 and 723 mg kg body weight^{-1} day^{-1}) for 13 weeks	Significant reduction (relative to the controls) in number of germinal epithelial cells and presence of synctial giant cells in seminiferous tubules at highest test dose (10000 ppm or 723 mg kg body weight^{-1} day^{-1}) after 13 weeks Significant reduction (relative to the controls) in epididymal sperm density and sperm head count at the highest dose (10000 ppm or 723 mg kg body weight^{-1} day^{-1}) after 13 weeks *(NOEL for effects on endocrine glands and hormone sensitive tissues = 342 mg kg body weight^{-1})*	Dunnick *et al* (1994)	Medium	Valid
	Females	Dietary exposure to 0, 625, 1250, 2500, 5000 and 10000 ppm 0, 44, 82, 164, 335 and 680 mg kg body weight^{-1} day^{-1}) for 13 weeks	No significant effects (relative to the controls) on gross histopathology of the uterus and ovaries at any test dose after 13 weeks No discernable oestrous cycle in animals at the highest dose (10000 ppm or 680 mg kg body weight^{-1}) *(NOEL for effects on endocrine glands and hormone sensitive tissues = 680 mg kg body weight^{-1})*			
Mice (B6C3F1)	Males	Dietary exposure to 0, 625, 1250, 2500, 5000 and 10000 ppm (0, 131, 212, 439, 813 and 1491 mg kg body weight^{-1} day^{-1}) for 13 weeks	No significant effects (relative to controls) on gross histopathlogy of the testis at any test dose after 13 weeks *(NOEL for effects on endocrine glands and hormone sensitive tissues = 1491 mg kg body weight^{-1})*	Dunnick *et al* (1994)	Medium	Valid
	Females	Dietary exposure to 0, 625, 1250, 2500, 5000 and 10000 ppm (0, 164, 320, 625, 1075 and 1634 mg kg body weight^{-1} day^{-1}) for 13 weeks	No significant effects (relative to the controls) on gross histopathology of the uterus and ovaries at any test dose after 13 weeks *(NOEL for effects on endocrine glands and hormone sensitive tissues = 1634 mg kg body weight^{-1})*			

Table 8.4 Continued

Species	Life stage of test organism	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Rats (F344/N)	Males	Dietary exposure to 0, 1250, 2500 and 5000 ppm (0, 55, 110 and 240 mg kg body weight^{-1} day^{-1}) for 2 years	Significant germinal epithelial atrophy (relative to the controls) at 5000 ppm (240 mg kg body weight^{-1} day^{-1}) *(NOEL for effects on endocrine glands and hormone sensitive tissues = 110 mg kg body weight^{-1})*	NTP (2001)	Medium	Valid
	Females	Dietary exposure to 0, 1250, 2500 and 5000 ppm (0, 60, 125 and 265 mg kg body weight^{-1} day^{-1}) for 2 years	No significant effects (relative to the controls) on reproductive organs at any test dose *(NOEL for effects on endocrine glands and hormone sensitive tissues = 265 mg kg body weight^{-1})*			
Mice (B6C3F1)	Males	Dietary exposure to 0, 1250, 2500 and 5000 ppm (0, 170, 345 and 690 mg kg body weight^{-1} day^{-1}) for 2 years	No significant effects (relative to the controls) on reproductive organs at any test dose *(NOEL for effects on endocrine glands and hormone sensitive tissues = 690 mg kg body weight^{-1})*	NTP (2001)	Medium	Valid
	Females	Dietary exposure to 0, 1250, 2500 and 5000 ppm (0, 155, 315 and 660 mg kg body weight^{-1} day^{-1}) for 2 years	No significant effects (relative to the controls) on reproductive organs at any test dose *(NOEL for effects on endocrine glands and hormone sensitive tissues = 660 mg kg body weight^{-1})*			

E. General conclusions on the potential endocrine mediated responses in laboratory mammals in in vivo studies

Table 8.5 summarises the data on potential endocrine mediated responses in laboratory mammals including both rats and mice in a range of study types. These include a range of studies assessing effects on reproductive parameters, however data of definitive significance from a multi-generation reproduction study is not available.

A 3 month oral exposure study (Ciss 1980) has indicated that 4-nitrotoluene affected testicular morphology only at a high, generally toxic dose without significant effects on reproduction or the offspring. A reproduction/development study is currently being carried out by Bayer which will provide data relevant to this finding. No conclusions can be drawn at present regarding the effects of 4-nitrotoluene on the development of laboratory mammals due to an absence of test data.

In both a 13 week subchronic study in rats and mice (Dunnick *et al* 1994) and a two year carcinogenicity study in rats and mice (NTP 2001) effects were evident on reproductive tissues which could be affected by the action of endocrine active substances. However, the germinal epithelial atrophy observed in the testes of male rats at exposure concentrations >110 mg kg body weight^{-1} day^{-1} could be due to endocrine mediated responses or to direct cytotoxic effects. Interpretation of the mechanism is complicated by the absence of haemotological data in these studies which may have implicated a direct cytotoxic effect if responses were evident in tissues with rapidly dividing cells.

The lowest doses tested in the oral and dermal exposure studies with mammals were in the region of 40 mg kg body weight^{-1} day^{-1} and no effects which may be endocrine mediated were evident at these doses. Available evidence from a Uterotrophic screening assay (Smith and Quinn 1992) indicates that in a series of three studies no effects on uterine weight occurred after intra-peritoneal injections from 0.01 – 1000 mg kg body weight^{-1}.

8.4.1.3 Human studies

No information on potential endocrine mediated responses of workers or consumers following exposure to 4-nitrotoluene has been identified. However, the closed production process and the use of 4-nitrotoluene as an intermediate means that these groups should not be exposed to the substance under normal circumstances (see Section 8.6).

Concern has been expressed over potential incidences of sexual dysfunction among men working in the production of stilbene derivative 4,4'-diaminostilbene-2,2'-disulphonic acid, an intermediate in the manufacture of fluorescent whitening agents. Two reports of the United States National Institute for Occupational safety and Health (NIOSH) by Grajewski *et al* (1995) and Whelan *et al* (1996) concluded that occupational DAS exposure may be associated with alterations in male reproductive hormone levels and there may be a possible effect on the sexual function of males working in a DAS manufacturing area. However, these effects are not the consequence of exposure to 4-nitrotoluene which is used in the process but rather the stilbene component.

Table 8.4 Summary of the potential endocrine mediated responses reported in *in vivo* studies with laboratory mammals

Type of study	Species and exposure route used	Dose series used	NOEL (mg kg body weight^{-1} day^{-1})		Reference
			Potential endocrine mediated responses	Systemic toxicity	
Sub-chronic oral toxicity (OECD 408)	Rats (F344/N) - dietary	0, 42, 82, 165, 342 and 723 mg kg body weight^{-1} day^{-1} (males) 0, 44, 82, 164, 335 and 680 mg kg body weight^{-1} day^{-1} (females)	342 (Histopathology) 335 (Reproductive function)	42 (NOAEL)	Dunnick *et al* (1994)
	Mice (B6C3F1) - dietary	0, 131, 212, 439, 813 and 1491 mg kg body weight^{-1} day^{-1} (males) 0, 164, 320, 625, 1075 and 1634 mg kg body weight^{-1} day^{-1} (females)	1491 (Histopathology) 1634 (Histopathology)	131 (NOAEL)	
Reproduction – One generation (OECD 415)	No data	-	-	-	-
Reproduction – Two generation (OECD 416)	No data	-	-	-	-
Reproduction (3 month study)	Rats (Wistar) - oral gavage	400 mg kg body weight^{-1} day^{-1}	400 (Reproduction) <400 (Histopathology)	<400	Ciss (1980)
Reproduction/ Development (OECD 421)	On going	-	-	-	Bayer (2002)
Development/ Teratogenicity (OECD 414)	No data	-	-	-	-
Carcinogenicity	Rats (F344/N) - dietary	0, 55, 110 and 240 mg kg body weight^{-1} day^{-1} for 2 years (males) 0, 60, 125 and 265 mg kg body weight^{-1} day^{-1} for 2 years (females)	110 (Histopathology – males) 265 (Histopathology – females)	55 (LOAEL)	NTP (2001)
	Mice (B6C3F1) - dietary	0,170, 345 and 690 mg kg body weight^{-1} day^{-1} for 2 years (males) 0, 155, 315 and 660 mg kg body weight^{-1} day^{-1} for 2 years (females)	690 (Histopathology – males) 660 (Histopathology – females)	155 (LOAEL)	NTP (2001)

8.4.2 Studies relevant to the assessment of potential endocrine disrupting effects in wildlife

8.4.2.1 In vitro studies

Jobling *et al* (1995) measured the *in vitro* receptor binding activity of tritiated 17β-oestradiol (5 nM) to the fish oestrogen receptor in rainbow trout hepatocytes at concentrations up to 1 mM (137 mg l^{-1}). The study found that the activity of 4-nitrotoluene was 10^4 times lower than that measured for 17β-oestradiol, though it was not determined whether this inhibitory effect was due to direct competition.

An investigation of the *in vitro* data on the binding of 4-nitrotoluene to the rainbow trout oestrogen receptor by the University of Kiel on behalf of the German Federal Office for the Environment concluded "4-nitrotoluene cannot be attributed an estrogenic potential (Bruhn *et al* 1999).

8.4.2.2 In vivo studies

A. Studies on aquatic organisms

The only data that has been located on the potential endocrine disrupting effects of 4-nitrotoluene on aquatic species is a 21 day reproduction test using *Daphnia magna* . This study investigated the effects of 4-nitrotoluene on the survival of adults and juvenile production of surviving adults (Canton *et al* 1985). No details were given on the concentration series used. The No Observed Effect Concentration (NOEC) for effects on juvenile production was 0.7 mg l^{-1}, though there is no information on the mechanism of action. It is not clear whether the effects observed in the *Daphnia magna* reproduction test are caused by direct oestrogenic effects. Other studies have shown an absence of reproductive impairment at 0.39 mg l^{-1} when animals are exposed to the synthetic steroid 17α-ethinylestradiol (Schweinfurth *et al* 1986).

B. Studies on terrestrial organisms

No data has been located on the potential endocrine disrupting effects of 4-nitrotoluene on terrestrial organisms. Given that 4-nitrotoluene is not expected to strongly sorb to organic carbon (see Section 8.1) the absence of data on potential endocrine disrupting effects in terrestrial organisms does not represent a key area of uncertainty. It should also be recognised that there are currently no internationally agreed methods specifically developed to assess potential endocrine disrupting effects in terrestrial organisms.

C. Studies on aerial organisms

No data has been located on the potential endocrine disrupting effects of 4-nitrotoluene on aerial organisms. Given that 4-nitrotoluene is considered to be volatile (see Section 8.1) the absence of data on potential endocrine disrupting effects in aerial organisms represents an area of uncertainty. However, it should be recognised that there are currently no internationally agreed methods specifically developed to assess potential endocrine disrupting effects in aerial organisms.

D. General conclusions on potential endocrine mediated responses in in vivo studies with wildlife species

The data that has been located on the potential endocrine disrupting effects of 4-nitrotoluene on wildlife is limited to one study on the effects on the reproduction of the water flea *Daphnia magna* (see Table 8.6) but there is no information on the mechanism of action. There is an absence of data for other wildlife species, particularly in relation to reproduction and development in fish and effects on aerial organisms.

8.5 Comparison of data for studies assessing potential endocrine disrupting effects and/or general toxicity

The general toxicity data in this section has largely been obtained from the IUCLID data set for 4-nitrotoluene and has been taken as accurate. Individual source documents have not been checked unless they are considered to be key studies which have a major influence on the outcome of the review. All information taken from IUCLID as been referenced as being from that source and individual references have not been given in the references.

8.5.1 Studies relevant to the assessment of general toxicity in humans

8.5.1.1 Acute studies

A series of acute mammalian toxicity studies on 4-nitrotoluene have been carried out in which various laboratory mammals (rats, mice and rabbits) have been exposed via the oral, dermal and inhalation routes and intra-peritoneal injection (IUCLID 2000). Experimental details for these studies (including information on strains of animals used and exposure and dose regimes) are generally absent (see Table 8.6).

A. Oral exposure

The acute oral exposure studies in rats resulted in LD_{50} values ranging from 1960 to 7100 mg kg body weight^{-1} (Sisa *et al* 1959, Back *et al* 1972, Vasilenko *et al* 1975, Groning and Kimmerle 1976, Vernot *et al* 1977). Ciss (1980) reported LD_{50} values of 3200 mg kg body weight^{-1} for female rats and 4700 mg kg body weight^{-1} for male rats. The acute oral exposure data for mice showed LD_{50} values of 1230 to 1280 mg kg body weight^{-1} (Back *et al* 1972, Vasilenko *et al* 1975, Vernot *et al* 1977) while for rabbits an value of LD_{50} value of 1750 mg kg body weight^{-1} was derived by Vasilenko *et al* (1975).

B. Dermal exposure

Acute dermal LD_{50} values of >750 mg kg body weight^{-1} (Kinkhead *et al* 1976) and >16000 mg kg body weight^{-1} (Sisa *et al* 1959) were derived in studies on rats. In both studies no mortalities were evident at the highest exposure concentrations tested.

C. Inhalation exposure

For the inhalation route by Groning and Kimmerle (1976) reported 1 hour LC_{50} value of >4.17 mg l^{-1} for both rats and mice (information on strains not given). RTECS (1978) reported values of 0.98 mg l^{-1} for rats and 0.42 mg l^{-1} for mice though no timescales were given.

Table 8.6 Summary of the studies assessing potential endocrine mediated responses in wildlife

Environmental compartment	Taxonomic group	Type of study	Species and exposure route used	Concentration series used	Lowest reported NOEC	Reference
Aquatic	Amphibians	No data	-	-	-	-
	Fish	No data	-	-	-	-
	Invertebrates	Reproduction (OECD TG 211)	*Daphnia magna* – aqueous exposure	No data	0.7 mg l^{-1} (a)	Canton *et al* (1985)
Terrestrial	Birds	No data	-	-	-	-
	Invertebrates	No data	-	-	-	-
Aerial	Invertebrates	No data	-	-	-	-

a – No information is available on the mechanism of action

D. Other routes of exposure

Magos *et al* (1958) exposed rats to doses of 280, 420, 621, 940 1400 and 2098 mg kg^{-1} by a single intra-peritoneal injection. All animals given the three highest doses died within 2-4 days after treatment. The levels of methomeglobin measured at the different doses were 6.6, 6.9, 21.7, 23.6, 16.0 and 27.1%.

Sisa *et al* (1959) reported an LD_{50} value of 940 mg kg body $weight^{-1}$ for rats exposed to 4-nitrotoleune via an intra-peritoneal injection.

8.5.1.2 Repeat dose studies

A large number of repeat dose studies are listed in IUCLID (2000) with varying degrees of information on the findings being reported. Table 8.7 summarises these studies but this section focuses on the results with the lowest NOELs or information on mechanisms of action which are relevant to this review.

A. Oral exposure

Ciss (1978, 1980) investigated the short-term (4 week) and longer-term (24 weeks) effects of 4-nitrotoluene on male and female Wistar rats. In the short-term study rats received doses of 500 and 100 mg kg body $weight^{-1}$ as a suspension in 1% methylcellulose once per day, 5 days per week for 4 weeks. For both male and female rats no deaths were reported at any test dose.

The systemic effects observed in other repeat dose studies (NTP 1992, Dunnick *et al* 1994 are described in Section 8.4.1.2.

B. Dermal exposure

No data has been located on the repeat dose toxicity of 4-nitrotoluene to laboratory mammals following dermal exposure.

C. Inhalation exposure

No data has been located on the repeat dose toxicity of 4-nitrotoluene to laboratory mammals following inhalation exposure.

D. Other routes of exposure

No data has been located on the repeat dose toxicity of 4-nitrotoluene to laboratory mammals following exposure by sub-cutaneous or intra-peritoneal injection.

Table 8.7 Summary of general mammalian toxicity data (Information from IUCLID 2000)

Test type	Test species	Exposure period	Test dose series used	Endpoint	Effect dose	Reference	Study validity
Acute Oral Toxicity	Rat	Not relevant	No data	Median lethal dose (LD_{50})	7100 mg kg body weight^{-1}	Sisa *et al* (1959)[1]	Use with care[2]
		Not relevant	No data	Median lethal dose (LD_{50})	2144 mg kg body weight^{-1}	Back *et al* (1972)[1]	Use with care[2]
		Not relevant	No data	Median lethal dose (LD_{50})	1960 mg kg body weight^{-1}	Vasilenko *et al* (1975)[1]	Use with care[2]
		Not relevant	No data	Median lethal dose (LD_{50})	>2250 mg kg body weight^{-1}	Groning and Kimmerle (1976)[1]	Use with care[2]
	Rat (Sprague-Dawley)	Not relevant	No data	Median lethal dose (LD_{50})	2140 mg kg body weight^{-1}	Vernot *et al* (1977)[1]	Valid[2]
	Rat (Males)	Not relevant	Oral gavage at 1000, 3000, 5000, 7000 and 9000 mg kg^{-1}	Median lethal dose (LD_{50})	4700 mg kg body weight^{-1}	Ciss (1980)[1]	Valid[2]
	Rat (Females)	Not relevant	Oral gavage at 1000, 3000, 5000 and 7000 mg kg^{-1}	Median lethal dose (LD_{50})	3200 mg kg body weight^{-1}	Ciss (1980)[1]	Valid[2]
	Mouse	Not relevant	No data	Median lethal dose (LD_{50})	1231 mg kg body weight^{-1}	Back *et al* (1972)[1]	Use with care[2]
		Not relevant	No data	Median lethal dose (LD_{50})	1280 mg kg body weight^{-1}	Vasilenko *et al* (1975)[1]	Use with care[2]
	Mouse (CF1)	Not relevant	No data	Median lethal dose (LD_{50})	1230 mg kg body weight^{-1}	Vernot *et al* (1977)[1]	Valid[2]
	Rabbit	Not relevant	No data	Median lethal dose (LD_{50})	1750 mg kg body weight^{-1}	Vasilenko *et al* (1975)[1]	Use with care[2]
Acute Dermal Toxicity	Rat	Not relevant	No data	Median lethal dose (LD_{50})	>16000 mg kg body weight^{-1}	Sisa *et al* (1959)[1]	Use with care[2]
		Not relevant	No data	Median lethal dose (LD_{50})	>750 mg kg body weight^{-1}	Kinkead *et al* (1976)[1]	Use with care[2]

Table 8.7 Continued

Test type	Test species	Exposure period	Test dose series used	Endpoint	Effect dose	Reference	Study validity
Acute Inhalation Toxicity	Rat	1 hour	No data	LC_{50}	>4.17 mg l^{-1}	Groning and Kimmerle (1976) [1]	Use with care[2]
		No data	No data	LC_{50}	0.98 mg l^{-1}	RTECS (1978) [1]	Use with care[2]
	Mouse	1 hour	No data	LC_{50}	>4.17 mg l^{-1}	Groning and Kimmerle (1976) [1]	Use with care[2]
		No data	No data	LC_{50}	0.42 mg l^{-1}	RTECS (1978) [1]	Use with care[2]
Acute Toxicity (Intra-peritoneal injection)	Rat	No data	No data	Median lethal dose (LD_{50})	940 mg kg body $weight^{-1}$	Sisa *et al* (1959) [1]	Use with care[2]
Repeated Dose Toxicity (Oral)	Rat (Male and Female Wistar)	4 weeks	0, 500, 1000 mg kg^{-1} body weight	NOAEL	No data	Ciss (1980)	Valid
		24 weeks	0, 400 mg kg^{-1} body weight	NOAEL	No data		
	Rat (Male Fischer 344)	13 weeks	0, 90, 180, 360 mg kg^{-1} body weight	NOAEL	No data	Morrissey *et al* (1988)	Valid
	Rat (Male and Female F344/N)	14 days	0, 1250, 2500, 5000, 10000 and 20000 ppm	NOAEL	20000 ppm	Dunnick *et al* (1994	Valid
		13 weeks	0, 650, 1250, 2500, 5000 and 10000 ppm	NOAEL	No data		
	Mouse (Male B6C3F1)	13 weeks	0, 40, 80 and 160 mg kg^{-1} body weight	NOAEL	No data	Morrissey *et al* (1988)	Valid
	Mouse (Male and Female B6C3F1)	14 days	0, 675, 1250, 2500, 5000 and 10000 ppm	NOAEL	No data	Dunnick *et al* (1994)	Valid
		13 weeks	0, 675, 1250, 2500, 5000 and 10000 ppm	NOAEL	No data		
	Mouse (Female B6C3F1)	14 days	0, 200, 400 and 600 mg kg^{-1} body weight	NOAEL	No data	Lysy (1988) Munson (1991)	Use with care

[1] – Cited In IUCLID (2000), [2] – Assessment based on the data in IUCLID (2000)

8.5.1.3 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity in mammals

A 3 month oral exposure study (Ciss 1980) has indicated that 4-nitrotoluene affected testicular morphology only at a high, generally toxic dose without significant effects on reproduction or the offspring. A reproduction/development study is currently being carried out by Bayer which will provide data relevant to this finding. No conclusions can be drawn at present regarding the effects of 4-nitrotoluene on the development of laboratory mammals due to an absence of test data.

In both a 13 week subchronic study in rats and mice (Dunnick *et al* 1994) and a two year carcinogenicity study in rats and mice (NTP 2001) effects were evident on reproductive tissues which could be affected by the action of endocrine active substances. However, the germinal epithelial atrophy observed in the testes of male rats at exposure concentrations >110 mg kg body weight^{-1} day^{-1} could be due to endocrine mediated responses or to direct cytotoxic effects. The available studies provide no consideration of changes in endocrine function.

Toxicity studies in rats and mice have indicated that the major systemic response to exposure to 4-nitrotoluene after acute or repeat exposure is methemoglobinemia leading to anaemia, Heinz body formation, reticulocytosis and increased haematopoesis (BUA 1989, NTP 1992). Effects observed in the spleen (haemosiderin disposition and congestion) are considered to be secondary responses to the erythrocyte damage induced by methemoglobinemia. These effects have been observed at exposure doses of 42 mg kg body weight^{-1} day^{-1} in rats and 131 mg kg body weight^{-1} day^{-1} in mice which represent markedly lower doses than those shown to cause potential endocrine mediated responses (>110 mg kg body weight^{-1} day^{-1} in rats and >660 mg kg body weight^{-1} day^{-1} in mice). As a result it is probable that the most toxic effects observed in mammals following exposure to 4-nitrotoluene are not endocrine mediated.

8.5.2 Studies relevant to the assessment of general toxicity in wildlife

8.5.2.1 Studies on aquatic organisms

Table 8.8 summarises the general toxicity data for aquatic organisms exposed to 4-nitrotoluene.

A. Fish

Acute toxicity

A number of acute studies have been carried out by Bayer AG where different species of fish were exposed to 4-nitrotoluene, but no experimental details have been provided. These studies were not carried out to GLP, had no analytical monitoring and no information on study conditions were provided so should, therefore, be used with care (IUCLID 2000).

The acute 96-hour LC_{50} values reported for fathead minnows (*Pimephales promelas*), golden orfe (*Leuciscus idus*) and Japanese medaka (*Oryzias latipes*) were 49.7 mg l^{-1}, 73 mg l^{-1} and 51 mg l^{-1} respectively (IUCLID, 2000). A higher 48 hour LC_{50} of 74 mg l^{-1} was found for the same species in another study (IUCLID, 2000).

A 96-hour LC_{100} value of 100 mg l^{-1} was calculated for zebrafish (*Brachydanio rerio*) in a static test and in the same study an LC_0 of 75 mg l^{-1} was obtained.

Chronic toxicity

A number of chronic studies have been carried out by Bayer where different species of fish were exposed to 4-nitrotoluene, but no experimental details have been provided. A 14 day LC_{50} (mortality) of 49 mg l^{-1} and an EC_{50} (behaviour) of 21 mg l^{-1} were found for guppy (*Poecilia reticulata*) when tested under semi-static conditions (IUCLID 2000). A 28 day LC_0 of 10 mg l^{-1} was found for the same species in another study (IUCLID, 2000).

A 40 day LC_0 value of 1 mg l^{-1} was calculated for Japanese medaka (*Oryzias latipes*) in one study, while in another study with the same species a 28-day NOEC value of 0.8 mg l^{-1} and a 28-day LOEC value of 2.8 mg l^{-1} was derived (IUCLID, 2000).

Canton *et al (*1985) investigated the effects of exposure of 4-nitrotoluene on the behaviour and lethality of Japanese medaka (*Oryzias latipes*) and showed an effect concentration of >1 mg l^{-1} (IUCLID, 2000).

B. Invertebrates

Acute toxicity

Only limited toxicity data with few experimental details are available for freshwater invertebrates, mostly for the water flea *Daphnia magna.* These studies were not carried out to GLP, had no analytical monitoring and no information on study conditions were provided so should, therefore, be used with care (IUCLID 2000).

The 24 hour EC_{50} (immobilisation) values reported for *Daphnia magna* range from of 7 to 11 mg l^{-1} with corresponding EC_0 values ranging from 3 to 5.6 mg l^{-1} (IUCLID 2000).

Chronic toxicity

In a 21-day life cycle toxicity study carried out with *Daphnia magna* by Canton *et al* (1985) the LC_{50} value for lethality was calculated to be 3.2 mg l^{-1}.

C. Summary of the general toxicity data for aquatic organisms

From the available toxicity data for 4-nitrotoluene it was noted that the most sensitive trophic levels based on freshwater data would appear to be fish. The lowest valid NOEC value was 0.8 mg l^{-1} for mortality in the Japanese medaka (*Oryzias latipes*) after 21 days exposure.

8.5.2.2 Studies on terrestrial organisms

No general toxicity data for terrestrial organisms following exposure to 4-nitrotoluene has been located*.*

8.5.2.3 Studies on aerial organisms

No general toxicity data for aerial organisms following exposure to 4-nitrotoluene has been located*.*

Table 8.8 Summary of general toxicity data for aquatic organisms (Information from IUCLID 2000)

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study validity
Acute Fish Toxicity	Fathead minnow (*Pimephales promelas)*	96h	No data	LC_{50}	49.7 mg l^{-1}	BUA (1989)[1]	Use with care[2]
	Golden orfe (*Leuciscus idus)*	96h	No data	LC_{50}	73 mg l^{-1}	BUA (1989)[1]	Use with care[2]
	Japanese Medaka *(Oryzias latipes)*	48h	No data	LC50	74 mg l^{-1}	MITI (1992)[1]	Use with care[2]
		96h	No data	LC_{50} (Mortality)	51 mg l^{-1}	BUA (1989)[1]	Use with care[2]
		96h	No data	EC_{50} (Behaviour)	18 mg l^{-1}		
	Zebrafish *(Danio rerio)*	96h	No data	LC_0	75 mg l^{-1}	BUA (1989)[1]	Use with care[2]
		96h	No data	LC_{100}	100 mg l^{-1}		
Chronic Fish Toxicity	Guppy *(Poecilia reticulata)*	14d	No data	LC_{50}	49 mg l^{-1}	BUA (1989)[1]	Use with care[2]
		14d	No data	LC_{50}	21 mg l^{-1}		
		28d	No data	LC_0	10 mg l^{-1}	BUA (1989)[1]	Use with care[2]
	Japanese Medaka *(Oryzias latipes)*	21d	No data	Behaviour and lethality	> 1 mg l^{-1}	Canton *et al* 1985	Valid
		28d	No data	NOEC	0.8 mg l^{-1}	BUA (1989)[1]	Use with care[2]
		28d	No data	EC_{50}	2.8 mg l^{-1}		
		40 d	No data	LC_0	1 mg l^{-1}	BUA (1989)[1]	Use with care[2]

Table 8.8 Continued

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study validity
Acute Invertebrate Toxicity	*Daphnia magna*	24h	No data	EC_0	3 – 4 mg l^{-1}	Hoechst AG (1982) [1]	Use with care[2]
		24h	No data	EC_{50}	7 – 11 mg l^{-1}		
		24h	No data	EC_0	5.6 mg l^{-1}	BUA (1989) [1]	Use with care[2]
		24h	No data	EC_{50}	9 mg l^{-1}		
		24h	No data	EC_{100}	12 mg l^{-1}		
		24h	No data	EC_{50}	7.5 mg l^{-1}	BUA (1989) [1]	Use with care[2]
Chronic Invertebrate Toxicity	*Daphnia magna*	21d	No data	LC_{50}	3.2 mg l^{-1}	BUA (1989) [1]	Use with care[2]

[1] – Cited In IUCLID (2000), [2] – Assessment based on the data in IUCLID (2000)

8.2.4 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity in wildlife

Comparison of the limited data on potential endocrine mediated responses in the aquatic invertebrate *Daphnia magna* with the acute and chronic data for this species indicates that mortalities ($\leq$3.2 mg l^{-1}) were evident at higher concentration than the threshold concentration for effects on reproduction (0.7 mg l^{-1}). However, no comparisons could be made for fish due to the absence of data on potential endocrine mediated responses in this key taxa.

8.6 Current classification of the substance against European Commission and national regulations

Table 8.9 summarises the current classification of the substance against Council Directives in order to assess the regulations to which 4-nitrotoluene is subject. Under Directive 67/548/EEC the R phrases indicate that 4-nitrotoluene is toxic to aquatic organisms (R51) and may cause long-term adverse effects in the aquatic environment (R51).

Table 8.9 Current classifcation of 4-nitrotoluene against Council Directives

Directive	Status (listed or not)
67/548/EEC - Classification, packaging and labelling of dangerous substances	Classified: T, N R phrases: 23/24/25-33-51/53

No national environmental quality standards for 4-nitrotoluene have been derived in any European country for the protection of aquatic or terrestrial ecosystems.

8.7 Exposure data

8.7.1 Worker exposure

Data on the concentrations of 4-nitrotoluene to which workers are potentially exposed during the production and use of this substance has been sought from the relevant CEFIC Sector Group. In the workplace exposure to 4-nitrotoluene can occur by inhalation of vapour and absorption through intact skin. Basic atmospheric monitoring carried out in the United Kingdom indicated that exposure by inhalation was generally well below the Occupational Exposure Standard (OES) of 30 mg/m^3 (IUCLID 2000).

Measurements within the scope of the monitoring duty according to the Gefahrstoff-Verordnung (German Dangerous Substances Regulations) were below 1 mg/m^3 for production and processing in 1995 to 1999 at a German production site.

8.7.2 Consumer exposure

The indications from the relevant CEFIC Sector Group are that 4-nitrotoluene is only used as a chemical intermediate and no products are produced which should result in consumer exposure.

In Germany indoor air from different sources was analysed for 86 target chemicals including 4-nitrotoluene and 4-nitrotoluene could not be measured above the detection limit of 0.0127 µg per sample in any of the air samples tested (Rudel 2001).

8.7.3 Environmental exposure data

At present only limited measured environmental exposure data has been obtained based on searches of the COMMPS database and literature sources.

8.7.3.1 Aquatic environment

Information from RIWA (1998) indicated that levels of 4-nitrotoluene in surface waters were between <0.1 and 1.0 μg l^{-1}.

Throughout Germany a comprehensive monitoring programme on several chemicals in various smaller rivers and streams has been conducted. The limits of determination were 0.02 to 0.5 μg l^{-1} depending on the State institute where the analysis was conducted. The 90 percentile values for the period 1995 to 1999 were as follows:

River Elbe: <0.01 to 0.49 μg l^{-1} (with the highest values at the eastern border of Germany)

River Rhine: <0.02 to 0.12 - <0.5 μg l^{-1}

River Donau: < 0.02 μg l^{-1}

River Main: <0.02 to 0.08 μg l^{-1}

For tributaries to the above rivers 4-nitrotoluene was only detected in samples from the tributary to the River Main.

8.7.3.2 Terrestrial environment

No information on terrestrial environmental concentrations of 4-nitrotoluene has been located.

8.7.3.3 Aerial environment

No information on aerial environmental concentrations of 4-nitrotoluene has been located.

8.7.3.4 Comparison of environmental monitoring data and exposure concentrations causing potential endocrine mediated responses

The limited data on the concentrations of 4-nitrotoluene in European surface waters (see Section 8.5.2.2) indicates that typical levels are in the range <0.01 – 1 μg l^{-1}, though most values are probably at the lower end of the range. The only data on potentially endocrine mediated responses in aquatic organisms is a 21 day NOEC for reproduction in *Daphnia magna* of 700 μg l^{-1}, though there is no information on the mechanism of action. The use of a margin of safety (MOS)[4] approach on the assumption that effects on *Daphnia magna* reproduction were endocrine mediated would result in values of 700 - 70000. On the basis that an MOS of 100 should be required for the risk to be acceptable then 4-nitrotoluene does not apparently present a risk to aquatic organisms in terms of endocrine disrupting effects. However, there is considerable uncertainty associated with the MOS due to the absence of

[4] Margin of safety (MOS) = (Lowest NOEC for endocrine mediated responses)/Environmental concentration

data on potential endocrine mediated effects on the reproduction and development of a range of aquatic species principally fish.

8.8 Overall Conclusions on 4-Nitrotoluene

The following conclusions have been drawn from a review of the data for 4-nitrotoluene:

8.8.1 Data from studies assessing potential endocrine disrupting effects

8.8.1.1 Human related studies

- A 3 month oral exposure study (Ciss 1980) has indicated that 4-nitrotoluene affected testicular morphology only at a high, generally toxic dose without significant effects on reproduction or the offspring. However, a reproduction/development study is currently being carried out by Bayer which will provide data relevant to this finding.

- No conclusions can be drawn at present regarding the effects of 4-nitrotoluene on the development of laboratory mammals due to an absence of test data. There is also no data of definitive significance from a multi-generation reproduction study.

- In both a 13 week sub-chronic study in rats and mice (Dunnick *et al* 1994) and a two year carcinogenicity study in rats and mice (NTP 2001) effects were evident on male reproductive tissues. However, the germinal epithelial atrophy observed in the testes of male rats at exposure concentrations >110 mg kg body weight^{-1} day^{-1} could be due to endocrine mediated responses or to direct cytotoxic effects. Interpretation of the mechanism is complicated by the absence of haematological and other data in these studies which may have implicated a direct cytotoxic effect if, for example, responses were evident in other tissues with rapidly dividing cells.

- Available evidence from a Uterotrophic screening assay (Smith and Quinn 1992) indicates that no effects on uterine weight occurred in a series of three studies involving intra-peritoneal injections of 0.01 – 300 mg kg body weight^{-1}.

- *In vitro* assays using mammalian cells and tissues assessing oestrogenic mechanisms of action. The data indicates no or weak binding of 4-nitrotoluene to the human oestrogen receptor and no substance-induced proliferation of human breast cancer cells. No data is available on the androgenic and anti-androgenic effects of 4-nitrotoluene and effects on thyroid function and hormone synthesis and secretion and steroidogenesis in mammalian cells and tissues.

8.8.1.2 Wildlife studies

- The data on potential endocrine disrupting effects in wildlife was limited to a reproduction study in the water flea *Daphnia magna* which showed a NOEL of 0.7 mg l^{-1}, though no information was available on the mechanism of action. No data was available on the effects of 4-nitrotoluene on the reproduction and development of a range of aquatic species principally fish.

- No data on potential endocrine mediated responses in terrestrial or aerial species could be located. Given that 4-nitrotoluene is considered to be volatile (see Section 8.1) the absence of data on potential endocrine disrupting effects in aerial organisms represents an area of uncertainty.

- *In vitro* data from assays using cells and tissues from wildlife species showed a weak capacity of 4-nitrotoluene to bind to the rainbow trout oestrogen receptor.

8.8.2 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity

8.8.2.1 Human related studies

- Toxicity studies in rats and mice have indicated that the major systemic response to exposure to 4-nitrotoluene after acute or repeat exposure is methemoglobinemia leading to anaemia, Heinz body formation, reticulocytosis and increased haematopoesis (BUA 1989, NTP 1992). Effects observed in the spleen (haemosiderin disposition and congestion) are considered to be secondary responses to the erythrocyte damage induced by methemoglobinemia.

- Effects in mammals have been observed at exposure doses of 42 mg kg body weight^{-1} day^{-1} in rats and 131 mg kg body weight^{-1} day^{-1} in mice which represent markedly lower doses than those shown to cause potential endocrine mediated responses (>125 mg kg body weight^{-1} day^{-1} in rats and >660 mg kg body weight^{-1} day^{-1} in mice).

8.8.2.2 Wildlife studies

- In wildlife studies data was only available for aquatic species (invertebrates and fish) and not for terrestrial or aerial species. The lowest NOEC for survival in invertebrates was <3.2 mg l^{-1} (21 day NOEC value for the water flea *Daphnia magna*) was higher than the NOEC for effects on reproduction in *Daphnia magna* which could have been endocrine mediated. However, no comparisons could be made for fish due to the absence of data on endocrine mediated responses in this taxonomic group, which represents an area of uncertainty.

8.8.3 Exposure data

8.8.3.1 Workers

- Information from the relevant CEFIC Sector Group indicates that the closed production process and use of the substance as an intermediate means that worker exposure should be minimal. This is consistent with the occupational exposure monitoring data which has been identified.

8.8.3.2 Consumers

- Information from the relevant CEFIC Sector Group indicates that 4-nitrotoluene is only used as an intermediate and no products are produced which should result in consumer exposure.

8.8.3.3 Environmental

- The limited data on the concentrations of 4-nitrotoluene in European surface waters (see Section 8.5.2.2) indicates that typical levels are in the range <0.01 – 1 μg l^{-1}, though most values are probably at the lower end of the range. The only data on potentially endocrine mediated responses in aquatic organisms is a 21 day NOEC for reproduction in *Daphnia magna* of 700 μg l^{-1} though there is no information on the mechanism of action. The use of a margin of safety (MOS)[5] approach on the assumption that effects on *Daphnia magna* reproduction were endocrine mediated would result in values of 700 - 70000. On the basis that an MOS of 100 should be required for the risk to be acceptable then 4-nitrotoluene does not apparently present a risk to aquatic organisms in terms of endocrine disrupting effects. However, there is uncertainty associated with the MOS due to the absence of data on effects on reproduction and development of fish which may be endocrine mediated.

- Given that 4-nitrotoluene is considered to be volatile there is the potential for aerial organisms to be exposed to the substance.

8.9 Summary of the weight of evidence for endocrine disrupting effects in humans and wildlife and associated uncertainties

The summary of the weight of evidence for endocrine disrupting effects of 4-nitrotoluene in humans and wildlife along with associated uncertainties are given in Table 8.10.

[5] Margin of safety (MOS) = (Lowest NOEC for endocrine mediated responses)/Environmental concentration

Table 8.10 Summary of the weight of evidence conclusion and uncertainties associated with the assessment of the endocrine disrupting effects of 4-nitrotoluene

	Target group	
	Humans	Wildlife
Weight of evidence	The available data from *in vivo* studies in laboratory mammals (using oral or dermal exposure routes) indicates that 4-nitrotoluene does not cause adverse effects on reproductive endpoints (which may be endocrine mediated) at exposure levels where general systemic toxic effects are observed. The lowest NOEL in the *in vivo* studies was 110 mg kg body weight $^{-1}$ day^{-1} for histopathological effects on reproductive tissues, though the observed effects at higher doses may have resulted from direct cytotoxic action. The available data indicate that current exposure patterns to 4-nitrotoluene do not represent a risk to workers or consumers (including children).	The available aquatic effects data shows that the threshold exposure concentration of 4-nitrotoluene above which reproduction of the invertebrate *Daphnia magna* is reduced (NOEC = 0.7 mg l^{-1}) is lower than the threshold level for general toxic effects (i.e. lethality). However, there is no information on the mechanism of action for effects on the reproduction of *Daphnia magna*. The available exposure data indicate that 4-nitrotoluene does not represent a risk to aquatic organisms.
Uncertainties	There are uncertainties with regard to the evaluation of potential adverse effects of 4-nitrotoluene on reproductive and developmental endpoints since data is not available from a definitive multi-generation study as well as developmental/teratogenicity studies. A reproduction screening test has been conducted and was due to be reported in late 2002. Mechanistic uncertainties exist because the available studies provide no direct measurement of changes in endocrine function (for example changes in hormone levels).	There are uncertainties with regard to potential adverse effects of 4-nitrotoluene on reproduction and development in wildlife due to the absence of key data for: • A wider range of aquatic taxa, particularly fish • Aerial organisms The absence of data on terrestrial organisms is not a major uncertainty since 4-nitrotoluene does not strongly sorb to organic carbon and the potential for these organisms to be exposed is limited. No environmental exposure data for 4-nitrotoluene in the terrestrial and aerial compartments has been located.

8.10 References

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9. REVIEW OF DATA FOR O-PHENYLPHENOL

Table of Contents

Notes:

This section contains information collected and collated from a range of sources including published papers, reports of studies conducted by industrial companies or sector groups and data compliations such as IUCLID (2000) and a recent WHO/FAO toxicological review of o-phenylphenol (WHO 2000). The data from IUCLID has been taken as accurate and individual source documents have not been checked unless they are considered to be key studies which have a major influence on the outcome of the review. All information taken from IUCLID has been referenced as being from that source and individual references have not been given in the references.

This review has been carried out in accordance with the evaluation framework described in Section 2. In the review the International Programme for Chemical safety (IPCS) definition of an endocrine disrupter has been adopted, namely that it is "*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*".

In the context of the review it is recognised that there are various laboratory-based *in vivo* and *in vitro* methods utilising a range of (eco)toxicological endpoints that are claimed by different sources to be relevant to the assessment of endocrine disruption in humans and wildlife. However, since this field is still in an early stage of development there is uncertainty regarding the significance of many of the current findings.

From the numerous recent reviews of potential test methods (such as the Detailed Review Paper prepared by OECD in 1997) there is a clear consensus in terms of the hierarchy of the relevance of test methods. In this hierarchy longer-term *in vivo* studies considering effects on reproduction and/or development (and including mechanistic information) are of greater relevance than short-term *in vivo* screening tests which are of greater relevance than *in vitro* assays. The greater relevance of chronic *in vivo* tests or those assessing effects during critical windows of sensitivity is also evidenced by the fact that these are the key (eco) toxicological methods being developed in the OECD Endocrine Disruption Testing and Assessment (EDTA) Programme. This hierarchy approach to data relevance has been adopted in the review along with a weight of evidence consideration of the available data.

The review has been carried out to address three key questions:

1. Does the available data indicate there is evidence that a chemical causes endocrine disrupting effects in target groups of humans/mammals and/or wildlife?

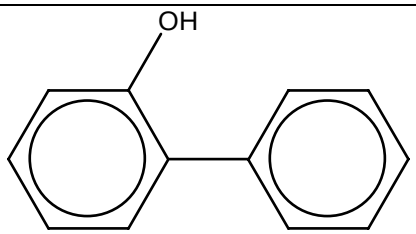
2. Do endocrine disrupting effects of the chemical in target groups of humans/mammals and/or wildlife occur at lower concentrations than those causing effect on general toxicological endpoints?

3. Are particular target groups of consumers or organisms in the environment likely to be exposed to concentrations of chemicals which exceed effects thresholds due to current emission patterns.

It should be recognised that this review is not designed to be a full Risk Assessment of a substance under the Existing Substances Regulation 793/93.

9.1 Physico-chemical data for o-phenylphenol

9.1.1 Summary details on the substance

CAS Number	90-43-7
EINECS Number	201-993-5
IUPAC Name	1,1'-Biphenyl-2-ol
Other names	o-phenylphenol, 2-phenyphenol, 2-biphenyol, 2-hydroxybiphenyl, 2-hydroxydiphenyl, o- hydroxybiphenyl, o-hydroxydiphenyl, ortho-hydroxydiphenyl
Molecular weight	170.2
Chemical formula	$C_{12}H_{10}O$
Chemical structure	

9.1.2 Physico-chemical properties and environmental fate information (from IUCLID 2000)

The data on the physico-chemical properties of o-phenylphenol and its environmental fate (see Table 9.1) indicate that the substance is to be readily biodegradable in a modified OECD Screening Test (OECD 301E).

Volatilisation is unlikely to represent a major removal process from the aquatic environment based on the Henry's Law Constant of 4.35×10^{-4} – 4.24×10^{-3} Pa-m^3 mol^{-1} (4.41×10^{-9} to 4.3×10^{-8} atm-m^3 mol^{-1}) being lower than a value range of 1-100 Pa-m^3 mol^{-1} which is considered to indicate volatility.

The low organic carbon water partition coefficient (log K_{oc}) of 2.5 indicates moderate adsorption. In soil o-phenylphenol undergoes rapid degradation with a half-life in soil between 1 and 7 days.

Table 9.1 Physico-chemical properties and environmental fate data (from IUCLID 2000)

Physico-chemical property	Value (and comments)
Physical state at ambient temperature	Solid
Water solubility	200 mg l^{-1} at 20°C
Octanol-water partition coefficient (log Kow)	3.18
Organic carbon water partition coefficient (Koc)	2.5
Henry's Law Constant	4.35 x 10^{-4} to 4.24 x 10^{-3} Pa-m^3 mol^{-1} (4.41 x 10^{-9} to 4.3 x 10^{-8} atm-m^3 mol^{-1})
Type of degradation	Information
Aquatic - abiotic	o-phenylphenol has been reported to be hydrolytically stable in the pH range 4 - 9
Aquatic - biotic	o-phenylphenol was reported to be readily biodegradable (100% loss after 10-14 days) in a modified OECD Screening Test (OECD 301E)
Terrestrial	o-phenylphenol was reported to have a half-life in soil between 1 and 7 days
Atmospheric	o-phenylphenol has a calculated hydroxyl reactivity constant (k_{OH}) of 29 x 10^{-12} cm^3 $molecule^{-1}$ s^{-1}

A Mackay Level 1 fugacity model has shown that for a discharge of 1000 tonnes of o-phenylphenol 53.8% of the substance will partition into the soil (Table 9.2), with 40.1% partitioning into the water. Amounts present in other compartments are minimal.

Table 9.2 Summary of the results of a Mackay Level 1 fugacity model

Compartment	Volumes of different compartments	% of substance present in different compartments
Water	2 x 10^{11}	40.1
Suspended sediment	10^6	0.037
Bottom sediment	10^8	1.19
Fish	2 x 10^5	0.003
Air	10^{14}	4.9
Aerosol	2000	3.6 x 10^{-4}
Soil	9 x 10^9	53.8

9.2 Production and Uses

9.2.1 Production Patterns

Information from IUCLID (2000) indicates that 2000 - 3000 tonnes of o-phenylphenol was produced in 1998 in the EU.

9.2.2 Use Patterns

o-Phenylphenol is mainly used as a biocide (preservative) in a number of applications including:

- preservation of aqueous products such as glues and adhesives, thickener solutions, concrete additives, filler suspensions and pigment slurries
- as a preservative for the leather industry
- preservation of textile auxilliaries
- preservation of whole citrus fruit

It is also used as an active ingredient for disinfectants and as a chemical synthesis intermediate for the manufacture of other chemical compounds.

9.3 Toxicokinetics, metabolism and bioaccumulation

9.3.1 Toxicokinetics and metabolism

A large range of studies have been carried out to investigate the metabolism of o-phenylphenol (OPP) in isolated tissues and whole organisms (IUCLID 2000, WHO 2000).

In the WHO (2000) review of o-phenylphenol it was stated that after oral administration to mice and rats, o-phenylphenol and its sodium salts are rapidly and extensively absorbed (95%) and distributed. Excretion is also rapid in these species, being almost complete within 48 hours, and occurs mainly in the urine (about 90%) and in the faeces (about 5%). Little radiolabel (<1%) is retained in organs and tissues including the urinary bladder. After dermal application of o-phenylphenol to humans, about 43% of the applied dose was adsorbed through the skin and about 58% was recovered in skin rinse and the protective enclosure. Most of the adosrbed radiolable was recovered in urine (99% and only 1% was recovered in the faeces). The absorption half-time was 10 hours and the elimination half-time was 0.8 hours. The rapid excretion of radiolabel into urine indicates that o-phenylphenol is unlikely to accumulate in humans exposed repeatedly. The metabolic profiles of o-phenylphenol is similar in mice, rats and humans at the various doses tested. The main metabolic pathways are conjugation of o-phenylphenol or hydroxylation at the 5 position of the phenol ring, followed by conjugation with glucuronide or sulphate. The parent compound was only detected in very small amounts (0.4%) in urine.

9.3.2 Bioaccumulation

The data from biokinetic and metabolism studies in mammals (see Section 9.3.1) as well as experimental long-term toxicological studies indicates that bioaccumulation does not occur (WHO 2000).

In a bioaccumulation study conducted by Bayer (1999) in zebrafish (*Danio rerio*) a bioconcentration factor (BCF) of 21.7 was reported, indicating that o-phenylphenol does not apparently bioaccumulate in aquatic organisms.

9.4 Studies relevant to the assessment of potential endocrine disrupting effects

9.4.1 Studies relevant to the assessment of potential endocrine disrupting effects in humans

9.4.1.1 In vitro studies

A. Receptor competitive binding assays

Blair *et al* (2000) used an oestrogen receptor competitive binding assay using uterine cytosol preparations from adult Sprague-Dawley rats to assess the relative binding affinity of o-phenylphenol to the human oestrogen receptor (ERα). No IC_{50} value could be established for o-phenylphenol over a concentration range up to 0.1 mM (17 mg l^{-1}) and the substance was classified as a non-binder in the assay.

B. Recombinant yeast assays

Routledge and Sumpter (1997) used recombinant yeast cells transfected with the human oestrogen receptor to assess the oestrogen-type response of o-phenylphenol. It was found that o-phenylphenol caused a weakly positive response with effects only being evident at concentrations 10^6 - 10^7 higher than those for 17β-oestradiol.

Rehmann *et al* (1999) assessed the activity of o-phenylphenol in yeast cells expessing a protein carrying the hormone binding domain of the human oestrogen receptor. Concentrations of 10^{-5} mM, 10^{-2} mM and 1 mM did not induce any response in the assay, whereas diethylstilbestrol was active at a concentration of 10^{-5} mM.

In a yeast oestrogen screen Vingaard *et al* (2000) determined a maximum 17β-oestradiol equivalent of o-phenylphenol of 3 x 10^{-15}. Miller *et al* (2001) have also used yeast cells transfected with human oestrogen receptor (ERα) to investigate the oestrogenic activity of o-phenylphenol. The relative potency of o-phenylphenol was 2 x 10^6 times lower than that of 17β-oestradiol based on a comparison of the exposure concentrations required to produce a half-maximal response.

C. Mammalian cell growth assays

In the E-Screen assay using MCF-7 human breast cancer cells Soto *et al* (1997) found that o-phenylphenol caused a weakly positive response with cell proliferation only being evident at concentrations 10^6 - 10^7 higher than those required for 17β-oestradiol. The maximum cell yield induced by o-phenylphenol was 30% when compared to the maximum cell yield induced by 17β-oestradiol.

In another assay using MVLN cells (MCF cells stably transfected with the Vit-Luc reporter gene) Itoh *et al* (2000) determined the transcriptional activity of o-phenylphenol by measuring the activity of luciferase in a cell lystate. The relative oestrogenic potency of o-phenylphenol was 5.4 x 10^3 times lower when compared to 17β-oestradiol. The maximum relative luciferase activity stimulated by o-phenylphenol was 23% of that for 17β-oestradiol.

Summary of *in vitro* data

Table 9.3 summarises the available *in vitro* data for o-phenylphenol which primarily relates to *in vitro* assays assessing the oestrogenic mechanism of action in mammalian cells and tissues. The data indicates no or weak binding affinity to the human oestrogen receptor and limited proliferation of mammalian cells following exposure to o-phenylphenol. No data is available on the androgenic and anti-androgenic effects of o-phenylphenol and effects on thyroid function and hormone synthesis and secretion and steroidogenesis in mammalian cells and tissues.

Table 9.3 Summary of the *in vitro* data in isolated mammalian cells and tissues relating to different mechanisms of action of o-phenylphenol

Mechanism of endocrine disruption	Responses observed in *in vitro* systems
Oestrogenicity/anti-oestrogenicity	Data indicates no or weak binding affinity to the oestrogen receptor and limited proliferation of mammalian cells following exposure to o-phenylphenol.
Androgenicity/anti-androgenicity	No data identified
Thyroid effects	No data identified
Effects on hormone synthesis or secretion	No data identified
Effects on steroidogenesis	No data identified

9.4.1.2 In vivo studies

Tables 9.4 and 9.5 summarises the information on potential endocrine mediated responses in laboratory mammals following oral and dermal exposure respectively.

A. Effects on endocrine glands and hormone sensitive tissues

Hodge *et al* (1952) investigated the toxicological properties of o-phenylphenol in both human subjects (skin sensitisation) and laboratory mammals. A series of longer-term oral exposure studies in rats and dogs were carried out which are summarised in the table below. These studies were clearly not designed to evaluate the endocrine disrupting effects of o-phenylphenol and the dates of the studies have to raise some concerns regarding the quality of the data. However, these studies do provide data that can be used for comparisons with more recent and relevant data.

Study type in Hodge *et al* (1952)	Test organism	Duration	Dose range	Relevant endpoints measured

Repeat dose – Oral gavage	Young male and female albino rats (5 of each sex per group)	6 months	0, 50, 100, 200 and 500 mg kg^{-1}, 5 days per week	Endocrine gland or hormone sensitive tissue weights and histo-pathology
Repeat dose – Oral gavage	Male and female rats (12 of each sex group)	3 months	0, 0.1, 0.3, 1.0 and 2.0% by weight , daily	"
Two year - Oral feed	28 day weanlings into 4 matched groups of 25 males and 25 females	24 months	0, 0.02, 0.2 and 2% by weight, daily	"
One year – Oral feed	2 dogs per group	1 year	0, 20, 200 and 500 mg kg^{-1} daily	"

Male and female rats (25 of each sex per group) that were maintained for two years on diets containing 0 (control), 0.02 and 0.2% o-phenylphenol showed no adverse effects at any test dose as judged by mortality, growth, gross appearance, haematology, urinary sugar and protein values, organ weights (including the testis) and histopathological examination of various tissues (including the adrenal and testes). A similar group of rats maintained for two years on a diet containing 2% o-phenylphenol deviated from the control by exhibiting increased testes weight as well as slight retardation of growth and histological kidney changes (with marked tubular dilation). Dogs (two per group) that received oral doses of 0 (control), 20, 200 and 500 mg kg body $weight^{-1}$ day^{-1} for a period of one year showed no adverse effects as judged by body weights, gross appearance, haematology, urinary sugar and protein values, organ weights (including the testis) and histopathological examination of various tissues (including the adrenal, thyroid, testes or uterus and ovaries).

In a study conducted by Cosse *et al* (1990) groups of beagle dogs (4 per sex per group) were given o-phenylphenol doses of 0, 30, 100 and 300 mg kg body $weight^{-1}$ day^{-1} in a study conducted to US EPA FIFRA Section 158.340, Guideline 83-1 and GLP. The doses were given in a peanut oil solution via gastric intubation 5 days per week for one year. They had been selected based on a palatability/probe and a 4 week study wherein repeated emesis was observed after administration of 400 mg kg body $weight^{-1}$ day^{-1} and above via capsules or gastric intubation, or when dietary rejection occurred when o-phenylphenol was administered in the feed.

In the main study, a treatment-related increase in emesis occurred in males at 300 mg kg body $weight^{-1}$ day^{-1} and females at 100 and 300 mg kg body $weight^{-1}$ day^{-1}. The emetic activity was characterised as a local transitory response of the upper alimentary duct rather than being initiated via the central nervous system.

No histopathological findings were noted in organs and tissues potentially susceptible to endocrine active compounds including the adrenals, cervix, epididymids, mammary glands, ovaries, oviducts, parathyroids, pituitary, prostate, seminal vesicles, testes, thyroid, uterus and vagina). No adverse toxicological effects were also noted on a range of parameters, including body weight, food consumption, haematology, urinalysis, clinical chemistry, opthalmology, organ weights, gross necropsy and histopathology of a complete set of tissues from all dogs, up to the highest dose tested. The chronic no-observed adverse effect-level (NOAEL) for o-phenylphenol in beagle dogs was reported as 300 mg kg body $weight^{-1}$ day^{-1}.

B. Reproduction and fertility studies

Eigenberg (1990) tested o-phenylphenol in a two generation reproduction toxicity study in rats involving oral dosing applied via the feed at nominal levels of 0, 40, 140 and 490 mg kg body weight^{-1} day^{-1}, and carried out to US EPA FIFRA Section 158.340, Guideline 83-4 and GLP. The actual doses given were 0, 35, 125 and 457 mg kg body weight^{-1} day^{-1} based on the mean analytical concentrations in the diet. OPP was administered 15 weeks prior to mating and continued throughout mating, gestation and lactation, with further exposure of the offspring after weaning.

No treatment-related effects were observed for clinical signs, gestation and lactation body weight gain and reproductive parameters. There were also no treatment-related clinical signs in pups and no effects on pup viability. Gross and histopathological examination of the pups did not reveal any treatment-related lesions, and for adults no treatment related lesions of the reproductive tract were observed. However, the following significant findings were identified:

- statistically significant decreases in weight were evident in the highest dose (457 mg kg body weight^{-1} day^{-1}) F_{1b}, F_{2a} and F_{2b} pups on days 14 and/or 21 of lactation and were considered to be compound related. However, a decrease was not observed during the first week of lactation, which indicated that there was no early effect on early pup development. These decreases corresponded to the time period in which neonatal rats would be expected to consume the treated diets.

- treatment-related decreases in body weight were evident in the F_0 and F_1 adults exposed to the 457 mg kg body weight^{-1} day^{-1} dose.

- statistically significant elevations in adult relative kidney weight in the F_0 and F_1 adults exposed to the 457 mg kg body weight^{-1} day^{-1} dose.

- statistically significant elevations in transitional cell hyperplasia/papillomatosis in the urinary bladder of F_0 males and females and F_1 males exposed to the 457 mg kg body weight^{-1} day^{-1} dose. Hyperplasia was quantitated by cell layer and micrometer measurements of epithelial depth. These measurements confirmed the microscopic finding at the 457 mg kg body weight^{-1} day^{-1} and also showed a significant increase in these measurements in the 125 mg kg body weight^{-1} day^{-1} F_0 males and females.

- Other adult urinary tract changes were elevated above the controls but were either not found in both F_0 and F_1 generations or were not statistically significant. These lesions in themselves would not be significant but together indicate a compound-related effect on the urinary tract system at the 125 and 457 mg kg body weight^{-1} day^{-1} doses, which confirms previously published findings.

The reproductive no-observed-effect-level (NOEL) was 457 mg kg body weight^{-1} day^{-1} for both adults and resulting offspring. The overall NOEL for systemic organ toxicity in the adults, based on morphological changes, was 35 mg kg body weight^{-1} day^{-1}.

In a further two generation reproduction study rats (Sprague-Dawley) were subjected to oral dosing of o-phenylphenol applied via the feed at doses of 0, 20 100 and 500 mg kg body weight^{-1} day^{-1} and carried out to US EPA FIFRA Section 158.340, Guideline 83-4 and GLP (Eigenberg and Lake 1995). Application of OPP started 10 weeks prior to mating and continued throughout mating, gestation and lactation, with further exposure of the offspring after weaning. F_0 adults were mated to produce F_{1a} and F_{1b} litters and F_1 adults (randomly selected F_{1b} pups) were mated to produce F_{2a} and F_{2b} litters.

There were no statistically significant effects at any test dose on adult reproductive parameters and litter size, gender, the number of stillborn pups, pup viability, clinical signs or pup gross lesions. No compound-related lesions were found in the histopathology of the reproductive organs and pituitary of adult F_0 and F_1 males and females.

The following significant conclusions were drawn from the study:

- a compound-related decrease in body weight in F_0 and F_1 males and females in the 500 mg kg body weight^{-1} day^{-1} was evident.
- a compound-related increase in food consumption was evident in the females in the 500 mg kg body weight^{-1} day^{-1} during the lactation phase but not in males.
- a compound-related decrease in adult terminal body weight was evident in the F_0 and F_1 males and females in the 500 mg kg body weight^{-1} day^{-1} dose.
- no compound-related effects on adult organ weights were evident.
- one high dose (500 mg kg body weight^{-1} day^{-1}) F_0 male died from kidney failure, which may have been compound-related.
- urinary bladder calculi were noted at necropsy in high dose group F_1 adult males and were considered to be compound-related. Compound-related urine staining was observed in the high-dose group F_0 and F_1 males.
- Micropathology examination of the kidney showed debris in the renal pelvis, chronic active inflammation and increased severity of the background lesions in high dose group F_0 and F_1 males that were considered to be treatment-related. Treatment-related transitional cell hyperplasia (simple and /or nodular/papillarty) calculi and chronic inflammation were found in the urinary bladder of the high-dose group F_0 and F_1 males. Dilutation and hyperplasia (combined) of the ureter in high-dose group F_0 and F_1 males were also considered to be compound-related.

The reproductive NOEL in the study was reported as 500 mg kg body weight^{-1} day^{-1}. The parental and neonatal toxicity NOEL was 100 mg kg body weight^{-1} day^{-1} based on:

1. a decrease in body weight for males and females in the 500 mg kg body weight^{-1} day^{-1};
2. morphologic (gross and/or microscopic) lesions in the kidney, urinary bladder and ureter of 500 mg kg body weight^{-1} day^{-1} group males ;
3. a possible compound-related death in the high-dose group males;
4. a decrease in pup body weights in the 500 mg kg body weight^{-1} day^{-1}.

C. Developmental and teratogenicity studies

Ogata *et al* (1978) reported that oral administration of OPP to the JCL-ICR strain of mouse from gestation days 7 to 19 at levels of 0, 1450, 1740 and 2100 mg kg body weight^{-1} day^{-1} produced evidence of maternal toxicity and delayed foetal development, but was not teratogenic. Negative results were also obtained in mice upon administration of the sodium salt of o-phenylphenol at levels up to 400 mg kg body weight^{-1} day^{-1}.

Kaneda *et al* (1978) investigated the potential teratogenic and mutagenic effects of o-phenylphenol in pregnant Wistar rats which were given OPP by oral gavage on days 6-15 of gestation. The dose range used was 0, 150, 300, 600 and 1200 mg kg^{-1} and the animals were killed on day 20 of gestation and their uterine contents were examined for implantation sites and live or dead foetuses. Living young were inspected externally for anomalies and weighed individually.

At doses of 300 mg kg^{-1} or above pregnant rats fell into ataxia for several hours and the severity was enhanced in a dose-related manner. Suppression of a gain in maternal weights began 3 days after initiation of the treatment and continued to the end of the gestation period. Ten of eleven dams given the highest dose of 1200 mg kg^{-1} died after 3 to 9 days of the treatment. In the 150 mg kg^{-1} group neither signs of toxicity nor deaths were seen in the dams. Mean numbers of implantation sites and live foetuses, resorption rates and foetal weights for groups treated with 150 and 300 mg kg^{-1} were comparable to those for the control animals. At 600 mg $kg^{-1,}$ foetal resorptions increased and the weights of survivors decreased significantly. A few anomalies were observed in the rat foetuses in both control and treatment groups. Of the foetuses from the 300 and 600 mg kg^{-1} group only 1 or 2 showed concurrent occurrence of anomalies such as cranial or sacral meningocele and diaphragmatic hernia. However, the level of anomalies were too low to be analysed for whether they were caused by o-phenylphenol. In the 600 and 1200 mg kg^{-1} dose groups o-phenylphenol was foetotoxic but not teratogenic, even in the presence of severe maternal toxicity. The reported NOAELs for foetotoxicity and teratogenicity were 300 and 600 mg kg^{-1} respectively while the NOAEL for maternal toxicity was 150 mg kg^{-1}.

In an associated dominant lethal study by Kaneda *et al* (1978) o-phenylphenol was given to groups of 15 male mice by daily oral intubation of 0, 100 or 500 mg kg^{-1} (0.2 ml per 10g body weight) for 5 successive days. In addition, a single intra-peritoneal dose of 300 mg kg^{-1} of ethylmethanesulphonate (EMS) was used as the concurrent positive control. Immediately after the administration, each male was mated with 2 untreated virgin females. Female mice were inspected every morning for the presence of vaginal plugs. They were caged separately when the plugs were found, and the day was designated as day 0 of gestation. At the end of a week, females remaining in the male cage were replaced by 2 other untreated females. These mating procedures were repeated for 6 successive weeks. As to the females revealing no plugs, mating was presumed to have occurred on the mid-week of pairing with the males. The female mice were killed on day 12 or 13 of gestation and scored for the numbers of corpora lutea, implants, living embryos and early or late embryonic deaths. Values for each treatment group were compared with those from the corresponding control group every week and the frequency of induced dominant-lethal mutations (%) were calculated using the equation:

Dominant-lethal mutations = [(1-live embryos per test female)/(live embryos per control female)] x 100

In the assay, no dominant-lethal mutations were induced in any group tested.

A similar study was subsequently carried out by John *et al* (1981) in which o-phenylphenol was administered to pregnant Sprague-Dawley rats by oral gavage at exposure concentrations of 0, 100, 300 and 700 mg kg body $weight^{-1}$ day^{-1} on days 6 to 15 of gestation. The rats (35 in the control group and 25-27 in the treatment groups) were sacrificed on gestation day 21. Body weight gain and liver weight of dams were measured and data was collected on the numbers of implantions, live foetuses, resorptions, the foetal sex ratios, foetal body weights and foetal crown-rump lengths. All foetuses were examined externally and skeletally and the soft tissue of approximately one third of the foetuses were examined.

At the 700 mg kg body weight^{-1} day^{-1} dose body weight gain of the dams was significantly reduced on the first 3 days of administration (day 6 to 9 of gestation) as was the food intake on the following days 9 to 11. The transitory reduction in body weight gain and the reduced food intake in the 700 mg kg body weight^{-1} day^{-1} dose were regarded as treatment-related. Absolute liver weight but not relative liver weight (compared to body weight) was decreased in animals in the 700 mg kg body weight^{-1} day^{-1} dose. No treatment-related effects were observed on the number of implantations, the number of pups per litter, the frequency of resorbed embryos or the body weights of foetuses. A slight retardation of ossification of the sternebrae of 10 foetuses (4%) in the 700 mg kg body weight^{-1} day^{-1} dose group was within the historical control range observed in Sprague-Dawley rats in the test laboratory. Furthermore, these effects were considered to be a possible secondary consequence of the reduced body weight development and food intake of the dams. All other changes observed were within the range of variation of historical controls. As a result of the absence of foetotoxic or teratogenic effects at the highest dose the no-observed-effect-level (NOEL) for foetoxicity and developmental toxicity was reported as 700 mg kg body weight^{-1} day^{-1}. The no observed adverse effect level for maternal toxicity was reported as 300 mg kg body weight^{-1} day^{-1}.

Zablotny *et al* (1991a) conducted a developmental toxicity probe study using pregnant New Zealand White rabbits (7 per group) following oral gavage administration to o-phenylphenol doses of 0, 250, 500 and 750 mg kg body weight^{-1} day^{-1} on days 7 through 19 of gestation. Dose-related signs of maternal toxicity were evident at all the dose levels with one rabbit dying at the 250 mg kg body weight^{-1} day^{-1} dose, two rabbits dying at the 500 mg kg body weight^{-1} day^{-1} dose and 6 rabbits dying at the 750 mg kg body weight^{-1} day^{-1} dose. Gross histopathologic examination showed dose-related increases in the incidences of haemorrhage, gaseous distension and erosions of the stomach or decreased/soft ingesta of the gastrointestinal tract. Body weight gain was decreased in a dose-related manner in all groups.

No treatment related effects were observed on reproductive or foetal parameters at 250 and 500 mg kg body weight^{-1} day^{-1} doses whereas at the 750 mg kg body weight^{-1} day^{-1} dose the high level of mortality and small sample size precluded an adequate evaluation. Kidney weights were decreased in the animals in the 250 and 500 mg kg body weight^{-1} day^{-1} doses but could not be evaluated at the highest dose. Histopathological examination of the kidneys showed a treatment-related increase in the incidence and/or severity of tubular degeneration and inflammation at all dose levels. Examination of the stomach revealed haematogenous pigment in the mucosa in some rabbits in the 500 and 750 mg kg body weight^{-1} day^{-1} doses, Focal erosions of the mucosa were only noted in the animals in the 750 mg kg body weight^{-1} day^{-1} dose. Overall in the probe study maternal toxicity was observed at all dose levels but no foetal effects were observed up to an including the 500 mg kg body weight^{-1} day^{-1} dose. The reported NOAELs for foetotoxicity and teratogenicity was 500 mg kg body weight^{-1} day^{-1} while the NOAEL for maternal toxicity was <250 mg kg body weight^{-1} day^{-1}.

Based on the results of the probe study, Zablotny *et al* (1991b) investigated the maternal toxicity, embryonal/foetal toxicity and teratogenic effects of o-phenylphenol in pregnant rabbits in a study carried out to US EPA FIFRA Section 158.340, Guideline 83-3b and GLP. Groups of 16-24 artificially inseminated adult female New Zealand White rabbits were exposed to targeted doses of 0, 25, 100 and 250 mg kg body weight^{-1} day^{-1} between gestation days 7-19 by oral gavage. In life parameters evaluated included clinical observations, body weight and body weight gain. On day 28 of gestation, all surviving rabbits were euthanathised prior to necropsy. Liver, kidney and gravid uterine weights, and the number of corpora lutea, implantations, resorptions and live/dead foetuses were then recorded at necropsy. All

foetuses were removed from the uterus, weighed, sexed and examined for external, visceral and skeletal abnormalities. The kidneys from all rabbits were examined microscopically.

No adverse embryonal/foetal effects were observed at any of the doses tested. In addition no significant maternal effects were observed at 25 or 100 mg kg body weight^{-1} day^{-1}. However, maternal effects were evident at the highest dose tested (250 mg kg body weight^{-1} day^{-1}) as evidenced by:

- treatment-related increased mortality (13%);
- gross pathologic alterations (ulceration and haemorrhage of the gastric mucosa, haemolised blood in the intestinal tract and decreased ingesta);
- histopathologic alterations (renal tubular degeneration and inflammation).

In the study the no-observed-effect-level (NOAEL) for foetotoxicity and developmental toxicity was reported as 250 mg kg body weight^{-1} day^{-1}, whereas the NOAEL for maternal toxicity was given as 100 mg kg body weight^{-1} day^{-1}.

D. Carcinogenicity and oncogenicity studies

In a 2 year National Toxicology Programme study (TR 301) (NTP 1986) groups of 50 male and 50 female Swiss CD-1 mice per treatment were exposed to o-phenylphenol alone or as a promoter following initiation with 7,12-dimethylbenz(a)-anthracene (DMBA) by repeated dermal application (3 days per week) of 55.5 mg of o-phenylphenol in 0.1 ml acetone on the shaved area on the back. No significant effect of o-phenylphenol on body weights or survival was evident. Furthermore no skin neoplasms were recorded with o-phenylphenol alone or after initiation with DBMA, whereas non-neoplastic changes (inflammation, ulceration, hyperkeratosis, acanthosis) were found in both groups. Histopathological examinations revealed no neoplastic changes in any tissue in the o-phenylphenol groups including those organs and tissues potentially susceptible to endocrine active compounds (adrenals, mammary glands, ovaries, parathyroid, pituitary, prostate, testes, thyroid and uterus).

Quast and McGuirk (1995) carried out a two year combined Chronic Toxicity/Oncogenicity study to OECD Test Guideline 453 (FIFRA Guideline Number 83-5) and GLP using B6C3F1 mice. Groups of 50 mice of each sex were fed diets supplying 0, 250, 500 and 1000 mg kg body weight^{-1} day^{-1} for 2 years. A satellite group of 10 mice per sex per dose level was maintained on diets for 12 months after which time they were necropsied and evaluated for general chronic toxicity.

In-life clinical observations and mortality were unaffected by exposure dose. However, body weights and weight gains of mice in all treatment groups were decreased (6-20% and 10-38% respectively) except for the males in the 250 mg kg body weight^{-1} day^{-1} dose. Food consumption data suggested that the treated mice consumed more than their respective controls though it was suggested that the difference were due to undetected food wastage.

Haematology, clinical chemistry and urinalyses values of the mice necropsied at 12 and 24 months lacked any consistent toxicologically significant findings indicative of target organ toxicity. Organ weight data for adrenal glands, brain, heart, kidneys, liver, spleen and testes which were identified as statistically significant were confounded by the marked decrease in the body weights of the mice. However, the consistent absolute and/or relative liver weight increases at all doses suggested a treatment-related effect, even though they were not always

identified as statistically significant. Gross necropsy observations in the 1000 mg kg body weight^{-1} day^{-1} dosed male mice at 12 months, and in the 500 and 1000 mg kg body weight^{-1} day^{-1} dosed males at 24 months suggested a slight increase in the number of mice with a liver mass/nodule.

The male adrenals, kidneys, lungs, oral tissues, pancreas, peripheral nerve, spleen and testes and female kidneys, lungs and nasal tissues showed a decreased incidence of the normal background microscopic observations compared to the controls. However, these observations were interpreted to be reflective of normal variation and the decreased body weights of the mice and not a primary response due to o-phenylphenol exposure.

Microscopic examination of the liver of male and female mice at 12 and 24 months revealed treatment-related effects at all dose levels. The cytoplasm of hepatocytes stained more homogeneously, however, there was no evidence of degeneration or necrosis. The microscopic changes were dose-related and were considered to be associated with adaptation to metabolic demands. An increased incidence of eosinophilic hepatocellular foci was also observed in the 500 and 1000 mg kg body weight^{-1} day^{-1} male mice from the oncogenecity study. In male high dose mice necropsied at 12 months a slight increase in the number with an hepatocellular adenoma was observed. At 24 months a statistically significant increase in the number of male mice with an hepatocellular adenoma was noted in the 500 and 1000 mg kg body weight^{-1} day^{-1} groups (40 and 41 in 50 animals respectively compared to 27 in 50 control animals) . A low incidence of a variant form of hepatocellular carcinoma (hepatoblastoma) was observed in all treatment groups of male mice. However, there was no significant increase in the incidence of hepatocellular carcinoma in any dose group. The combined incidence of hepatoblastoma and/or hepatocellular carcinoma was also not significantly increased in the male mice. The primary non-tumorous microscopic changes in the male mice liver, which appeared to have been adaptive in nature, ultimately resulted in the promotion of hepatocellular adenomas. There were no other tissues in male mice with a statistically increased incidence of tumours. The female mice livers showed microscopic adaptive changes comparable to the males, however, no female mice had an hepatoblastoma. In addition, no statistically identified increases in the incidences of liver tumours, or tumours in any other tissue, was observed in females.

In the study the maximum tolerated dose was exceeded and a no-observed-effect-level (NOEL) for toxicity was not established at the lowest o-phenylphenol dose level of 250 mg kg body weight^{-1} day^{-1} in either male or female mice. The livers in animals of each sex were the primary target organ affected by ingestion of o-phenylphenol. A statistically significant increased incidence of hepatocellular adenomas was observed in male mice in the 500 and 1000 mg kg body weight^{-1} day^{-1} groups. There was no significant increased incidences of tumours in female mice. The microscopic liver change in the low dose (250 mg kg body weight^{-1} day^{-1}) was the most sensitive indication of a treatment-related effect. The NOEL was estimated to be approximately 100 mg kg body weight^{-1} day^{-1}.

In a subsequent two year combined Chronic Toxicity/Oncogenecity study conducted to OECD Test Guideline 453 (US EPA FIFRA Guideline Number 83-5) and GLP, Wahle and Christenson (1996) investigated the effect of o-phenylphenol on groups of F344 rats (50 animals per sex per treatment) for 2 years. In this dietary exposure study males were administered concentrations of 0, 800, 4000 and 8000 ppm (resulting in an average dose of 0, 39, 200 and 402 mg kg body weight^{-1} day^{-1}) while females received 0, 800, 4000 and 10000 ppm (resulting in an average dose of 0, 49, 248 and 647 mg kg body weight^{-1} day^{-1}). Determination of body weight and food consumption as well as detailed clinical examination of each animal were conducted weekly. Standard haematologic, clinical chemistry, and

urinalysis endpoints were evaluated from blood (drawn from the orbital sinus) and urine collected at 3, 6, 12, 18 and 24 nominal months into the study. Pre-exposure ophthalmologic exams were conducted on all acclimatised animals and then again on all surviving animals just prior to termination of the 1- and 2- year in-life segments of the study. All animals placed on study were subject to a postmortem examination which included: documenting and saving all gross lesions, weighing designated organs and collecting representative tissue specimens for histopathologic evaluation.

Mean body weights decreased in both sexes at the mid (200 and 248 mg kg body weight^{-1} day^{-1}) doses (5%) and the high (402 and 647 mg kg body weight^{-1} day^{-1}) doses (11%) while food consumption remained unchanged. Mortality was increased in the males exposed to the 402 mg kg body weight^{-1} day^{-1} dose. No histopathological effects were found in organs and tissues potentially susceptible to endocrine active compounds including the adrenals, cervix, epididymis, mammary gland, ovaries, parathyroid, pitiutary, prostate, seminal vesicles, testes, thyroid and uterus. Clinical observations included abnormal urine colour and urine stains in mid and high dose groups. Ophthalmology, haematology and clinical chemistry showed no effects while urinalyses showed an increased incidence of blood in the urine of high-dose males. No evidence of an alteration in organ weights, attributable to o-phenylphenol exposure, was suggested in either sex at any dose tested. Postmortem findings included wet ventrum with staining, urinary bladder masses, as well as pitted zones and abnormal texture in the kidney in mid and high dose groups. In males, and to a lesser extent females, exposed to the highest dose (402 and 647 mg kg body weight^{-1} day^{-1}) an increased incidence of simple urinary bladder hyperplasia (males 84% and females 12%) and nodular/papillary hyperplasia (males 86% and females 2%) was observed. Increased incidences of urinary bladder neoplasia (papilloma 12%, transitional cell carcinoma 68%) were noted in males fed diets of 8000 ppm. In contrast, no neoplastic changes were observed in females at an approximately 60% higher level of exposure over 2 years. At 4000 ppm, males showed marginal and non-statistical increase in both urinary bladder hyperplasia and transitional cell carcinoma which were considered border-line. Non-neoplastic lesions occurred in the kidney of high dose males and females.

Table 9.4 Summary of the data on potential endocrine mediated responses in laboratory mammals following oral exposure

Species	Life stage of the test organism at start of test	Exposure route and dose series	Description of endocrine disruption measurement parameter(s) and effect doses	Reference	Test Relevance	Study Validity
Beagle dog	Adults	Oral gavage at 0, 30, 100 and 300 mg kg body weight^{-1} day^{-1} for 5 days per week for 1 year	No significant histopathological effects (relative to the controls) on endocrine glands or hormone sensitive tissues (adenals, epididymus, mammary glands, ovaries, parathyroid, pituitary, prostate, seminal vesicles, testes, thyroid, uterus and vagina) at any test dose *(NOEL for effects on endocrine glands or homone sensitive tissues = 300 mg kg body weight^{-1} day^{-1})*	Cosse *et al* (1990)	Medium	Valid
Rat	Adult males and females	Dietary exposure at 0, 35, 125 and 457 mg kg body weight^{-1} day^{-1} for two generations	No significant effects (relative to the controls) on mating performance, gestation period or the ability to rear offspring to weaning at any test dose No significant effects (relative to the controls) on the reproductive tract in either sex of adults at any test dose *(NOEL for reproductive effects = 457 mg kg body weight^{-1} day^{-1})*	Eigenberg (1990)	High	Valid
Rat (Sprague-Dawley)	Adult males and females	Dietary exposure at 0, 20, 100 and 500 mg kg body weight^{-1} day^{-1} for two generations	No significant effects (relative to the controls) on mating performance, gestation period or the ability to rear offspring to weaning at any test dose No significant effects (relative to the controls) on the reproductive tract in either sex of adults at any test dose *(NOEL for reproductive effects = 500 mg kg body weight^{-1} day^{-1})*	Eigenberg and Lake (1996)	High	Valid
Rat (Wistar)	Pregnant females	Oral gavage at 0, 150, 300, 600 and 1200 mg kg body weight^{-1} day^{-1} on gestation days 6 to 15	Significant effects (relative to the controls) on embryo and foetal development at higher test doses *(NOEL for foetotoxicty = 300 mg kg body weight^{-1} day^{-1})* *(NOEL for teratogenicity = 600 mg kg body weight^{-1} day^{-1})*	Kaneda *et al* (1978)	Medium	Valid

Table 9.4 Continued

Species	Life stage of the test organism at start of test	Exposure route and dose series	Description of endocrine disruption measurement parameter(s) and effect doses	Reference	Test Relevance	Study Validity
Rat (Sprague-Dawley)	Pregnant females	Oral gavage at 0, 100, 300 and 700 mg kg body weight^{-1} day^{-1} on gestation days 6 to 15	No significant effects (relative to the controls) on embryo and foetal development at any test dose *(NOEL for foetotoxicty and developmental toxicity = 700 mg kg body weight^{-1} day^{-1})*	John *et al* (1981)	Medium	Valid
Mice (JCL-ICR)	Pregnant females	Oral administration at 0, 1450, 1740 and 2100 mg kg body weight^{-1} day^{-1} on gestation days 7 to 19	No significant effects (relative to the controls) on embryo and foetal development at any test dose *(NOEL for teratogenicity = 2100 mg kg body weight^{-1} day^{-1})*	Ogata *et al* (1978)	Medium	Valid
Rabbit (New Zealand White)	Pregnant females	Oral gavage at 0, 250, 500 and 750 mg kg body weight^{-1} day^{-1} on gestation days 7 to 19	No significant effects (relative to the controls) on embryo and foetal development in survivors at any test dose *(NOEL for foetotoxicty and developmental toxicity = 500 mg kg body weight^{-1} day^{-1})*	Zablotny *et al* (1991a)	Medium	Valid
	Pregnant females	Oral gavage at 0, 25, 100 and 250 mg kg body weight^{-1} day^{-1} on gestation days 7 to 19	No significant effects (relative to the controls) on embryo and foetal development at any test dose *(NOEL for foetotoxicty and developmental toxicity = 250 mg kg body weight^{-1} day^{-1})*	Zablotny *et al* (1991b)	Medium	Valid

Table 9.5 Summary of the data on potential endocrine mediated responses in laboratory mammals following dermal exposure

Species	Life stage of the test organism at start of test	Exposure route and dose series	Description of endocrine disruption measurement parameter(s) and effect doses	Reference	Test Relevance	Study Validity
Mice (Swiss CD-1)	Adult males and females	Exposure by dermal application at 55.5 mg in 0.1 ml acetone (3 days per week) for 2 years	No significant histopathological effects (relative to the controls) on endocrine glands or hormone sensitive tissues (adenals, epididymus, mammary glands, ovaries, parathyroid, pituitary, prostate, seminal vesicles, testes, thyroid, uterus and vagina) at any test dose *(NOEL for effects on endocrine glands or hormone sensitive tissues = 55.5 mg ml^{-1})*	NTP (1986)	Medium	Valid

The NOEL for effects on endocrine glands and hormone sensitive tissues was 402 mg kg body weight^{-1} day^{-1} for males and 647 mg kg body weight^{-1} day^{-1} for females. A systemic chronic toxicity no-observed-effect-level (NOEL) of 39 mg kg body weight^{-1} day^{-1} was established (specifically 39 mg kg body weight^{-1} day^{-1} for male rats and 248 mg kg body weight^{-1} day^{-1} for female rats).

E. General conclusions on the potential endocrine mediated responses in laboratory mammals in in vivo studies

A series of oral and dermal exposure studies in rats and mice (see Table 9.6) have investigated whether o-phenylphenol affects reproduction or developmental endpoints which may be endocrine mediated even in the presence of (high dose) toxicity of parental animals. A large number of the studies have been conducted to OECD Test Guidelines and to GLP.

Exposure of o-phenylphenol to pregnant rats and rabbits during the period of organogenesis does not induce any embryo or foetotoxicity or malformations at maternally toxic doses. The lowest NOEL recorded from these studies is a value of 250 mg kg body weight^{-1} day^{-1} for foetotoxicity and developmental toxicity in rabbits. Two generation reproduction toxicity studies in rats found no effects on reproductive parameters in adults and offspring at doses of 457 – 500 mg kg body weight^{-1} day^{-1} (Eigenberg 1990, Eigenberg and Lake 1995). In these studies NOELs for systemic organ toxicity in the adults based on morphological changes were 35 – 100 mg kg body weight^{-1} day^{-1}.

The lowest doses tested in the oral and dermal exposure studies were in the range of 25 - 30 mg kg body weight^{-1} day^{-1} and no effects which may be endocrine mediated were evident at these doses.

9.4.1.3 Human studies

At present no information on endocrine mediated responses of workers or consumers following exposure to o-phenylphenol have been identified. The information that is available primarily relates to skin sensitisation and irritation (IUCLID 2000, WHO 2000).

Table 9.5 Summary of potential endocrine mediated responses reported for o-phenylphenol in *in vivo* studies with laboratory mammals

Type of study	Species and exposure route used	Dose series used	NOEL (mg kg body weight^{-1} day^{-1})		Reference
			Endocrine mediated responses	Systemic toxicity	
Subchronic oral toxicity (OECD 408)	Beagle dogs - oral gavage	0, 30, 100 and 300 mg kg body weight^{-1} day^{-1}	300 (Histopathology)	300 (NOAEL)	Cosse *et al* (1990)
Reproduction – One generation (OECD 415)	No data	-	-	-	-
Reproduction – Two generation (OECD 416)	Rats	0, 35, 125 and 457 mg kg body weight^{-1} day^{-1}	457 (Reproduction)	35	Eigenberg (1990)
	Rats (Sprague-Dawley) - dietary exposure	0, 20, 100 and 500 mg kg body weight^{-1} day^{-1}	500 (Reproduction)	100	Eigenberg and Lake (1995)
Development/ Teratogenicity (OECD 414)	Rats (Wistar) - oral gavage	0, 150, 300, 600 and 1200 mg kg body weight^{-1} day^{-1}	300 (NOAEL – Foetotoxicity) 600 (NOAEL - developmental toxicity	150 (NOAEL – Maternal toxicity)	Kaneda *et al* (1978)
	Rats (Sprague-Dawley) - oral gavage	0, 100, 300 and 700 mg kg body weight^{-1} day^{-1}	700 (NOAEL - Foetotoxicity and developmental toxicity)	300 (NOAEL – Maternal toxicity)	John *et al* (1981)
	Mice (JCL-ICR)	0, 1450, 1740 and 2100 mg kg body weight^{-1} day^{-1}	2100 (Teratogenecity)	<2100 (Maternal toxicity)	Ogata *et al* (1978)
	Rabbit (New Zealand White) - oral gavage	0, 250, 500 and 750 mg kg body weight^{-1} day^{-1}	500 (NOAEL - Foetotoxicity and developmental toxicity	<250 (NOAEL – Maternal toxicity)	Zablotny *et al* (1991a)
	Rabbit (New Zealand White) - oral gavage	0, 25, 100 and 250 mg kg body weight^{-1} day^{-1}	250 (NOAEL - Foetotoxicity and developmental toxicity)	100 (NOAEL – Maternal toxicity)	Zablotny *et al* (1991b)
Chronic Toxicity/ Oncogenicity (OECD 453)	Mice (B6C3F1) - dietary exposure	0, 250, 500 and 1000 mg kg body weight^{-1} day^{-1}	No value defined	100	Quast and McGuirk (1995)
	Rats (F344) - dietary exposure	0, 39, 200 and 402 mg kg body weight^{-1} day^{-1} (males) 0, 49, 248 and 647 mg kg body weight^{-1} day^{-1} (females)	402 (Histopathology - males) 647 (Histopathology - females)	39 (Males) 248 (Females)	Wahle and Christenson (1996)

9.4.2 Studies relevant to the assessment of potential endocrine disrupting effects in wildlife

9.4.2.1 In vitro studies

Petit *et al* (1997) used a series of complementary bioassays to screen the oestrogenic potency of xenobiotics. The oestrogen like potential for OPP was assessed using assays concerning vitellogenin gene expression in rainbow trout hepatocyte cell cultures as well as an oestradiol binding assay. It was found that o-phenylphenol was able to induce vitellogenin gene expression in rainbow trout hepatocyte aggregate cultures. The induction rate of equimolar concentrations was roughly two orders of magnitude lower than that for 17β-oestradiol. In the oestradiol binding assay OPP was not found to be an efficient competitor to oestradiol even at the highest test concentration and showed 10000 x less binding affinity to the oestrogen receptor than oestradiol. The study was carried out at high (17 mg l^{-1}) concentrations and no clear mechanism of action was proposed.

9.4.2.2 In vivo studies

A. Studies on aquatic organisms

Fish

A study investigating the effects of o-phenylphenol on reproduction in fathead minnows has been carried out (AstraZeneca 2002) using the paired breeding assay procedure (Harries *et al* 2000). The study assessed the effects of 21 day exposure to Preventol O extra (99.9% purity o-phenylphenol) via a flow through system at nominal concentrations of 1.0, 5.0, 50 and 500 μg l^{-1} (actual concentrations = not detectable, 4.0, 36 and 293 μg l^{-1}). A positive control of 10 ng l^{-1} 17α-ethinylestradiol was used. The test was initiated with mature adults that had a record of reproductive success as measured by fecundity (number of spawnings, number of eggs, number of eggs per brood) and by embryo viability (hatchability of eggs). This was established during a pre-exposure period of 21 days in the same system/tanks utilised for the chemical exposure. Each replicate tank contained a pair of male and female fathead minnows and eight breeding pairs were used at each concentration. Measurements of fecundity were assessed daily and the viability of resultant embryos was assessed in animals held in the same treatment regime to which the adults were exposed. At the conclusion of the test, blood samples were collected from the adults for determination of vitellogenin (VTG)[1] and the gonads were sampled for measurement of the gonadosomatic index (GSI) and histological analyses (if required).

The results obtained from the study are summarised in Table 9.7 and the parameters which were significantly affected by o-phenylphenol were the number of spawnings, egg production, egg batch size and egg hatching. The results were taken to show that Preventol O extra does not exhibit any adverse effects on reproductive parameters up to a measured test concentration of 36 μg l^{-1} (nominal test concentration of 50 μg l^{-1}). With regard to the induction of vitellogenin and changes in gonadosomatic index and early indication of possible oestrogenic effects no substance-related effects were noted even at the highest concentration (293 μg l^{-1}) compared to the effects observed in the positive control 17α-ethinylestradiol.

From the data the overall no observed effect concentration (NOEC) has been reported as 36 μg l^{-1} based on measured concentrations (50 μg l^{-1} based on nominal concentrations). The

[1] Vitellogenin has been identified as a biomarker of exposure of fish to oestrogenic substances

overall lowest effect concentration (LOEC) has been reported as 293 μg l^{-1} based on measured concentrations (500 μg l^{-1} based on nominal concentrations).

Table 9.7 Summary of the results for the fathead minnow paired breeding assay

Biological parameter	Effect concentration (μg l^{-1}) based on measured levels	
	NOEC	LOEC
F_0 - Number of spawnings	36	293
F_0 - Egg production	36	293
F_0 - Egg batch size	36	293
F_0 - GSI (males)	293	>293
F_0 - GSI (females)	293	>293
F_0 - Vitellogenin (males)	293	>293
F_0 - Vitellogenin (females)	293	>293
F_1 - Egg hatch	36	293

Invertebrates

Two 21 day reproduction tests using *Daphnia magna* has been carried out which investigated the effects of o-phenylphenol on the survival of adults and juvenile production of surviving adults (Bayer 1989, 2001). In the original study the No Observed Effect Concentration for effects on juvenile production was 0.075 mg l^{-1} based on nominal concentrations. In the later juvenile production study no mortality of parental females was observed even at the highest test concentration (0.1 mg l^{-1}) and the decrease in juvenile production at 0.1 mg l^{-1} was only 18.5% (118 juveniles per female compared to 140 juveniles per female in the control). The NOEC and LOEC values reported in the study were 0.01 and 0.03 mg l^{-1} respectively based on nominal concentrations (0.009 and 0.022 mg l^{-1} based on measured concentrations). However, no information is available on the mechanism of action for the observed effects.

The effects observed in *Daphnia magna* reproduction tests are probably not caused by direct oestrogenic effects since other studies have shown an absence of reproductive impairment at 0.39 mg l^{-1} when animals are exposed to the synthetic steroid 17α-ethinyestradiol (Schweinfurth *et al* 1986).

B. Studies on terrestrial organisms

No data has been located on the potential endocrine disrupting effects of o-phenylphenol on terrestrial organisms. However, given the rapid degradation of o-phenylphenol in soil (see Section 9.1) the likelihood of potential endocrine mediated effects in terrestrial organisms is reduced. It should also be recognised that there are currently no internationally agreed methods to assess potential endocrine disrupting effects in terrestrial organisms.

C. Studies on aerial organisms

No data has been located on the potential endocrine disrupting effects of o-phenylphenol on aerial organisms. However, given the rapid degradation of o-phenylphenol in air (see Section 9.1) the likelihood of potential endocrine mediated effects in aerial organisms is reduced. It

should also be recognised that there are currently no internationally agreed methods to assess potential endocrine disrupting effects in aerial organisms.

D. General conclusions on potential endocrine mediated responses in in vivo studies with wildlife species

The data that has been located to assess the potential endocrine disrupting effects of o-phenylphenol is limited to studies on the effects on the reproduction of aquatic invertebrates (the water flea *Daphnia magna*) and fish (fathead minnow *Pimephales promelas*). The data (see Table 9.8) indicates that NOECs are in the measured concentration range of 9 - 36 μg l^{-1}.

The NOECs observed in the reproduction study using *Pimephales promelas* for the parameters vitellogenin and gonadosomatic index were 293 μg l^{-1}, which represents the highest concentration tested. The fact that no adverse effects on VTG and GSI were evident at the highest exposure concentration whereas the NOEC for the parameters egg production and hatchability were 36 μg l^{-1} indicates that these effects of o-phenylphenol are probably not due to oestrogenic effects. The effects on *Daphnia magna* reproduction may not be the result of an oestrogenic mechanism of action, but no information is available on the mechanism of action.

9.5. Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity

The general toxicity data in this section has largely been obtained from the IUCLID data set for chlorocresol and has been taken as accurate. Individual source documents have not been checked unless they are considered to be key studies which have a major influence on the outcome of the review. All information taken from IUCLID as been referenced as being from that source and individual references have not been given in the references.

9.5.1 Studies relevant to the assessment of general toxicity in humans

Table 9.9 summarises the general toxicity data from acute and repeat dose studies with o-phenylphenol.

9.5.1.1 Acute studies

A. Oral exposure

In oral toxicity tests with o-phenylphenol LD_{50} values of greater than >5000 mg kg body $weight^{-1}$ have been recorded in studies with rats, mice, rabbits guinea pigs and cats (see Table 9.8). For rats LD_{50} values ranged from 1049 – 2980 mg kg body $weight^{-1}$ for the sodium salt of o-phenylphenol (Na-OPP), 2118 – 2573 mg kg body $weight^{-1}$ for the potassium salt of o-phenylphenol (Na-OPP) and 2000 – 4000 mg kg body $weight^{-1}$ for o-phenylphenol (OPP). Mice showed similar LD_{50} values to rats with values of 683 – 1018 mg kg body $weight^{-1}$ being found for Na-OPP and values of 2000 – 3499 mg kg body $weight^{-1}$ for OPP. The LD_{50} values

Table 9.8 Summary of the studies assessing potential endocrine mediated responses in wildlife

Environmental compartment	Taxonomic group	Type of study	Species and exposure route used	Concentration series used	Lowest reported NOEC	Reference
Aquatic	Amphibians	No data	-	-	-	-
	Fish	Draft OECD Reproduction Screening Procedure	Fathead minnow (*Pimephales promelas*) - aqueous exposure	0, 0.001, 0.005, 0.05 and 0.5 mg l^{-1} (Nominal)	0.05 mg l^{-1} (Nominal) 0.036 mg l^{-1} (Measured)	AstraZeneca (2002)
	Invertebrates	Reproduction (OECD TG 211)	*Daphnia magna* – aqueous exposure	0, 0.01, 0.03 and 0.1 mg l^{-1} (Nominal)	0.01 mg l^{-1} (Nominal) [(a)] 0.009 mg l^{-1} (Measured) [(a)]	Bayer (2001)
Terrestrial	Birds	No data	-	-	-	-
	Invertebrates	No data	-	-	-	-
Aerial	Invertebrates	No data	-	-	-	-

a - No information is available on the mechanism of action

for the guinea pig and cat were 3500 and 500 mg kg body weight^{-1} respectively. While the studies may not satisfy all the conditions of a GLP guideline investigation many of them meet the scientific principals for assessment of an oral LD_{50} in rats.

B. Dermal exposure

A number of studies have investigated the acute toxicity in rats, mice and rabbits following dermal exposure to OPP (see Table 9.9). The values recorded for rats were all > 2000 mg kg body weight^{-1}, while the values for rabbits was > 5000 mg kg-1 body weight^{-1}.

C. Inhalation exposure

Mihail (1977) recorded 1 hour LC_{50} values of 0.95 to 1.13 mg l^{-1} in rats following exposure via the inhalation route (see Table 9.9).

D. Other routes of exposure

The LD_{50} data for acute studies involving exposure of OPP via intra-peritoneal injection showed values of 500 - 1500 mg kg body weight^{-1} for rats and 50 - 100 mg kg body weight^{-1} for mice (see Table 9.9).

9.5.1.2 Repeat dose studies

A. Oral exposure

A large number of repeat dose studies using oral exposure have been conducted in rats, mice, guinea pigs, golden hamsters and dogs and reported to varying degrees of detail in IUCLID (2000). These are summarised in Table 9.9 but in the majority of these a no observed (adverse) effect level was not reported. Information on general systemic toxicity in reproduction and developmental toxicity studies has been reported in Section 9.4.1.2 and these provide the lowest NO(A)ELs following oral exposure.

B. Dermal exposure

As part of the National Toxicology Programme a 4 week dermal exposure study in male and female Swiss Webster CFW mice was conducted at doses of 5.95, 11.4, 20.8, 35.7 and 55.5 mg OPP per animal (NTP 1986). The OPP was administered 3 times per week in 0.1ml acetone. No compound related changes in body weight or survival of animals at any dose were evident. However, dose-related ulcerative lesions at the site of application were evident. Dow (1993) conducted a dermal exposure in male and female Fischer 344 rats in which doses of 0, 100, 500 and 1000 mg kg^{-1} body weight^{-1} were administered once per day, 5 days per week. The study was conducted to OECD TG 411 (Subchronic Dermal Toxicity: 90 day Study) and GLP using OPP of >99% purity. Slight skin alterations at the application sites were evident in the 500 and 1000 mg kg^{-1} body weight^{-1} dose groups, but no changes were evident in any parameters indicative of systemic toxicity associated with administration of OPP.

C. Inhalation studies

No data has been located on the repeat dose toxicity of o-phenylphenol to laboratory mammals following inhalation exposure.

D. Other routes of exposure

No data has been located on the repeat dose toxicity of o-phenylphenol to laboratory mammals following exposure by intra-peritoneal or sub-cutaneous injection.

9.5.1.3 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity in laboratory mammals

The lowest NOEL identified from the review of data on endocrine mediated responses in laboratory mammals was apparently 250 mg kg body weight^{-1} day^{-1} based on effects on pregnancy rate and foetal sex ratio (in a teratogenicity study in New Zealand White rabbits by Zablotny *et al* (1991b). A NOEL of 300 mg kg body weight^{-1} day^{-1} (the highest dose tested) was found based on an assessment of histopathological effects in endocrine glands and hormone sensitive tissues of beagle dogs (Cosse *et al* 1990). In two generation reproduction studies in rats (Eigenberg 1990, Eigenberg and Lake 1995) and developmental/teratogenicity studies in rats and rabbits (Ogata *et al* 1978, Kaneda *et al* 1978, John *et al* 1981, Zablotny *et al* 1991a,b) no effects on reproduction and development which may be endocrine mediated have been identified even at the highest doses tested. However, in the one and two generation reproduction studies and development/teratogenicity studies lower NOELs were identified for parental/maternal toxicity.

In acute and repeat-dose studies the general systemic toxicity data indicates that the threshold in rats for an absence of effects which are not endocrine mediated occurs at a dose of approximately 35 mg kg body weight^{-1} day^{-1} for adult rats in a two generation reproduction study (Eigenberg 1990). The NOEL of 35 mg kg body weight^{-1} day^{-1} reported in the study relates to transitional cell hyperplasia/papillomatosis in the urinary bladder of animals at 125 mg kg body weight^{-1} day^{-1}. In a two year chronic toxicity/oncogenicity study in rats a NOEL of 39 mg kg body weight^{-1} day^{-1} was reported based on histopathological changes in the livers, kidneys and urinary bladders of males at 200 mg kg body weight^{-1} day^{-1}. As a result it appears from the available data that endocrine mediated responses are probably not be the mechanism responsible for lowest observed toxicity in laboratory mammals and that the kidney and liver are the main target sites for o-phenylphenol (see Section 9.3.1).

Table 9.9 Summary of the general mammalian toxicity data (Information from IUCLID 2000)

Test type	Test species	Exposure period	Test dose series used	Endpoint	Effect dose	Reference	Study validity
Acute Oral Toxicity	Rat	Not relevant	No data	Median lethal dose (LD_{50})	3000 mg kg^{-1} body $weight^{-1}$	MacIntosh (1945)[1]	Use with care[2]
	Rat (Males)	Not relevant	No data	Median lethal dose (LD_{50})	2700 mg kg^{-1} body $weight^{-1}$	Hodge (1952) [1]	Use with care[2]
	Rat	Not relevant	Na salt: No data	Median lethal dose (LD_{50})	1250 mg kg^{-1} body $weight^{-1}$	Gucklhorn (1969) [1]	Use with care[2]
	Rat (Males)	Not relevant	No data	Median lethal dose (LD_{50})	>2500 mg kg^{-1} body $weight^{-1}$	Kimmerle (1969) [1]	Use with care[2]
	Rat	Not relevant	No data	Median lethal dose (LD_{50})	2480 mg kg^{-1} body $weight^{-1}$	Martin (1974) [1]	Use with care[2]
	Rat	Not relevant	No data	Median lethal dose (LD_{50})	2000 - 4000 mg kg^{-1} body $weight^{-1}$	Kaneda (1978) [1]	Use with care[2]
	Rat	Not relevant	Na- salt: No data	Median lethal dose (LD_{50})	1096 mg kg^{-1} body $weight^{-1}$ (males) 1049 mg kg^{-1} body $weight^{-1}$ (females)	Tayama (1979) [1]	Use with care[2]
	Rat	Not relevant	Na-salt: No data	Median lethal dose (LD_{50})	1720 mg kg^{-1} body $weight^{-1}$	Loeser (1980) [1]	Use with care[2]
	Rat (Males and Females)	Not relevant	Na-salt: No data	Median lethal dose (LD_{50})	2600 mg kg^{-1} body $weight^{-1}$ (males) 2850 mg kg^{-1} body $weight^{-1}$ (females)	Tayama (1980) [1]	Use with care[2]
	Rat (Males)	Not relevant	No data	Median lethal dose (LD_{50})	2980 mg kg^{-1} body $weight^{-1}$	Loeser (1981) [1]	Use with care[2]

	Rat (Males and Females)	Not relevant	Na-salt: No data	Median lethal dose (LD_{50})	1650 mg kg^{-1} body $weight^{-1}$ (males) 1550 mg kg^{-1} body $weight^{-1}$ (females)	Taniguchi (1981) [1]	Use with care[2]

Table 9.9 Continued

Test type	Test species	Exposure period	Test dose series used	Endpoint	Effect dose	Reference	Study validity
Acute Oral Toxicity	Rat (Males and Females)	Not relevant	K-salt: No data	Median lethal dose (LD_{50})	2573 mg kg^{-1} body $weight^{-1}$ (males) 2118 mg kg^{-1} body $weight^{-1}$ (females)	Bayer (1988) [1]	Use with care[2]
	Mouse	Not relevant	No data	Median lethal dose (LD_{50})	2000 – 3000 mg kg^{-1} body $weight^{-1}$	Kaneda (1978) [1]	Use with care[2]
	Mouse (Females)	Not relevant	No data	Median lethal dose (LD_{50})	2000 mg kg^{-1} body $weight^{-1}$	Yanagisawa (1978) [1]	Use with care[2]
	Mouse	Not relevant	Na-salt: No data	Median lethal dose (LD_{50})	1018 mg kg^{-1} body $weight^{-1}$ (males) 857 mg kg^{-1} body $weight^{-1}$ (females)	Ogata (1979) [1]	Use with care[2]
	Mouse	Not relevant	Na-salt: No data	Median lethal dose (LD_{50})	683 mg kg^{-1} body $weight^{-1}$ (males) 812 mg kg^{-1} body $weight^{-1}$ (females)	Ogata (1979) [1]	Use with care[2]
	Mouse (Males and Females)	Not relevant	No data	Median lethal dose (LD_{50})	1200 mg kg^{-1} body $weight^{-1}$ (males) 1050 mg kg^{-1} body $weight^{-1}$ (females)	Taniguchi (1981) [1]	Use with care[2]
	Mouse (Males and Females)	Not relevant	No data	Median lethal dose (LD_{50})	3499 mg kg^{-1} body $weight^{-1}$ (males) 3152 mg kg^{-1} body $weight^{-1}$ (females)	Tayama (1983) [1]	Use with care[2]

	Guinea pig	Not relevant	No data	Median lethal dose (LD_{50})	3500 mg kg^{-1} body $weight^{-1}$	Fawell (1988) [1]	Use with care[2]
	Cat	Not relevant	No data	Median lethal dose (LD_{50})	500 mg kg^{-1} body $weight^{-1}$	MacIntosh (1945) [1]	Use with care[2]

Table 9.9 Continued

Test type	Test species	Exposure period	Test dose series used	Endpoint	Effect dose	Reference	Study validity
Acute Dermal Toxicity	Rat	Not relevant	K-salt: No data	Median lethal dose (LD_{50})	>2000 mg kg^{-1} body $weight^{-1}$	Bomhard (1990) [1]	Use with care[2]
	Rat	Not relevant	No data	Median lethal dose (LD_{50})	>2000 mg kg^{-1} body $weight^{-1}$	Bayer (1991a) [1]	Use with care[2]
	Rat	Not relevant	K-salt: No data	Median lethal dose (LD_{50})	>2000 mg kg^{-1} body $weight^{-1}$	Bayer (1991b) [1]	Use with care[2]
	Rabbit	Not relevant	No data	Median lethal dose (LD_{50})	>5000 mg kg^{-1} body $weight^{-1}$	Carreon (1981) [1]	Use with care[2]
Acute Inhalation Toxicity	Rat (Males)	1 hour	No data	LC_{50}	0.95 -1.13 mg l^{-1}	Mihail (1977) [1]	Use with care[2]
Acute Toxicity (Intra-peritoneal injection)	Rat	No data	No data	Median lethal dose (LD_{50})	1500 mg kg^{-1} body $weight^{-1}$	MacIntosh (1945) [1]	Use with care[2]
	Rat	No data	No data	Median lethal dose (LD_{50})	500 mg kg^{-1} body $weight^{-1}$	MacIntosh (1945) [1]	Use with care[2]
	Mouse	No data	No data	Median lethal dose (LD_{50})	50 - 100 mg kg^{-1} body $weight^{-1}$	IUCLID (2000) [1]	Use with care[2]
Repeated Dose Toxicity (Oral)	Rat (Male)	32 days	0, 2, 20, and 200 mg kg^{-1}	NOAEL	200 mg kg^{-1}	MacIntosh (1945) [1]	Use with care[2]
	Rat (Males and Females)	26 weeks	0, 50, 100, 200 and 500, mg kg bw^{-1}	NOAEL	No adverse effects observed	Hodge (1952) [1]	Valid[2]

	Rat (Females)	4 weeks	0, 20000, 30000, 40000, 50000 and 100000 mg kg^{-1} in diet	NOAEL	No data	Hodge (1952) [1]	Valid[2]
	Rat (Males and Females)	13 weeks	0, 1000, 3000, 10000 and 20000 mg kg^{-1} in diet	NOAEL	No data	Hodge (1952) [1]	Valid[2]

Table 9.9 Continued

Test type	Test species	Exposure period	Test dose series used	Endpoint	Effect dose	Reference	Study validity
Repeat Dose Toxicity (Oral)	Rat (F344/DuCrj Males and Females)	13 weeks	0, 1250, 2500, 5000, 10000, 20000 and 40000 mg kg^{-1} in diet	NOAEL	No data	Iguchi (1979) Kishiyama (1989) [1]	Valid[2]
	Rat	36 Weeks	0, 2500, 5000, 10000 and 20000 mg kg^{-1} in diet	NOAEL	No data	Fukushima *et al* (1982) [1]	Valid[2]
	Rat (F344 Males)	12 weeks	0, 2500, 5000, 10000 and 20000 mg kg^{-1} in diet	NOAEL	No data	Fukushima *et al* (1985) [1]	Valid[2]
	Rat (F344 Males)	64 weeks	0 and 20000 mg kg^{-1} in diet	NOAEL	No data	Fukushima *et al* (1985) [1]	Use with care[2]
	Rat (F344/DuCrj Males and Females)	159 days	0 and 20 mg kg^{-1} in diet	NOAEL	No data	Kobayashi (1983) [1]	Use with care[2]
	Rat (Male)	91 weeks	0, 6250, 12500 and 25000 mg kg^{-1} in diet	NOAEL	No data	Nakamura (1983) [1]	Valid[2]
	Rat (Male F344)	90 days	0 and 20000 mg kg^{-1} in diet	NOAEL	No data	Reitz *et al* (1983) [1]	Use with care[2]

	Rat (F344/DuCrj Males and Female)	13 weeks	0, 1560, 3130, 6250, 12500 and 25000 mg kg^{-1} in diet	NOAEL	6250 mg kg^{-1}	Hiraga (1984) [1]	Valid [2]
	Rats (Males)	4 weeks	20000 mg kg^{-1} in diet	NOAEL	No data	Fukumori (1983) [1]	Use with care [2]
	Rat (F344/DuCrj Males and Females)	20 weeks	0 and 20000 mg kg^{-1} in diet	NOAEL	No data	Nagai and Nakao (1984) [1]	Use with care [2]
		136 days	0 and 20000 mg kg^{-1} in diet	NOAEL	No data	Nakagawa and Nakao (1984) [1]	Use with care [2]
	Rat (F 344 Males)	32 Weeks	0 and 20000 mg kg^{-1} in diet	NOAEL	No data	Fukushima (1985), Ito (1984) [1]	Use with care [2]

Table 9.9 Continued

Test type	Test species	Exposure period	Test dose series used	Endpoint	Effect dose	Reference	Study validity
Repeat Dose Toxicity (Oral)	Rat	4 weeks	0 and 20000 mg kg^{-1} in diet	NOAEL	No data	Fukumori (1984) [1]	Use with care [2]
	Rat (Males)	3 days	0 and 1500 mg kg^{-1} in diet	NOAEL	No data	Fukumori (1985) [1]	Use with care [2]
	Rat	3 days	0 and 1.5 mg kg^{-1} in diet	NOAEL	No data	Fukumori (1986) [1]	Use with care [2]
	Rat (F344 Males)	24 weeks	0 and 20000 mg kg^{-1} in diet	NOAEL	No data	Fukushima (1986) [1]	Use with care [2]
	Rat (Males)	13 weeks	0 and 5000, 7000, 10000 mg kg^{-1} in diet	NOAEL	No data	Fujii *et al* (1986) [1]	Valid [2]
	Rat	13 weeks	0 and 10000 mg kg^{-1} diet	NOAEL	No data	Mikuriya (1987) [1]	Use with care [2]
	Rat (F344 Males)	13 weeks	0, 377, 763 and 1554 mg kg^{-1} day^{-1} in diet	NOAEL	No data	Kishiyama (1989), Nakamura (1982) [1]	Valid [2]
	Rat (F344 Males)	8 weeks	0 and 20000 mg kg^{-1} in diet	NOAEL	No data	Shibata (1989a) [1]	Use with care [2]
	Rat (F344 Males)	24 weeks	0 and 20000 mg kg^{-1} in diet	NOAEL	No data	Shibata (1989b) [1]	Use with care [2]

	Rats (Males)	48 weeks	0 and 20000 mg kg^{-1} in diet	NOAEL	No data	Hasegawa *et al* (1990) [1]	Use with care [2]
	Rat (F344 Males and Females)	8 weeks	0 and 12500 mg kg^{-1} in diet	NOAEL	No data	Hasegawa *et al* (1991) [1]	Use with care [2]
	Rat (F344 Males)	13 weeks	0, 800, 4000, 8000 and 125000 ppm	NOAEL	No data	Bayer (1996) [1]	Valid [2]
	Mouse (B6C3F1 Females)	2 weeks	0, 1.0, 10 and 200 mg kg^{-1} bw^{-1}	NOAEL	No data	Luster (1981) [1]	Valid [2]

Table 9.9 Continued

Test type	Test species	Exposure period	Test dose series used	Endpoint	Effect dose	Reference	Study validity
Repeat Dose Toxicity (Oral)	Mouse (B6C3F1 Males)	36 weeks	0 and 20000 mg kg^{-1} in diet	NOAEL LOAEL	No effects observed	Fukushima (1982) [1]	Use with care [2]
	Mouse (Males)	48 weeks	0 and 20000 mg kg^{-1} in diet	NOAEL	No effects observed	Hasegawa (1990) [1]	Use with care [2]
	Mouse (B6C3F1 Male and Females)	13 weeks	0, 2500, 5000, 10000, 20000 and 40000 mg kg^{-1} in diet	NOAEL	No data	Shibata (1981), Shibata (1985) [1]	Valid [2]
	Mouse (B6C3F1 Males)	52 weeks	0, 6500, 13000 and 26000 mg kg^{-1} in diet	NOAEL	No data	Mikuriya (1989a) [1]	Valid [2]
	Guinea pig (Hartley Males)	36 weeks	0 and 20000 mg kg^{-1} in diet	NOAEL	No effects observed	Fukushima (1982) [1]	Use with care [2]
	Guinea pig (Hartley Males)	48 weeks	0 and 20000 mg kg^{-1} in diet	NOAEL	No effects observed	Hasegawa (1990) [1]	Use with care [2]
	Golden hamster (Syrian Males)	36 weeks	0 and 20000 mg kg^{-1} in diet	NOAEL	No effects observed	Fukushima (1982) [1]	Use with care [2]
	Golden hamster (Syrian Males)	48 weeks	0 and 20000 mg kg^{-1} in diet	NOAEL	No effects observed	Hasegawa (1990) [1]	Use with care [2]

	Dog (mongrel)	4 weeks	0, 100 and 1000 mg kg bw^{-1} day^{-1}	NOAEL	No data	Hodge (1952) [1]	Valid[2]
	Dog (Male and Female)	52 weeks	0, 20, 200 and 500 mg kg bw^{-1} day^{-1}	NOAEL	No data	Hodge (1952) [1]	Valid[2]
	Dog (Male beagles)	5 days	0 and 300 mg kg^{-1} day^{-1}	NOAEL	No data	Cosse (1990) [1]	Use with care[2]

Table 9.9 Continued

Test type	Test species	Exposure period	Test dose series used	Endpoint	Effect dose	Reference	Study validity
Repeat Dose Toxicity (Oral)	Dog (Male and Female beagles)	9 days	0, 300-1000 mg kg^{-1} day^{-1}	NOAEL	No data	Cosse (1990) [1]	Valid[2]
	Dog (Male and Female beagles)	4 weeks	0, 100, 200 and 300 mg kg^{-1}	NOAEL	300 mg kg^{-1}	Cosse (1990) [1]	Valid[2]
	Dog (Male and Female beagles)	52 weeks	0, 30, 100 and 300 mg kg^{-1} day^{-1}	NOAEL	300 mg kg^{-1}	Cosse (1990) [1]	Valid[2]
	Dog (Male and Female beagles)	1 to 2 days	0, 400-700 mg kg^{-1}	NOAEL	No data	Cosse (1990) [1]	Valid[2]
Repeat Dose Toxicity (Dermal)	Rat (F344 Males and Females)	90 days	0, 100, 500 and 1000 mg kg^{-1}	NOAEL	No data	Dow (1993) [1]	Valid[2]
	Mouse (Swiss Webster CFW Males and Females)	4 weeks	0, 5.95, 11.4, 20.8, 35.7 and 55.5 mg $animal^{-1}$ in 0.1ml acetone	NOAEL	No data	NTP (1986)	Valid[2]

[1] – Cited in IUCLID (2000), [2]- Assessment based on data in IUCLID (2000)

9.5.2 Studies relevant to the assessment of general toxicity in wildlife

9.5.2.1 Studies on aquatic organisms

Table 9.10 summarises the general toxicity data for aquatic organisms exposed to o-phenylphenol.

A. Fish

Acute toxicity

Acute toxicity tests with fish have shown 96-hour LC_{50} values of 2.8 to 6.2 mg l^{-1} for fathead minnows depending on the test exposure system (static or flow-through) adopted (Haas *et al* 1974, Hall *et al* 1984, Holcombe *et al* 1984, Dill *et al* 1985, Geiger *et al* 1985, Simmons *et al* 1988). Corresponding 96-hour LC_{50} values for other species were 4.6 mg l^{-1} for bluegill sunfish (*Lepomis macrochirus*), 4.8 mg l^{-1} for chinook salmon (*Onchorhynchus tschawytscha*), 44.7 mg $^{l-1}$ for golden orfe (*Leuciscus idus*), 3.0 mg l-1 for guppy (Poecilia reticulata) , 4.0 mg l^{-1} for rainbow trout (*Onchorhynchus mykiss)* and 4.5 mg l^{-1} for zebrafish *(Danio rerio)*.

Chronic toxicity

No toxicity data for fish following chronic exposure to o-phenylphenol has been located.

B. Invertebrates

Acute toxicity

Acute toxicity tests with *Daphnia magna* have shown 48-hour EC_{50} values of 1.5 to 2.9 mg l^{-1} (Dill *et al* 1985, Kuhn *et al* 1988, Ramos *et al* 1998). Acute toxicity data has been located for another aquatic invertebrate species, the freshwater mollusc *Lymnea stagnalis* which showed a 96 h LC_{50} value of 4.5 mg l^{-1} (Ramos *et al* 1998).

Chronic toxicity

Two chronic *Daphnia magna* reproduction studies have been conducted by Bayer AG (1989, 2001). In the original study conducted to a Draft OECD Guideline the NOEC for mortality was 0.075 mg l^{-1} based on nominal concentrations. In the later study conducted to OECD TG 211 the NOEC for mortality was $\geq$ 0.07 mg l^{-1} based on measured concentrations (0.1 mg l^{-1} based on nominal concentrations).

9.5.2.2 Studies on terrestrial organisms

Data on the toxicity of o-phenylphenol to birds following dietary exposure has been located. Unpublished data from Dow Corporation indicates the LC_{50} values for bobwhite quail and mallard ducks were both > 5620 ppm. For the mallard duck study a NOEL of 2250 ppm was reported.

9.5.2.3 Studies on aerial organisms

No general toxicity data for aerial organisms following exposure to of o-phenylphenol has been located.

Table 9.10 Summary of general toxicity data for aquatic organisms (Information from IUCLID 2000)

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration (mg l^{-1})	Reference	Study validity
Acute Fish Toxicity	Bluegill sunfish (*Lepomis macrochirus*)	96h	No data	LC_{50}	4.6	Dill *et al* (1985)[1]	Use with care[2]
	Chinook salmon (*Oncorhynchus tschawytscha*)	96h	No data	LC_{50}	4.8	BC Research Corp (1991)[1]	Use with care[2]
	Fathead minnow *(Pimephales promelas)*	96h	No data	LC_{50}	20	Haas *et al* (1974)[1]	Use with care[2]
		96h	Flow-through	LC_{50}	6.0	Hall *et al* (1984)[1]	Valid[2]
		24h	No data - Flow through	LC_{50}	6.2	Holcombe *et al* (1984)[1]	Valid[2]
		48h	"	LC_{50}	6.1		
		72h	"	LC_{50}	6.1		
		96h	"	LC_{50}	6.0		
		96h	No data - Static	LC_{50}	5.1	Dill *et al* (1985)[1]	Use with care[2]
		96h	No data	LC_{50}	6.2	Gieger *et al* (1985)[1]	Use with care[2]
		96h	No data	LC_{50}	2.8	Simmons *et al* (1988)[1]	Use with care[2]
	Golden orfe (*Leuciscus idus*)	96h	No data	LC_{50}	44.7	Bayer AG (1976)[1]	Valid[2]
	Guppy (*Poecilia reticulata*)	96h	No data	LC_{50}	3.0	Ramos *et al* (1998)[1]	Valid[2]
	Rainbow trout *(Oncorhynchus mykiss)*	96h	No data	LC_{50}	4.0	Dill *et al* (1985)[1]	Use with care[2]
	Zebrafish *(Danio rerio)*	96h	No data	LC_0	2.3	Bayer AG (1990)[1]	Valid[2]

Table 9.10 Continued

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration (mg l^{-1})	Reference	Study validity
Acute Invertebrate Toxicity	Water flea (*Daphnia magna*)	48h	No data	EC_{50}	2.7	Dill *et al (*1985) [1]	Use with care[2]
		24h	No data	EC_{50}	2.1	Kuhn *et al* (1988) [1]	Valid[2]
		48h	No data	EC_{50}	1.5		
		48h	No data	EC_{50}	2.7	Ramos *et al* (1998) [1]	Valid[2]
	Mollusc *(Lymnea stagnalis)*	96h	No data	LC_{50}	4.5	Ramos *et al* (1998) [1]	Valid[2]
Chronic Invertebrate Toxicity	Water flea (*Daphnia magna*)	21d	0, 0.075 and 0.75 mg l^{-1}	NOEC (mortality)	0.075	Bayer AG (1989) [1]	Valid[2]
		21d	0, 0.1, 0.3 and 1.0 mg l^{-1}	NOEC (mortality)	$\geq$0.07	Bayer AG (2001) [1]	Valid[2]

[1] – Cited in IUCLID (2000), [2]- Assessment based on data in IUCLID (2000)

9.5.2.3 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity in wildlife

Comparison of the limited data on potential endocrine mediated responses in the aquatic invertebrate *Daphnia magna* with the acute and chronic mortality data for this species indicates that mortalities were evident at higher concentrations (>0.07 mg l^{-1}) than the threshold concentration for effects on reproduction (NOEC = 0.009 mg l^{-1}). For the fathead minnow effects on reproduction (egg production and hatchability) were observed above 0.036 mg l^{-1} whereas acute lethality effects were recorded at o-phenylphenol concentrations of 2.8 to 6.2 mg l^{-1}. In a longer-term exposure study concentrations of o-phenylphenol up to 0.29 mg l^{-1} did not result in lethality in fathead minnows.

9.6 Current classification of the substance against European Commission and national regulations

Table 9.11 summarises the current classification of the substance against Council Directives in order to assess the regulations to which o-phenylphenol is subject. o-Phenylphenol is listed as an evaluated and approved preservative in Annex VIA in Directive 76/768/EEC and Directive 95/2/EEC (E231 and E232).

Table 9.11 Current classification of the substance against Council Directives

Directive	Status (listed or not)
67/548/EEC - Classification, packaging and labelling of dangerous substances	Classified: Xi, C R phrases: 36/38
76/768/EEC - Approximation of laws relating to cosmetic products	Listed as an evaluated and approved preservative in Annex VIA

In 1999 the FAO/WHO evaluation of o-phenylphenol derived an Acceptable Daily Intake (ADI) value of 0.4 mg body $weight^{-1}$ day^{-1} for humans (WHO 2001).

No national environmental quality standards have been derived for o-phenylphenol for the protection of aquatic and terrestrial ecosystems.

9.7 Exposure data

9.7.1 Worker exposure data

Data on concentrations of o-phenylphenol to which workers are potentially exposed during the production and use of the substance has been sought from the relevant CEFIC Sector Group. Table 9.12 summarises the suggested addition doses of Preventol O extra/Dowicide 1/1E (>99.5% o-phenylphenol) and Preventol ON extra/Dowicide A (>95% sodium o-phenylphenol) for different applications.

Table 9.12 Suggested addition doses of Preventol O extra/Dowicide 1/1E and Preventol ON extra/Dowicide A for different applications

Application	Use	Suggested addition doses (%)	
		Preventol O extra	Preventol ON extra
Disinfection and cleaning agents	Disinfection		
Disinfection concentrates		10 - 15	16 – 24
Ready to use disinfectant concentrates		0.075 - 0.15	0.12 – 0.24
Timber	Temporary protection		
Sawn timber		1 - 3	1.6 – 4.8
Leather	Preservation		
Chrome leather		0.15 - 0.25	0.24 - 0.4
Glues and adhesives	Preservation		
Dextrin and cellulose glues		0.1 - 0.2	0.16 - 0.32
Starch glues (liquid)		0.1 - 0.2	0.16- 0.32
Starch glues (dry)		0.1 - 1.0	0.16 -1.6
Paper	Preservation		
Filler suspensions, coating compounds		0.07 - 0.15	0.11 - 0.24
Pigment slurries		0.025 - 0.05	0.04 - 0.08
Textile auxillaries	Preservation, ready to use dilution		
Print thickeners (dry)		1.0 - 2.0	1.6 - 3.2
Citrus fruits	Whole fruit preservation	0.15 - 1.3	0.24 - 2.1
Various	Preservation		
Polishes, wax emulsions		0.2 - 0.3	0.32 - 0.48
Concrete additives		0.1 - 0.3	0.16 - 0.48

The highest potential exposure levels of 4-chloro-3-methylphenol occur when handling the concentrates for disinfection and cleaning agents. However, given that appropriate safety equipment will be worn at this time the risk to workers should be minimised. The materials containing 4-chloro-3-methylphenol which are used for preservation in other areas have lower levels of the substance (typically <1% vol/vol or ~ 10 mg l^{-1})

Harke and Klein (1981) reported on a study in which an undiluted formulation of hand disinfectant containing 2% (approximately 60 mg) o-phenylphenol (OPP) were rubbed into the hands of 11 volunteers for 1 minute. Water was then added and the hands washed for a further minute. The hands were subsequently rinsed for 30 seconds under running water and dried on aper towels. The process was carried out 10 times by each volunteer so that the total quantity of o-phenylphenol applied was 600 mg. The daily urine production of all the volunteers was then collected over the next 4 days and the OPP content determined. The daily urine production of some volunteers was also tested over 4 weeks for the occurrence of OPP from other sources. Taking the recovery rate into account, the volunteers eliminated a mean of 6.2 mg OPP in the first 2 days after application of the preparation. In contrast, on days 3 and 4 only traces of the test substance were observed, which could not be determined quantitatively. No OPP was detected in the urine of untreated volunteers. From the data it was concluded that by far the greater part of the test substance (approximately 99%) is not adsorbed, but rinsed off the skin after use. This conclusion was confirmed by analysis of the washing water.

9.7.2 Consumer exposure data

No data on concentrations of o-phenylphenol to which consumers are potentially exposed during the use of the products containing the substance has been obtained.

9.7.3 Environmental exposure data

9.7.3.1 Aquatic environment

Treatment plant discharges and sewage sludges

Körner *et al* (1998) investigated the input/output balance of oestrogenic active compounds (including o-phenylphenol) in a major municipal sewage plant in Germany. In the study 24 hour samples of untreated and treated wastewater were taken from the modern sewage plant in Southern Germany in March and June 1998. The analysis detected o-phenylphenol in the influent samples at concentrations of 1.54 and 3.58 μg l^{-1} whereas o-phenylphenol was not detected in the effluent samples (concentrations < 0.01 μg l^{-1}).

Surface waters and sediments

No data has been located on the concentrations of o-phenylphenol in surface waters. However, given that o-phenylphenol is readily biodegradable and is almost completely eliminated within the treatment processes of a sewage treatment works low levels of o-phenylphenol would be expected in surface waters receiving treated effluents.

9.7.3.2 Terrestrial environment

No measured concentration data are available but information from Bayer AG indicates that o-phenylphenol or related products are not directly applied or released to the terrestrial environment and that residues would not be expected.

9.7.3.3 Aerial environment

No measured concentration data are available but the low volatility of o-phenylphenol means that the substance would not be expected to be present in the aerial compartment.

9.7.3.4 Comparison of environmental monitoring data and exposure concentrations causing endocrine mediated responses

At present the absence of data on concentrations of o-phenylphenol in surface waters precludes an assessment of the margin of safety relative to exposure concentrations causing endocrine mediated responses in aquatic organisms.

9.8 Overall Conclusions on o-phenylphenol

The following conclusions have been drawn from a review of the data for o-phenylphenol:

9.8.1 Data from studies assessing potential endocrine disrupting effects

9.8.1.1 Human related studies

- *In vivo* sub-chronic studies in rats using oral exposure up to doses of 300 mg kg body weight^{-1} day^{-1} resulted in no histopathological effects on endocrine glands and hormone sensitive tissues.

- Two generation reproduction studies carried out in rats using oral exposure showed no evidence of effects on reproductive parameters at the highest doses tested (457 and 500 mg kg body weight^{-1} day^{-1} respectively), even though there was evidence of maternal toxicity at lower doses.

- *In vivo* developmental studies in rats and rabbits indicated no teratogenic effects at the highest doses tested (250 – 1200 mg kg body weight^{-1} day^{-1}) even though maternal toxic effects were evident at lower doses[1].

- The lowest doses tested in the oral and dermal exposure studies were in the range of 25 - 30 mg kg body weight^{-1} day^{-1} and no effects which may be endocrine mediated were evident at these doses.

- *In vitro* data indicates no or weak binding affinity to the human oestrogen receptor and limited proliferation of mammalian cells following exposure to o-phenylphenol. No data is available on the androgenic and anti-androgenic effects of o-phenylphenol and effects on thyroid function and hormone synthesis and secretion and steroidogenesis in mammalian cells and tissues.

9.8.1.2 Wildlife studies

- The data on potential endocrine disrupting effects in wildlife was limited to a reproduction study in the water flea *Daphnia magna* which showed a NOEC of 0.009 mg l^{-1} and a reproduction study in fathead minnows (*Pimephales promelas*) which showed a NOEC of 0.036 mg l^{-1}. No effects on vitellogenin induction and gonadosomatic index were evident even at the highest exposure concentration (0.29 mg l^{-1}) indicating that the effects on reproductive parameters may not be oestrogen mediated.

- No data on potential endocrine mediated responses in terrestrial or aerial species has been located.

- The *in vitro* data from rainbow trout hepatocyte cells showed effects on vitellogenin gene expression but only at high exposure levels (17 mg l^{-1}).

9.8.2 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity

9.8.2.1 Human related studies

- In acute and repeat-dose studies the general systemic toxicity data for laboratory mammals indicates that the threshold in rats for an absence of effects which are not directly endocrine mediated occurs at a dose of approximately 35 mg kg body weight^{-1} day^{-1} for adult rats in a two generation reproduction study (Eigenberg 1990). The NOEL of 35 mg kg body weight^{-1} day^{-1} reported in the study relates to transitional cell hyperplasia/papillomatosis in the urinary bladder of animals at 125 mg kg body weight^{-1} day^{-1}. In a two year chronic toxicity/oncogenicity study in rats a NOEL of 39 mg kg body weight^{-1} day^{-1} was reported based on histopathological changes in the livers, kidneys and urinary bladders of males at 200 mg kg body weight^{-1} day^{-1}. As a result it appears that on the basis of the available data endocrine mediated responses may not be the mechanism responsible for lowest observed toxicity in laboratory mammals and that the kidney and liver are the main target sites for o-phenylphenol (see Section 9.3.1).

9.8.2.2 Wildlife studies

- In wildlife studies data was only available for aquatic species (invertebrates and fish) and not for terrestrial or aerial species. The lowest NOECs for survival in invertebrates and fish were $\geq$0.07 mg l^{-1} (21 day NOEC value for the water flea *Daphnia magna*). These values were higher than the NOEC for potential endocrine mediated responses in *Daphnia magna* of 0.009 mg l^{-1} and the NOEC for effects on reproduction (egg production and hatchability) of fathead minnows (*Pimephales promelas*) of 0.036 mg l^{-1}.

9.8.3 Exposure data

9.8.3.1 Worker exposure

- Limited data has been located on concentrations of o-phenylphenol to which workers or consumers are exposed.

9.8.3.2 Consumer exposure

- No data on concentrations of o-phenylphenol to which consumers are potentially exposed during the use of the products containing the substance has been obtained.

9.8.3.3 Environment

- No data has been located on environmental concentrations of o-phenylphenol in surface waters but data for a municipal treatment works in Germany indicated that o-phenylphenol was not discharged at detectable levels (<0.01 µg l^{-1}).

9.9 Summary of the weight of evidence for endocrine disrupting effects in humans and/or wildlife and associated uncertainty

The summary of the weight of evidence for endocrine disrupting effects of o-phenylphenol in humans and wildlife along with associated uncertainties are given in Table 9.12.

	Target group	
	Humans	Wildlife
Weight of evidence	The available data from *in vivo* studies in laboratory mammals (using oral or dermal exposure routes) indicates that o-phenylphenol does not cause adverse effects on reproductive and developmental endpoints (which may be endocrine mediated) at exposure levels where general systemic toxic effects are observed. The lowest NOEL in the *in vivo* studies was 250 mg kg body weight $^{-1}$ day^{-1} for foetotoxic and developmental effects. Limited exposure data for workers and consumers has been located.	The available aquatic effects data shows that the threshold exposure concentrations of o-phenylphenol above which reproduction of the invertebrate *Daphnia magna* and fish (fathead minnow) are reduced (NOECs = 0.036 mg l^{-1} and 0.009 mg l^{-1} respectively) are lower than the theshold levels for general toxic effects (i.e. lethality). The effects observed on reproduction in fish were evidently not oestrogen mediated. However, there is no information on the mechanism of action for the effects on reproduction observed in *Daphnia magna*.
Uncertainties	There are no major uncertainties with regard to the evaluation of potential adverse effects of o-phenylphenol on reproductive and developmental endpoints since data is available from a definitive multi-generation study as well as supporting reproduction and developmental studies. Mechanistic uncertainties exist because the available studies provide no direct measurement of changes in endocrine function (for example changes in hormone levels).	There is no data on potential adverse effects on reproduction and development in terrestrial and aerial organisms but this is not a major uncertainty since the physico-chemical properties of o-phenylphenol mean the potential for these organisms to be exposed is limited. No environmental exposure data for o-phenylphenol in the aquatic, terrestrial and aerial compartments has been located.

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10. REVIEW OF DATA FOR RESORCINOL

Table of Contents

Notes:

This section contains information collected and collated from a range of sources including published papers, reports of studies conducted by industrial companies or sector groups and data compilations such as IUCLID (2000). The data from IUCLID has been taken as accurate and individual source documents have not been checked unless they are considered to be key studies which have a major influence on the outcome of the review. All information taken from IUCLID has been referenced as being from that source and individual references have not been given in the references.

This review has been carried out in accordance with the evaluation framework described in Section 2. In the review the International Programme for Chemical Safety (IPCS) definition of an endocrine disrupter has been adopted, namely that it is "*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*".

In the context of the review it is recognised that there are various laboratory-based *in vivo* and *in vitro* methods utilising a range of (eco)toxicological endpoints that are claimed by different sources to be relevant to the assessment of endocrine disruption in humans and wildlife. However, since this field is still in an early stage of development there is uncertainty regarding the significance of many of the current findings.

From the numerous recent reviews of potential test methods (such as the Detailed Review Paper prepared by OECD in 1997) there is a clear consensus in terms of the hierarchy of the relevance of test methods. In this hierarchy longer-term *in vivo* studies considering effects on reproduction and/or development (and including mechanistic information) are of greater relevance than short-term *in vivo* screening tests which are of greater relevance than *in vitro* assays. The greater relevance of chronic *in vivo* tests or those assessing effects during critical windows of sensitivity is also evidenced by the fact that these are the key (eco)toxicological methods being developed in the OECD Endocrine Disruption Testing and Assessment (EDTA) Programme. This hierarchy approach to data relevance has been adopted in the review along with a weight of evidence consideration of the available data.

The review has been carried out to address three key questions:

1. Does the available data indicate there is evidence that a chemical causes endocrine disrupting effects in target groups of humans/mammals and/or wildlife?

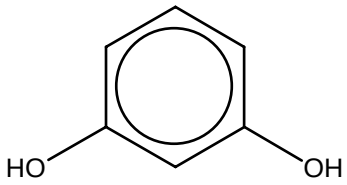
2. Do endocrine disrupting effects of the chemical in target groups of humans/mammals and/or wildlife occur at lower concentrations than those causing effect on general toxicological endpoints?

3. Are particular target groups of consumers or organisms in the environment likely to be exposed to concentrations of chemicals which exceed effects thresholds due to current emission patterns.

It should be recognised that this review is not designed to be a full Risk Assessment of a substance under the Existing Substances Regulation 793/93.

10.1 Physico-chemical data for Resorcinol

10.1. Summary details on the substance

CAS Number	108-46-3
EINECS Number	203-585-2
IUPAC Name	1,3-Benzenediol
Other names	Resorcinol, resorcin, 1,3-benzendiol, 1,3-dihydroxybenzene, m-dihydroxybenzene, m-hydroquinone, 3-hydroxyphenol, m-hydroxyphenol
Molecular weight	110.11
Chemical formula	$C_6H_6O_2$
Chemical structure	

10.1.2 Physico-chemical properties and environmental fate information (from IUCLID 2000)

The data on the physico-chemical properties of resorcinol and its environmental fate (see Table 10.1) indicate that in a series of aerobic Zahn Wellens tests the substance has been reported to be inherently biodegradable while in a modified MITI test resorcinol was reported to be readily biodegradable (66.7% loss after 14 days).

Volatilisation is unlikely to represent a major removal process from the aquatic environment based on the Henry's Law Constant of 8×10^{-6} Pa-m^3 mol^{-1} (8.1×10^{-11} atm m^3 mol^{-1}) being lower than a value range of 1-100 Pa-m^3 mol^{-1} which is considered to indicate volatility. Resorcinol is readily degraded by reaction with photochemically produced hydroxyl radicals (half life of < 1 day)

In soil, biodegradation is the major degradation process for resorcinol.

Table 10.1 Physico-chemical properties and environmental fate data (from IUCLID 2000)

Physico-chemical property	Value (and comments)
Physical state at ambient temperature	Solid
Water solubility	1400 g l^{-1} at 20°C
Octanol-water partition coefficient (log Kow)	0.93 at 20°C
Organic carbon water partition coefficient (Koc)	No data
Henry's Law Constant	8 x 10^{-6} Pa-m^3 mol^{-1} (8.1 x 10^{-11} atm m^3 mol^{-1})
Type of degradation	
Aquatic - abiotic	No data
Aquatic - biotic	In a series of aerobic Zahn Wellens tests resorcinol has been reported to be inherently biodegradable. In a modified MITI test resorcinol was reported to be readily biodegradable (66.7% loss after 14 days).
Terrestrial	Biodegradation is the major degradation process for resorcinol in soils.
Atmospheric	Resorcinol is readily degraded by reaction with photochemically produced hydroxyl radicals (half life of < 1 day).

A Mackay Level 1 fugacity model has shown that for a discharge of 1000 tonnes of resorcinol 99.2% of the substance will partition into the soil (Table 10.2). Amounts present in other compartments are minimal.

Table 10.2 Summary of the results of a Mackay Level 1 fugacity model

Compartment	Volumes of different compartments	% of substance present in different compartments
Water	2 x 10^{11}	99.2
Suspended sediment	10^6	5.2 x 10^{-4}
Bottom sediment	10^8	0.017
Fish	2 x 10^5	4.2 x 10^{-5}
Air	10^{14}	0.0016
Aerosol	2000	2.4 x 10^{-8}
Soil	9 x 10^9	0.75

10.2 Production and Uses

10.2.1 Production Patterns

Since the withdrawal from production of resorcinol by Hoechst in 1991, the demand for resorcinol in Europe has been satisfied exclusively by imports. The three main global producers are INDSPEC, Mitsui and Sumitomo. According to the recent report on Resorcinol by CEH (2001) there are also three small capacity plants located in China and four in India. The total imports into Europe for 2000 are estimated to be 14700 tonnes with 1100 tonnes

being re-exported, leaving a residual demand of 13600 tonnes. This demand has grown at an average rate of 6.4% in the period from 1995 to 2000, but is expected to decline at a rate of 1.2% per annum to 2005, primarily because of the projected elimination of use of resorcinol in flame retardants (current use 2100 tonnes). The projection for consumption in 2005 is therefore approximately 12700 tonnes.

10.2.2 Use Patterns

The application and use of resorcinol is relatively well understood because of the limited number of producers of resorcinol and the well-documented application areas supplied. Any confidentiality issues pertaining to the disclosure of supply data by producers have been overcome by a recent study conducted by CEH, which has updated the industry's statistics to the year 2000. Table 10.3 summarises the current situation which has been assembled using a combination of both CEH and producer sources.

Table 10.3 Annual consumption of resorcinol by application and region

	Europe		United States	Japan	Other Regions	Total	%
	Tonnes	%	Tonnes	Tonnes	Tonnes	Tonnes	
Rubber Products	6,480	48.0%	10,271	1,598	5,470	23,820	53.2%
Wood Adhesives	2,700	20.0%	1,820	572	2,280	7,373	16.5%
Flame Retardants	2,100	15.6%	1,222	250	500	4,072	9.1%
UV Stabilisers	1,000	7.4%	588	120	200	1,908	4.3%
Dyes	300	2.2%	350	230	750	1,630	3.6%
MAPs	0	0.0%	0	1,880	0	1,880	4.2%
Hair Dyes	150	1.1%	150	75	75	450	1.0%
Pharmaceuticals	75	0.6%	75	50	25	225	0.5%
Others	695	5.1%	323	875	1,550	3,443	7.7%
Accounted Total	13,500		14,800	5,651	10,850	44,801	
CEH declared total	13,500		14,800	5,400	11,100	44,800	
% Accounted	100.0%		100.0%	104.6%	97.7%	100.0%	

It should be noted that the data sets on the smaller uses of resorcinol (e.g. pharmaceutical uses) are less precise than the larger mainstream uses. Nonetheless, even where the regional data has been estimated, the global totals are believed to be sound.

Table 10.3 has been constructed in such a way as to highlight the differences in the use patterns in Europe in comparison with the global picture. For instance, it can be seen that the use of resorcinol is more prevalent in the wood adhesives sector in Europe than it is elsewhere in the world. From this data set, it is possible to assess the likely points of exposure in both human and environmental terms.

10.3 Toxicokinetics, metabolism and bioaccumulation

10.3.1 Toxicokinetics and metabolism

Following sub-cutaneous administration of a single dose of 50 or 100 mg kg body weight^{-1} ^{14}C labelled resorcinol to male CD rats, radioactivity was rapidly lost from plasma with approximately 90% being cleared in the first two hours. Elimination was biphasic and characterised by half-lives of 18-21 minutes and 9-11 hours. Twenty four hours after dosing of 10 mg kg^{-1} body weight^{-1}, 94% was excreted in the urine and <0.5% in the faeces primarily (84%) in the form of the glucuronide conjugate. Resorcinol equivalents were rapidly distributed to major tissues, but showed no tendency to accumulate. A 14 or 30 day daily sub-cutaneous administration of 100 mg kg body weight^{-1} did not affect the toxicokinetics of resorcinol (Merker *et al* 1982).

Kim and Mathews (1987) administered an oral dose of 112 mg kg^{-1} body weight^{-1} ^{14}C labelled resorcinol to F344 rats. It was found that resorcinol was quickly absorbed by the gastrointestinal tract and then rapidly metabolised and excreted. There was no significant sex difference in response. Twenty four hours after dosing, 91-93% of the material was excreted in the urine and 1-2% was detected in the faeces. The remaining radioactivity was distributed among various tissues with no indication of bioaccumulation in any single tissue. A large proportion of the material excreted in bile underwent enterohepatic circulation to be eventually excreted in the urine. The major metabolite present in the urine was the monoglucuronide conjugate (approximately 70%). Additional metabolites included a monosulphate conjugate, a mixed sulphate-glucuronide conjugate, and a diglucuronide conjugate, which was a minor metabolite. Generally the same results were obtained after a single dose of 225 mg kg^{-1} body weight^{-1} or daily doses of 225 mg kg body weight^{-1} for 5 consecutive days.

The percutaneous and metabolic disposition of 2% resorcinol applied in a hydroalcoholic vehicle has been determined in three human subjects with 150 μg per cm^2 applied to 2600 cm^2 of the body surface (30%) 2 times per day, 6 days per week (Yeung *et al* 1983). Resorcinol penetrated the skin at a rate of 0.37 μg per cm^2 per hour and after two weeks of bi-daily application an average of 1.64% of the dosage was being excreted in 24-hour urine samples as the glucuronide or sulphate conjugates. These results indicate that absorption through intact skin is low. Use of radiolabelled resorcinol in a hair dye formulation topically applied to human volunteers resulted in urinary excretion of 0.076% of the applied dose (Wolfram and Maibach 1985).

10.3.2 Bioaccumulation

In laboratory mammals no evidence of bioaccumulation has been found. No experimentally derived information on bioaccumulation in aquatic or terrestrial organisms is available, but the low log Kow (0.93) indicates that significant bioaccumulation is unlikely.

10.4 Studies relevant to the assessment of potential endocrine disrupting effects

10.4.1 Studies relevant to the assessment of potential endocrine disrupting effects in humans

A review of the human and animal toxicology of resorcinol with emphasis on thyroidal effects has been prepared by CANTOX Health Sciences International in collaboration with Dr E Delzell (Professor of Epidemiology at University of Alabama). In order to place the outcome of this review in context the conclusions of the CANTOX report (CANTOX 2000) have been presented below for comparison:

"The *in vitro* data demonstrate that resorcinol can inhibit thyroid peroxidase enzymes (TPO) and potentially block the synthesis of thyroid hormone. The results of older, non-Good Laboratory Practice (GLP), studies conducted in experimental studies show that resorcinol administered by routes or methods (e.g. dermal, subcutaneously in oil vehicle, in drinking water, and in feed) that allow for continuous exposure (i.e. continued presence of resorcinol in the blood) disrupts thyroid hormone synthesis and produces changes in the thyroid gland consistent with goitrogenesis. However, more recent, high quality, GLP-complaint studies conducted by the National Toxicology Program of the U.S. (NTP, 1992), showed that there were no effects of resorcinol on serum T3 and T4 concentrations[1] or on the gross histologic pathology of the thyroid gland, even at near lethal doses for 13 weeks to 2 years. This finding underscores the need for extreme doses and for continuous exposure to elicit thyroid effects in animals (i.e. in the NTP studies the NOAELs for thyroidal effects ranged up to 520 mg kg body weight^{-1} day^{-1}). In any case, the animal data must be evaluated in light of known susceptibility of the rodent to disruption of thyroid function due to species-specific differences in synthesis, binding, and transport of thyroid hormone. Several leading scientists have stated that hormonally-induced changes of the thyroid of rats may have little relevance to humans (Ames *et al* 1987, Alison *et al* 1994, McClain 1994). Despite the sensitivity of the rodent thyroid, there is no evidence in any of the animal studies of widespread disruption of other endocrine function. This is further supported by the lack of effect of resorcinol in reproductive and developmental toxicity studies or in tests to evaluate estrogenic and anti-estrogenic properties. Most substances considered classical "endocrine disrupters" can be expected to show a wide spectrum of effects in these types of tests.

The human data echo the results of the experimental animal findings in that the implications of an effect of resorcinol on thyroid function come from case study reports of individuals who applied copious amounts of resorcinol-containing skin ointments for periods of months to years. In most cases, application was to skin that was ulcerated or was otherwise compromised. This would have allowed for greater absorption and continuous exposure to the resorcinol from the ointment formulation. Effect levels have been estimated to be in the range 34 to 122 mg kg body weight^{-1} day^{-1}. The affected patients developed signs and symptoms of hypothyroidism that improved or disappeared after cessation of exposure to resorcinol.

The results of Dr Delzell's review of the available epidemiologic data indicate that beyond the case reports, no causal link has been established between resorcinol and thyroid disease in human populations. Occupational epidemiology studies have not provided any evidence that potential exposures to concentrations of resorcinol higher than found in the general environment causes thyroid dysfunction. A number of studies (Gaitan *et al*.) have investigated the relationship between endemic goiter in areas of Columbia and Eastern Kentucky and exposure to "phenolic substances" including resorcinol in drinking water. However, these

[1] T3 = Tri-iodothyronine, T4 = Thyroxine, TSH = Thyroid stimulating hormone

studies do not fulfil accepted scientific criteria for establishing resorcinol as a cause of thyroid disease. They did not qualify exposure to resorcinol in individual subjects, and they did not demonstrate dose-response or rule out confounding by the multiple other chemicals present in water supplies, by bacterial contamination of water or by nutritional factors. As a result, the studies of Gaitan *et al*. have not identified the specific agents causing endemic goitre in the areas investigated. Overall, epidemiologic research provides no consistent evidence that environmental or occupational exposures to resorcinol adversely affect thyroid function.

A risk assessment compared estimated worst-case environmental and occupational exposures to resorcinol with a value of 10 mg kg body weight^{-1} day^{-1} established for safe human exposure based on human and animal studies. The results of this risk assessment support the conclusion that under real world exposure conditions, resorcinol is not expected to cause adverse effects on thyroid function."

10.4.1.1 In vitro studies

A. Receptor competitive binding assays

Saito *et al* (1999) investigated the oestrogenic and anti-oestrogenic activities of resorcinol using two *in vitro* receptor-mediated assays. A mammalian cell-based luciferase reporter gene assay was developed for detecting oestrogenic and anti-oestrogenic effects of chemicals on human oestrogen receptor (ER) mediated transactivation. Cells expressing luciferase constitutively were used for estimating effects on a transcriptional activity by a receptor-independent manner and cytotoxicity. Neither oestrogenic nor anti-oestrogenic effects were detected by the assays at concentrations from 10^{-3} to 10 μM (0.11 – 1101 μg l^{-1}). In the analysis of oestrogenic activity 100 pM of 17β-oestradiol was used as a positive control and showed 4 fold induction relative to the solvent control. The anti-oestrogen, 4-hydroxytamoxifen, markedly inhibited 17β-oestradiol luciferase induction.

B. Recombinant yeast assays

Saito *et al* (1999) used a yeast two hybrid assay to investigate the effects of resorcinol on ligand-dependent interaction between the human oestrogen receptor and a coactivator (TIF2: Transcriptional Intermediary Factor 2). In the assay 17β-oestradiol showed oestrogen activity (maximum induction of approximately 56 times). However, no significant effects were observed with resorcinol, indicating that the substance is not capable of affecting oestrogen receptor mediated transactivation *in vitro*.

C. Mammalian cell growth assays

No data has been located on the effects of resorcinol in mammalian cell growth assays.

Summary of in vitro data

Table 10.4 summarises the *in vitro* data for resorcinol which relates to assays using mammalian cells and tissues assessing oestrogenic and anti-oestrogenic mechanisms of action. In mammalian cell-based luciferase reporter gene assays resorcinol did not show oestrogenic or anti-oestrogenic effects. In a yeast two-hybrid assay resorcinol was not found to be capable of affecting oestrogen receptor mediated transactivation *in vitro*.

No data has been identified on the androgenic and anti-androgenic effects of resorcinol or effects on steroidogenesis in mammalian cells and tissues.

In vitro studies have been performed to assess the effects of resorcinol on thyroid function. In porcine thyroid gland slices the presence of 0.2 – 0.5 μM resorcinol ^{125}I-uptake to form precursors of T3/T4 (mono or diiodotyrosines) was significantly inhibited (Cooksey *et al* 1985). Porcine thyroid peroxidase was also inhibited following resorcinol (0.3 μM or 33 μg l^{-1}) exposure. A similar study was carried out by Divi and Doerge (1994) with lactoperoxidase (closely related to thyroid peroxidase) in which resorcinol (0.2 mM or 22 mg l^{-1}) resulted in the inhibition of activity. However, in a study with the rat thyroid cell line (FRTL-5) resorcinol (5-10 μM or 550 – 1101 μg l^{-1}) increased ^{125}I-uptake in a dose dependent manner with TSH (100 μU ml^{-1}) whilst without TSH resorcinol did not affect ^{125}I-uptake (Gaitan *et al* 1995). In thyroid slice studies, aqueous extracts of coal-derived pollutants were found to be potent inhibitors of thyroid peroxidase or ^{125}I organification. Resorcinol, 2-methyl and 5-methyl resorcinols were reportedly more potent inhibitors than the anti-thyroid drug 6-propylthiouracil (Lindsay *et al* 1992) although the study design was limited.

Table 10.4 Summary of the *in vitro* data in isolated mammalian cells and tissues relating to different mechanisms of action of resorcinol

Mechanism of endocrine disruption	Responses observed in *in vitro* systems
Oestrogenicity/anti-oestrogenicity	In mammalian cell-based luciferase reporter gene assays resorcinol did not show oestrogenic or anti-oestrogenic effects. In a yeast two-hybrid assay resorcinol was not found to be capable of affecting oestrogen receptor mediated transactivation *in vitro*.
Androgenicity/anti-androgenicity	No data identified
Thyroid effects	Available data indicate that resorcinol inhibits thyroid function
Effects on hormone synthesis or secretion	No data identified
Effects on steroidogenesis	No data identified

10.4.1.2 In vivo studies

Tables 10.5 to 10.7 summarises the information on endocrine mediated responses in laboratory mammals following exposure via the oral (Table 10.5), dermal (Table 10.6) or sub-cutaneous injection (Table 10.7) route.

A. Effects on endocrine glands and hormone sensitive tissues

A series of older, non-Good Laboratory Practice (GLP), studies conducted in laboratory mammals have shown that resorcinol administered by routes or methods (e.g. dermal, subcutaneously in oil vehicle, in drinking water, and in feed) that allow for continuous exposure (that is the continued presence of resorcinol in the blood) disrupts thyroid hormone synthesis and produces changes in the thyroid gland consistent with goitrogenesis (Doniach and Logothetopoulos 1953, Samuel 1955)

Administration of 5% resorcinol via diet to rats for 2 weeks increased thyroid weight, decreased plasma T4 levels and a decreased T4 half-life (Berthezene *et al* 1979). The effect on T4 half-life may indicate a hepatic effect due to the excessively large constant dietary dose.

Merker *et al* (1982) administered multiple sub-cutaneous daily doses of 100 mg kg body weight^{-1} (2 x 50 mg kg body weight^{-1}) for 14 or 30 days to male Sprague-Dawley rats. It was found that exposure to resorcinol did not result in overt signs of toxicity or adverse changes in thyroid function (as serum T3 and T4 levels) organ weights, body weight gain, several haematological parameters (including number of red blood cells, haemoglobin and haematocrit), clinical chemistry parameters and histopathology.

In a study exposing Wistar rats to 5 mg kg body weight^{-1} day^{-1} for 30 days significant enlargement of the thyroid gland and decreased T3 and T4 levels was reported (Cooksey *et al* 1985). However, the experimental details reported for this study are limited.

Brandt (1986) reported a rat 4 week feeding study conducted at a dose level of 0-260 mg kg body weight^{-1} day^{-1} resulted in decreased relative adrenal weights in all exposed animals. Exposure did not induce clinical abnormalities, histopathology, body weight effects or mortalities.

In a study by Seffner *et al* (1995) administration of 0.004% resorcinol in drinking water (calculated intake[2] = 5 mg kg body weight^{-1} day^{-1}) to cross bred rats for 3 months resulted in significantly increased mean follicular epithelial cell height and significantly decreased mean follicular diameter. These changes were considered to be precursors of goitre though no NOAEL value for these effects was derived.

In National Toxicology Programme studies rats and mice were exposed to resorcinol in 13 week studies conducted to US EPA/FDA standards and GLP. Groups of 10 male and female F344 *rats* were treated with 0, 32, 65, 130, 260 or 520 mg kg^{-1} day^{-1} by oral gavage in deionized water for 13 weeks. All females (10 of 10) and 8 of 10 males at 520 mg kg^{-1} day^{-1} died as a result of treatment. Absolute and relative adrenal weights were significantly *increased* in *all* surviving males albeit without a clear dose-response relationship such that no NOEL not established. Increased absolute and relative liver weights were seen in the $\geq$65 mg kg^{-1} day^{-1} females and in $\geq$130 mg kg^{-1} day^{-1} males. There were no other gross or microscopic lesions (including the thyroid gland) attributable to treatment in animals surviving the exposure period and final bodyweights were similar across groups (NTP 1992). No effects on measures of thyroid function or the plasma concentrations of T3 and T4 in either males or females was recorded and there was an absence of histological evidence of goitrogenic activity.

Groups of 10 male and female B6C3F1 *mice* were treated with 0, 28, 56, 112, 225 or 420 mg kg^{-1} day^{-1} by oral gavage in deionized water for 13 weeks. Mortalities related to treatment were observed in 7 of 10 male and female mice at 420 mg kg^{-1} day^{-1} following exhibition of dyspnea, prostration and tremors. Absolute and relative adrenal gland weights were *decreased* in males at *all* dose levels although no dose-response relationship was evident and no NOEL could be established. There were no gross or microscopic lesions (including the thyroid gland) attributable to treatment in animals surviving the exposure period and final bodyweights were similar across groups (NTP 1992). No effects on measures of thyroid

[2] 5 mg kg body weight^{-1} day^{-1} = 0.04 mg ml^{-1} x 35 ml day^{-1} x 0.275 kg body weight^{-1}

function or the plasma concentrations of T3 and T4 in either males or females was recorded and there was an absence of histological evidence of goitrogenic activity.

No No-Effect-Levels were established in 13 week NTP rat or mouse studies due to adrenal weight effects at all treatment groups in both species. The significance of the adrenal effects observed by Brandt (1986) and NTP (1992) is unclear and represents an area of uncertainty.

Berthezene *et al* (1979) showed that intravenous resorcinol decreased radioiodine uptake which is consistent with thyroid peroxidase enzyme (TPO) inhibition (see Section 10.4.1.1). Subsequent studies by Divi and Doerge (1994, 1996) and Doerge and Divi (1995) have provided direct evidence that the anti-thyroid effect of resorcinol is mediated by inhibition of thyroid peroxidase enzymes.

B. Reproduction and fertility studies

At present no data has been located on the effects of resorcinol in reproduction or fertility studies by the oral route. A limited topical application study of hair colouring formulations applied twice weekly to Spague Dawley rats revealed no adverse effects on fertility or gestation, lactation or weaning indices (Burnett and Goldenthal 1988).

The Resorcinol Task Force is in the process of initiating a regulatory guideline compliant reproduction/ multi-generation study in the rat (OECD TG 415, 416; USEPA 870.3800/ 3550) to examine potential effects on the postnatal development of offspring and derive NOEL's for the endpoints of evaluation. It is anticipated that this will include histopathology of endocrine tissues.

C. Developmental and teratogenicity studies

Teratology studies in the rat and rabbit conducted to prevailing regulatory standards have been conducted (Osterburg 1982a,b). In the rat study, groups of 23 female Sprague-Dawley rats were treated by oral gavage with resorcin at dose levels of 0, 40, 80 or 250 mg kg^{-1} day^{-1} from days 6-15 of pregnancy. There were no adverse effects of treatment on gross embryo-foetal development and no effects on maternal toxicity. In the rabbit study, groups of 11-15 New Zealand White rabbits were treated by gastric intubation from day 6 to 18 of pregnancy with 0, 25, 50 or 100 mg kg^{-1} day^{-1} resorcin. There was a slight reduction in maternal bodyweights at 100 mg kg^{-1} day^{-1} but no evidence of gross teratogenicity or embryotoxicity.

DiNardo *et al* (1985) administered doses of 0, 125, 250 and 500 mg kg body $weight^{-1}$ resorcinol daily by oral gavage to Sprague-Dawley rats from days 6 to 15 of gestation. Exposure caused a slight reduction in maternal weight gain at the 500 mg kg body $weight^{-1}$ dose level. However, there was no evidence of foetal abnormalities or malformations, embryotoxicity or effects on the number of litters produced even at the highest dose (500 mg kg^{-1} day^{-1}).

A teratology study was conducted by the United States Environmental Protection Agency (Kavlock 1990) in which resorcinol was administered by oral gavage at doses of 0, 333, 667 and 1000 mg kg body $weight^{-1}$ day^{-1} to groups of Sprague-Dawley rats on day 11 of gestation. No effects of treatment were recorded on any of the developmental parameters including litter size, rate of perinatal loss, average pup weight and total litter weight. Maternal weight loss was reported the first day after dosing at all levels. However, no effect on maternal weights were found 72 hours after commencement of treatment.

Hogan *et al* (1977) assessed the effects of hair dye formulations (with 2 of 3 formulations tested containing 1.7% resorcinol) on mice following dermal application of 0.5 ml twice a week from 4 weeks prior to mating through the mating and gestation periods. The study showed no evidence of teratogenic effects. However, there was a suggestion of a retarding effect of the formulations on the ossification process. In addition, slightly lower foetal weights were noted in all formulation-treated groups although mean crown-rump distances were comparable to the controls. No overt signs of maternal toxicity were found.

Brandt (1986) also reviewed a series of developmental/teratogenicity studies in which hair dyes containing resorcinol were evaluated in rats and rabbits and shown to have no adverse effects.

D. Carcinogenicity and oncogenicity studies

Using information from the 17 day and 13 week studies (see Section on effects on endocrine glands and hormone sensitive tissues) to select dose levels, the National Toxicology Programme sponsored lifetime carcinogenesis studies in two species. The studies were unconventional and are not fully guideline compliant in that mice and male rats were only exposed to two dose levels (below the limit dose) and the female rats died requiring a second study to be started (NTP 1992).

Groups of 60 male F344 *rats* were treated with 0, 112 or 225 mg kg^{-1} day^{-1} by oral gavage in deionized water 5 times per week for 104 weeks. Groups of 60 female rats were initially treated with the same dose levels as male rats but by 22 weeks, 16 of 60 of the high dose females had died. The female study was re-started separately using doses of 0, 50, 100 or 150 mg kg^{-1} day^{-1}. At 15 months 10 animals per group were subject to interim evaluations that revealed no treatment related biological effects. In the studies mean bodyweights of high-dose male rats were 10-15% lower than the controls between weeks 87 to 104 of study indicating a maximum tolerated dose was achieved. Mean body weights of high dose females (150 mg kg body $weight^{-1}$ day^{-1}) were 11-14% lower than the controls between weeks 95 to 104 of study indicating a maximum tolerated dose was achieved. Survival was also reduced at the high dose. There were no effects on body weights at lower dose levels. There were no treatment-related increases in non-neoplastic or neoplastic lesions. Female rats showed dose-related reductions in mammary fibroadenoma's. At the higher doses in the male (112 and 225 mg kg body $weight^{-1}$ day^{-1}) and female studies (100 and 150 mg kg body $weight^{-1}$ day^{-1}) clinical signs associated with an effect on the central nervous system (for example ataxia or tremors) were seen.

Groups of 60 B6C3F1 male and female *mice* were treated with 0, 112 or 225 mg kg^{-1} day^{-1} by oral gavage in deionized water 5 times per week for 104 weeks. Clinical signs associated with an effect on the central nervous system (for example ataxia and tremors) were seen at both test doses. Mean body weights of high-dose female mice only were 10-15% lower than the controls between weeks 85 to 104 of study indicating a maximum tolerated dose was achieved in this sex. There were no effects of treatment on bodyweights at lower dose levels or on survival. There were no treatment-related increases in non-neoplastic or neoplastic lesions. The incidence of subcutaneous fibroma or sarcoma in high dose male mice was reduced.

Table 10.5 Summary of the data on potential endocrine mediated responses in laboratory mammals following oral exposure

Species	Life stage of the test organism at start of test	Exposure route and dose series	Description of endocrine disruption measurement parameter(s) and effect doses	Reference	Test Relevance	Study validity
Rat (Wistar)	No details	0 and 5 mg kg body weight^{-1} day^{-1} for 30 days	Significant enlargement of the thyroid gland and decreased T3 and T4 levels at 5 mg kg body weight^{-1} day^{-1}	Cooksey *et al* (1985)	Medium	Use with care
Rat (Fischer 344)	Males and Females	Oral gavage at 0, 27.5, 55, 110, 225 and 450 mg kg body weight^{-1} day^{-1} for 17 days	No significant effects (relative to the controls) on histopathology of tissues at any test dose *(NOAEL for effects on endocrine glands or hormone sensitive tissues = 450 mg kg^{-1} body weight^{-1}*	NTP (1992)	Medium	Valid
Rat (Fischer 344)	Males and Females	Oral gavage at 0, 32, 65, 130, 260 and 520 mg kg bw^{-1} day^{-1} for 13 weeks	Significant effects (relative to the controls) on adrenal weights at all test doses	NTP (1992)	Medium	Valid
Mouse (B6C3F1)	Males and Females	Oral gavage at 0, 37.5, 75, 100, 300 and 600 mg kg bw^{-1} day^{-1} for 17 days	No significant effects (relative to the controls) on histopathology of tissues at any test dose, but mortalities at 300 and 600 mg kg^{-1} body weight^{-1} *(NOAEL for effects on endocrine glands or hormone sensitive tissues = 100 mg kg^{-1} body weight^{-1}*)	NTP (1992)	Medium	Valid
Mouse (B6C3F1)	Males and Females	Oral gavage at 0, 28, 56, 112, 225 and 420 mg kg bw^{-1} day^{-1} for 13 weeks	Significant effects (relative to the controls) on adrenal weights at all test doses	NTP (1992)	Medium	Valid
Rats (Cross bred)	No details	O and 5 mg kg body weight^{-1} day^{-1} for 90 days in drinking water	Significant histomorphometric changes in structure of thyroid gland cells at 5 mg kg body weight^{-1} day^{-1}	Seffner *et al* (1995)	Medium	Use with care
Rat (Sprague-Dawley)	Pregnant females	Oral gavage at 0, 40, 80 and 250 mg kg body weight^{-1} day^{-1} on gestation days 6 to 15	No significant effects (relative to the controls) on embryo and foetal development at any test dose *(NOEL for embryotoxicity and foetal development = 250 mg kg body weight^{-1} day^{-1})*	Osterburg (1982a)	Medium	Valid

Table 10.5 Continued

Species	Life stage of the test organism at start of test	Exposure route and dose series	Description of endocrine disruption measurement parameter(s) and effect doses	Reference	Test Relevance	Study validity
Rat (Sprague-Dawley)	Pregnant females	Oral gavage at 0, 125, 250 and 500 mg kg body weight^{-1} day^{-1} on gestation days 6 to 15	No significant effects (relative to the controls) on embryo and foetal development at any test dose *(NOEL for embryotoxicity and foetal development = 500 mg kg body weight^{-1} day^{-1})*	DiNardo *et al* (1985)	Medium	Valid
Rat (Sprague-Dawley)	Pregnant females	Oral gavage at 0, 333, 667 and 1000 mg kg body weight^{-1} day^{-1} on gestation day 11	No significant effects (relative to the controls) on embryo and foetal development at any test dose *(NOEL for embryotoxicity and foetal development = 1000 mg kg body weight^{-1} day^{-1})*	Kavlock (1990)	Medium	Valid
Rabbit (New Zealand White)	Pregnant females	Oral at doses of 0, 25, 50 and 100 mg kg body weight^{-1} day^{-1} on gestation days 6 to 18	No significant effects (relative to the controls) on embryo and foetal development at any test dose *(NOEL for embryotoxicity and foetal development = 100 mg kg body weight^{-1} day^{-1})*	Osterburg (1982b)	Medium	Valid

Table 10.6 Summary of the data on potential endocrine mediated responses in laboratory mammals following dermal exposure

Species	Life stage of the test organism at start of test	Exposure route and dose series	Description of endocrine disruption measurement parameter(s) and effect doses	Reference	Test Relevance	Study validity
Rat (Albino)	Male and female	Twice daily application of a 12.5% resorcinol ointment (~308 mg kg body weight^{-1} day^{-1}) for 4 weeks	No significant effects (relative to the controls) on thyroid weight or histology *(NOAEL for thyroid effects = ~308 mg kg body weight^{-1} day^{-1})*	Doniach and Logothetopoulos (1953)	Low/ Medium	Use with care
Rat (Wistar)	No data	Twice daily application of a 12.5% resorcinol ointment (~750 mg kg body weight^{-1} day^{-1}) for 4 weeks	Increased thyroid weight and histological changes *(LOAEL for thyroid effects = ~750 mg kg body weight^{-1} day^{-1})*	Samuel (1955)	Low/ Medium	Use with care

Table 10.7 Summary of the data on potential endocrine mediated responses in laboratory mammals following sub-cutaneous injection

Species	Life stage of the test organism at start of test	Exposure route and dose series	Description of endocrine disruption measurement parameter(s) and effect doses	Reference	Test Relevance	Study validity
Rat (Albino)	Males and females	Sub-cutaneous injections of ~308 mg kg body weight^{-1} day^{-1} in an arachis oil base for periods up to 69 days	Increased thyroid weight and histological changes *(LOAEL for thyroid effects = ~308 mg kg body weight^{-1} day^{-1})*	Doniach and Logothetopoulos (1953)	Low/ Medium	Use with care
Rat (Wistar)	No data	Sub-cutaneous injections of ~400 mg kg body weight^{-1} day^{-1} in a peanut oil base for periods up to 69 days	Increased thyroid weight and histological changes *(LOAEL for thyroid effects = ~308 mg kg body weight^{-1} day^{-1})*	Samuel (1955)	Low/ Medium	Use with care

Several dermal exposure or skin painting studies have been conducted reporting no detectable changes. For example a twice weekly topical application of 0.02 ml of 5% to 50% resorcinol to the inner ear pinna of New Zealand White rabbits for 180 weeks failed to induce local or distant tumours or cause any compound related toxicity (Stenback 1977). However, the study design protocols do not comply with current standards.

Long-term toxicity and carcinogenicity studies in rats and mice on hair dyes containing 0.4-2% resorcinol did not reveal overt toxicity or carcinogenic effects following lifetime dermal application (Brandt 1986).

IARC (1999) has concluded that there is inadequate evidence in experimental animals for the carcinogenicity of resorcinol and therefore, resorcinol is not classifiable as to its carcinogenicity to humans.

The overall NOAEL for chronic toxicity in the 2 year NTP carcinogenicity studies was considered by the Committee on the Health Council of the Netherlands to be 50 mg kg body weight^{-1} day^{-1} (Gezondheidsraad 2001**)**.

E. General conclusions on potential endocrine mediated responses in laboratory mammals

Resorcinol has a limited package of regulatory mammalian toxicology data and there is currently no multi-generation reproduction or fertility study carried out to an internationally recognised test guideline (see Table 10.8), which represents a key area of uncertainty in terms of the assessment of endocrine disruption. However this uncertainty is going to be addressed by a Resorcinol Task Force which has already formulated a comprehensive test programme. This will comprise of an extensive dose range finding study and a test guideline compliant two-generation reproduction study with expanded thyroid endpoints. It will also provide additional observations on sub-chronic exposure NOEL's derived from the pre-mating dosing period.

In vitro studies indicate that the anti-thyroidal activity observed following resorcinol exposure is due to inhibition of thyroid peroxidase (TPO) enzymes, as evidenced by disruption of thyroid hormone synthesis and changes in the thyroid gland consistent with goitrogenesis.

Certain older *in vivo* laboratory animal studies (Doniach and Logothetopoulos 1953, Samuel 1995) have revealed reversible anti-thyroid activity. The thyroid effects in these studies resulted from continuous exposure to high resorcinol doses and required a vehicle (such as peanut oil) to establish a reservoir of resorcinol and to alter the pharmacokinetics such that resorcinol was continuously bioavailable.

Studies conducted as part of the National Toxicology Programme (NTP 1992) have shown no effects on the thyroid of rats or mice at doses of up to 520 mg kg body weight^{-1} day^{-1} in rats and 450 mg kg body weight^{-1} day^{-1} in mice for 13 weeks and 150 – 225 mg kg body weight^{-1} day^{-1} for 5 days per week over 2 years in rats and mice.

Table 10.8 Summary of the potential endocrine mediated responses in laboratory mammals

Type of study	Species and exposure route used	Dose series used	NOEL (mg kg body weight^{-1} day^{-1})		Reference
			Potential endocrine mediated responses	Systemic toxicity	
Sub-chronic oral toxicity (OECD 408 or equivalent)	Rat (13 week - oral gavage)	0, 32, 65, 130, 260 and 520 mg kg body weight^{-1} day^{-1}	< 32 (Adrenal weight *increased* in all treated males but not dose-dependent)	No data reported	NTP (1992)
	Mouse (13 week - oral gavage)	0, 28, 56, 112, 225 and 420 mg kg bodyweight^{-1} day^{-1}	< 28 (Adrenal weight *decreased* in all treated males but not dose-dependent)	No data reported	
Sub-chronic oral toxicity	Rat (30 day – oral)	0 and 5 mg kg body weight^{-1} day^{-1}	< 5 (Enlargement of thyroid gland)	No data reported	Cooksey *et al* (1985)
	Rat (30 day – oral)	0 and 5 mg kg body weight^{-1} day^{-1}	< 5	No data reported	Seffner *et al* (1995)
Reproduction – One generation (OECD 415)	No Data	-	-	-	-
Reproduction – Two generation (OECD 416)	No Data	-	-	-	-
Reproduction/ Development (OECD 421)	No data	-	-	-	-
Development/ Teratogenicity (OECD 414)	Rat (Oral gavage)	0, 40, 80 and 250 mg kg body weight^{-1} day^{-1}	250 (Embryotoxic and foetal development)	250 (Maternal toxicity)	Osterburg (1982a)
	Rat (Oral gavage)	0, 125, 250 and 500 mg kg body weight^{-1} day^{-1}	500 (Embryotoxic and foetal development)	250 (Maternal toxicity)	DiNardo *et al* (1985)
	Rat (Oral gavage)	0, 333, 667 and 1000 mg kg body weight^{-1} day^{-1}	1000 (Developmental)	1000 (Maternal toxicity)	Kavlock (1990)
	Rabbit (Oral gavage)	0, 25, 50 and 100 mg kg body weight^{-1} day^{-1}	100 (Embryotoxic and foetal development)	50 (Maternal toxicity)	Osterburg (1982b)
Carcinogenicity	Rat (Oral gavage – 104 weeks)	0, 112 and 225 mg kg body weight^{-1} day^{-1} (males) 0, 50, 100 and 150 mg kg body weight^{-1} day^{-1} (females)	225 150	- 50 (NOAEL)	NTP (1992)
	Mice (Oral gavage – 104 weeks)	0, 112 and 225 mg kg body weight^{-1} day^{-1}	225	No data reported	NTP (1992)

It also needs to be recognised that rodents, especially rats, have been reported to be particularly susceptible to goitrogens, primarily due to the lack of thyroid binding protein (TBP) which is the primary thyroid hormone binding and transport protein (Dohler *et al* 1979, Curran and DeGroot 1991, Alison *et al* 1994). In the rat the absence of TBP results in a much shorter half life of T4 and much higher levels of TSH (Dohler *et al* 1979, Capen *et al* 1991, Alison *et al* 1994). These differences suggest that the activity of the thyroid gland in rats is considerably higher than that of other species, including humans, and this increased activity correlates to a greater susceptibility to hormonally-induced thyroid effects (Alison *et al* 1994, McClain 1994). Given the relative insensitivity of humans to changes in the thyroid gland, it has been suggested by certain authors (Ames *et al* 1987, Alison *et al* 1994, McClain 1994) that high doses of substances such as resorcinol which cause hormonally-induced changes of the thyroid in rodents (particularly rats) have limited relevance to humans.

The available data from the 13-week NTP rat and mouse studies also provides evidence of effects on adrenal weights at all doses tested. However, the observed responses did not show dose-dependent relationships. Possible adrenal effects will be addressed in the test programme formulated by the Resorcinol Task Force.

No evidence of gross teratogenicity or embryotoxicity has been recorded in studies with rats and rabbits at the highest exposure concentrations tested even though maternal toxicity was evident.

In 2001 the Committee on the Health Council of the Netherlands produced a Health-based reassessment of current administrative occupational exposure limits in the Netherlands (Gezondheidsraad 2001**)**. The Committee on Updating of Occupational Exposure Limits considered current values in the light of available data. In the review the Committee considered that “the NTP studies were well performed and attaches more importance to these studies than to the studies of Cooksey *et al* (1985) and Seffner *et al* (1995). The Committee questions the relevance of the thyroid effects found by these authors”. The Committee also concluded that “resorcinol is not embryotoxic or teratogenic.”.

10.4.1.3 Human studies

In humans, resorcinol has effects (observed primarily after clinical use of resorcinol containing ointments) on the central nervous system (with symptoms such as dizziness, trembling, cramps and shortness of breath) and this can be considered the critical end-point at least temporally. It also has effects on red blood cells (methaemoglobinemia, haemolytic anaemia and haemoglobinuria). Effects on the thyroid have also been reported after prolonged exposure. In addition exogenous ochronosis, chronic myxoedema and cyanosis have been associated with resorcinol exposure. Human pathology reports from poisoning include siderosis of the spleen and kidney tubule damage.

The CANTOX review stated that in certain cases continuous dermal exposure to resorcinol for periods of months to years can result in thyroid abnormalities in humans when applied doses are in the range 34 – 122 mg kg body weight^{-1} day^{-1} (CANTOX 2000).

Yeung *et al* (1993) reported topical application of 20 ml of 2% resorcinol (2 times per day, 6 days per week equal to 150 ug per cm^2 per application) for 4 weeks to 2600 cm^2 of the body surface (daily dose 12 mg kg body weight^{-1} day^{-1}) to 3 human volunteers did not result in significant changes in thyroid function (as measured by T3, T4, and TSH) or haematological parameters.

Hypothyroidism was reported in a 70 year old male on chronic dialysis who applied large amounts of a resorcinol containing cream (3 x 2.5 g tubes of cream containing 2% resorcinol [calculated dose of 150 mg or 2.14 mg kg^{-1} day^{-1} topically assuming a 70 kg bodyweight] because of pruritus[3] (Katin *et al*, 1977). It was noted that the patient responded following cessation of treatment with the cream and that skin was unbroken. If the calculated dose of 2.1 mg kg^{-1} day^{-1} applied topically is corrected for 1.64% absorption (based on data of Yeung *et al* 1983) then the estimated systemic dose is 0.034 mg kg^{-1} day^{-1} which is very low, and this patient may be deemed particularly sensitive due to impaired renal function and excretion. There are several other case studies of hypothyroidism associated with the use of resorcinol creams for ulcerative skin conditions and the consensus is that hypothyroidism occurs following persistent use of relatively large amounts of cream, where ulcerated skin may assist absorption.

10.4.2 Studies relevant to the assessment of potential endocrine disrupting effects in wildlife

10.4.2.1 In vitro studies

No data has been located on the conduct of *in vitro* studies using cells and tissues from wildlife species.

10.4.2.2 In vivo studies

A. Studies on aquatic organisms

Limited data has been located on the potential endocrine disrupting effects of resorcinol on aquatic organisms. The only study assessing potential endocrine mediated responses involved early life stage studies of rainbow trout (*Oncorhynchus mykiss*) and zebrafish (*Danio rerio*) in which newly fertilised eggs of each species were exposed to resorcinol for periods of 60 and 7 days respectively (Van Leeuwen *et al* 1990). The tests involved the use of a control and five to seven toxicant concentrations at intervals of 3.2 times in the range 1-1000 mg l^{-1}. Test solutions were renewed three times a week, but no analytical confirmation of test concentrations was performed. The endpoints in the studies were total embryotoxicity (teratogenicity) and mortality. In the rainbow trout test the wet weights and lengths of macroscopically normal fish were determined at the end of the study.

Resorcinol induced teratogenic effects in both rainbow trout and zebrafish embryos with observed effects including vertebral abnormalities (including lateral flexures), ventral curvatures (lordosis), dorsal curvatures (kyphosis) and irregular dwarfed structures of the trunk. These terata greatly impaired swimming performance. The LOEC values for embryotoxicity in rainbow trout and zebrafish were 320 and 100 mg l^{-1} respectively. However, there is no information on the mechanism of action for these effects.

B. Studies on terrestrial organisms

The only data that has been located on the potential endocrine disrupting effects of resorcinol on terrestrial organisms relates to a study by Korhonen *et al* (1983) on chicken embryos.

[3] Pruritus is a symptom of chronic renal disease

Resorcinol was embryotoxic to 3 day old embryos with an ED_{50} of 2.4 μmol/egg (264 μg/egg), whereas the LD_{50} for embryo mortality was 2.7 μmol/egg (297 μg/egg).

Given that resorcinol is not expected to strongly sorb to organic carbon and be present in soils (see Section 10.1) the absence of data on potential endocrine disrupting effects in terrestrial organisms does not represent a key area of uncertainty. It should also be recognised that there are currently no internationally agreed methods specifically developed to assess potential endocrine disrupting effects in terrestrial organisms.

C. Studies on aerial organisms

No data has been located on the potential endocrine disrupting effects of resorcinol on aerial organisms. Given that resorcinol is not considered to be volatile (see Section 10.1) and is rapidly degraded by reaction with hydroxyl radicals the absence of data on potential endocrine disrupting effects in aerial organisms does not represent an area of uncertainty. It should also be recognised that there are currently no internationally agreed methods specifically developed to assess potential endocrine disrupting effects in aerial organisms.

D. General conclusions on potential endocrine mediated responses in wildlife

The limited data for potential endocrine mediated responses in wildlife precludes general assessments of the effects of resorcinol (see Table 10.9). In teratogenicity studies with rainbow trout and zebrafish embryos teratogenic effects were evident at exposure concentrations > 100 mg l^{-1}. Importantly, no data is available on the effects of resorcinol on the reproduction of invertebrates (such as *Daphnia magna*) or fish and these represent an area of uncertainty. The data gap for *Daphnia magna* reproduction is important given that 48h EC_{50} values of $\leq$1.3 mg l^{-1} have been reported (see Section 10.5.2.1).

10.5 Comparison of data from studies assessing endocrine disrupting effects and/or general toxicity

The general toxicity data in this section has largely been obtained from the IUCLID data set for resorcinol and has been taken as accurate. Individual source documents have not been checked unless they are considered to be key studies which have a major influence on the outcome of the review. All information taken from IUCLID as been referenced as being from that source and individual references have not been given in the references.

10.5.1 Studies relevant to the assessment of general toxicity in humans

Table 10.10 summarises the general toxicity data from acute and repeat dose studies with resorcinol.

10.5.1.1 Acute studies

A. Oral exposure

Resorcinol is of moderate to low acute oral toxicity with reported median lethal doses (LD_{50}) for rats being between 202 and 980 mg kg body $weight^{-1}$ (Flickinger 1976, Lloyd 1977, Hoechst 1979, 1981, Van den Heuvel, 1990). Corresponding values for mice and rabbits were 200 mg kg body $weight^{-1}$ (Summit Chemicals, 1992) and 750 mg kg body $weight^{-1}$ (Heyroth 1981).

Table 10.9 Summary of the potential endocrine mediated responses in wildlife

Environmental compartment	Taxonomic group	Type of study	Species and exposure route used	Concentration series used	Lowest reported NOEC	Reference
Aquatic	Amphibians	No data	-	-	-	-
	Fish	Developmental/ Teratogenicity 60 day 7 day	 Rainbow trout (Aqueous) Zebrafish (Aqueous)	 No data No data	 <320 mg l^{-1} (a) <100 mg l^{-1} (a)	Van Leeuwen *et al* (1990)
	Invertebrates	No data	-	-	-	-
Terrestrial	Birds	Embryo-toxicity	Chicken embryos	No data	264 μg/egg	Korhonen *et al* (1983)
	Invertebrates	No data	-	-	-	-
Aerial	Invertebrates	No data	-	-	-	-

a – No information is available on the mechanism of action

B. Dermal exposure

The only reported mammalian dermal LD_{50} is a value of 3360 mg kg body weight^{-1} reported for male rabbits (Flickinger 1976).

C. Inhalation exposure

Flickinger (1976) reported 1 hour LC_{50} values of 21.3 to 78 mg l^{-1} for resorcinol-water aerosol exposed female Wistar rats. Resorcinol-water aerosol concentrations up to 7.8 mg l^{-1} (1733 ppm) for a 1 hour period and up to 2.8 mg l^{-1} (625 ppm) for an 8 hour period caused no deaths or gross lesions at autopsy attributable to inhalation of the aerosol.

D. Other routes of exposure

In the rat and mouse LD_{50} values of 215 – 460 mg kg body weight^{-1} (Podlesnaya, 1966) and 213 mg kg body weight^{-1} (NIOSH 1992) have been reported following single intra-peritoneal injections of resorcinol. Following single sub-cutaneous injections of resorcinol, LD_{50} values of 400 mg kg body weight^{-1} have been reported for rats and guinea pigs, 100 mg kg body weight^{-1} for cats and 400 mg kg body weight^{-1} for dogs NIOSH (1992).

10.5.1.2 Repeat dose studies

A. Oral exposure

There is a lack of studies conducted to recent regulatory guidelines and GLP, but in range finding for carcinogenicity studies in rodents, NTP have conducted sub-acute and sub-chronic studies (se also Section 10.4.1.2).

Groups of 5 male and female F344 *rats* were initially treated with 0, 27.5, 55, 110, 225 or 450 mg kg^{-1} day^{-1} by oral gavage in deionized water for 17 days. There were no gross or microscopic lesions attributable to treatment and final body weights were similar across groups (NTP, 1992). Subsequently groups of 10 male and female F344 rats were treated with 0, 32, 65, 130, 260 or 520 mg kg^{-1} day^{-1} by oral gavage in deionized water for 13 weeks. All females (10 of 10) and 8 of 10 males at 520 mg kg^{-1} day^{-1} died as a result of treatment. Absolute and relative adrenal weights were significantly *increased* in *all* surviving males albeit without a clear dose-response relationship such that no NOEL was established. Increased absolute and relative liver weights were seen in the $\geq$65 mg kg^{-1} day^{-1} females and in $\geq$130 mg kg^{-1} day^{-1} males. There were no other gross or microscopic lesions attributable to treatment in animals surviving the exposure period and final bodyweights were similar across groups.

In the NTP studies groups of 5 male and female B6C3F1 *mice* were initially treated with 0, 37.5, 75, 100, 300 or 600 mg kg^{-1} day^{-1} by oral gavage in deionized water for 17 days. All female (5 of 5) and 4 of 5 males at 600 mg kg^{-1} day^{-1} and 1 of 5 males at 300 mg kg^{-1} day^{-1} died as a result of treatment. There were no gross or microscopic lesions attributable to treatment and final body weights were similar across groups (NTP 1992). Subsequently groups of 10 male and female B6C3F1 mice were treated with 0, 28, 56, 112, 225 or 420 mg kg^{-1} day^{-1} by oral gavage in deionized water for 13 weeks. Mortalities related to treatment were observed in 7 of 10 male and female mice at 420 mg kg^{-1} day^{-1} following exhibition of dyspnea, prostration and tremors. Absolute and relative adrenal gland weights were *decreased* in males at *all* dose levels although no dose-response relationship was evident and no NOEL was established. There were no gross or microscopic lesions attributable to

treatment in animals surviving the exposure period and final bodyweights were similar across groups (NTP 1992).

Brandt (1986) reported a rat 4 week feeding study conducted at a dose level of 0-260 mg kg body weight^{-1} day^{-1} resulted in decreased relative adrenal weights in all exposed animals. Exposure did not induce clinical abnormalities, histopathology, body weight effects or mortalities in resorcinol exposed animals.

Groups of 20 male and female Wistar rats were treated with 0 or 20 mg kg^{-1} day^{-1} by oral gavage for 12 weeks. There were no adverse effects related to treatment including an absence of adrenal or thyroid weight effects and the study NOEL was 20 mg kg^{-1} day^{-1} (Potokar and Pittermann, 1980). However, it should be noted that study design and conduct was not to GLP or current regulatory standards.

B. Dermal exposure

No reliable data has been located of the repeat dose effects of resorcinol via dermal exposure (see Section 10.4.2.2).

C. Inhalation exposure

Flickinger (1976) has reported studies on the repeat dose effects of resorcinol via inhalation exposure in rats, rabbits and guinea pigs. All species were given a dose of 8 ppm (or 34 mg m^{-3}) for 6 hours per day for 14 days and no adverse effects (although the parameters recorded were not specified) were evident in any species throughout the study.

D. Other routes of exposure

No data has been located of the repeat-dose effects of resorcinol via exposure by sub-cutaneous or intra-peritoneal injection.

10.5.1.3 Comparison of data from studies of endocrine disrupting effects and /or general toxicity in mammals

The primary signs of acute resorcinol intoxication (animals and humans) have been shown to include initial stimulation of the central nervous system followed by CNS depression, renal glomerular and tubule degeneration, central hepatic necrosis, myocardial depression, pruritis and reddening of the skin. Repeated exposure to resorcinol also results in microscopic changes in the kidneys and in the liver and changes in the function and morphology of the thyroid. The effects on the thyroid in mammals have been noted after oral, intravenous and sub-cutaneous administration and the lowest dose levels exerting thyroid effects were 5 mg kg body weight^{-1} day^{-1} for both oral (drinking water) and sub-cutaneous exposure. However, other NTP studies using the oral gavage exposure route have shown an absence of effects on the thyroid at concentrations above 150 mg kg body weight^{-1} day^{-1}. In these studies effects on the adrenals were observed at all test doses (lowest levels being 28 – 32 mg kg body weight^{-1} day^{-1}), though there is uncertainty as to the significance of this finding given the absence of data in other studies.

Table 10.10 Summary of general mammalian toxicity data (Information from IUCLID 2000)

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect dose	Reference	Study validity
Acute Oral Toxicity	Rat	Not relevant	No data	Median lethal dose (LD_{50})	980 mg kg^{-1} body $weight^{-1}$	Flickinger (1976)[1]	Valid[2]
	Rat	Not relevant	No data	Median lethal dose (LD_{50})	301 mg kg^{-1} body $weight^{-1}$	Lloyd (1977) [1]	Use with care[2]
	Rat	Not relevant	No data	Median lethal dose (LD_{50})	370 mg kg^{-1} body $weight^{-1}$	Lloyd (1977) [1]	Use with care[2]
	Rat (Females)	Not relevant	No data	Median lethal dose (LD_{50})	202 mg kg^{-1} body $weight^{-1}$	Hoechst (1979) [1]	Use with care[2]
	Rat	Not relevant	No data	Median lethal dose (LD_{50})	334 mg kg^{-1} body $weight^{-1}$	Hoechst (1981) [1]	Use with care[2]
	Rat	Not relevant	No data	Median lethal dose (LD_{50})	349 mg kg^{-1} body $weight^{-1}$	Hoechst (1981) [1]	Use with care[2]
	Rat	Not relevant	No data	Median lethal dose (LD_{50})	502 mg kg^{-1} body $weight^{-1}$	Hoechst (1981) [1]	Use with care[2]
	Rat	Not relevant	No data	Median lethal dose (LD_{50})	Male: 533 mg kg^{-1} body $weight^{-1}$ Female: 489 mg kg^{-1} body $weight^{-1}$	Van den Heuvel (1990) [1]	Use with care[2]
	Mouse	Not relevant	No data	Median lethal dose (LD_{50})	200 mg kg^{-1} body $weight^{-1}$	Summit Chemicals (1992) [1]	Use with care[2]
	Rabbit	Not relevant	No data	Median lethal dose (LD_{50})	750 mg kg^{-1} body $weight^{-1}$	Heyroth (1981) [1]	Use with care[2]
Acute Dermal Toxicity	Rabbit	Not relevant	No data	Median lethal dose (LD_{50})	3360 mg kg^{-1} body $weight^{-1}$	Flickinger (1976) [1]	Use with care[2]
Acute Inhalation Toxicity	Rat	1 hour	No data	LC_0	> 7.8 mg l^{-1}	Flickinger (1976) [1]	Valid[2]
		1 hour	No data	LC_{50}	21.3 - 78 mg l^{-1}		
		8 hours	No data	LC_0	> 2.8 mg l^{-1}		

Table 10.10 Continued

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect dose	Reference	Study validity
Acute Toxicity (Intra-peritoneal injection)	Rat	No data	No data	Median lethal dose (LD_{50})	450 mg kg^{-1} body $weight^{-1}$	Podlesnaya (1966) [1]	Use with care [2]
	Mouse	No data	No data	Median lethal dose (LD_{50})	215 mg kg^{-1} body $weight^{-1}$	Podlesnaya (1966) [1]	Use with care [2]
	Mouse	No data	No data	Median lethal dose (LD_{50})	213 mg kg^{-1} body $weight^{-1}$	NIOSH (1992) [1]	Use with care [2]
Acute Toxicity (Sub-cutaneous injection)	Rat	No data	No data	Median lethal dose (LD_{50})	400 mg kg^{-1} body $weight^{-1}$	NIOSH (1992) [1]	Use with care [2]
	Guinea pig	No data	No data	Median lethal dose (LD_{50})	400 mg kg^{-1} body $weight^{-1}$		
	Cat	No data	No data	Median lethal dose (LD_{50})	100 mg kg^{-1} body $weight^{-1}$		
	Dog	No data	No data	Median lethal dose (LD_{50})	700 mg kg^{-1} body $weight^{-1}$		
Repeat Dose Toxicity (Oral)	Rat	14 days	0 and 2500 mg kg^{-1} in feed	NOAEL	No data	Berthezene *et al* (1979) [1]	Use with care [2]
	Rat	28 days	0 – 260 mg kg^{-1} in feed	NOAEL	No data	FEMA (1979) [1]	Use with care [2]
	Rat (Fischer 344 Males)	8 weeks	0 and 0.8 % in feed	NOAEL	No data	Shibata (1990) [1]	Use with care [2]
	Rat (Fischer 344 Males and Females)	17 days	Oral gavage at 0, 27.5, 55,110, 225 and 450 mg kg bw^{-1} day^{-1}	NOAEL	450 mg kg body $weight^{-1}$	NTP (1992)	Valid
	Rat (Fischer 344 Males and Females)	13 weeks	Oral gavage at 0, 32, 65,130, 260 and 520 mg kg bw^{-1} day^{-1}	NOAEL	260 mg kg body $weight^{-1}$	NTP (1992)	Valid
	Mouse (B6C3F1 Males and Females)	17 days	Oral gavage at 0, 37.5, 75,100, 300 and 600 mg kg bw^{-1} day^{-1}	NOAEL	100 mg kg body $weight^{-1}$	NTP (1992)	Valid

	Mouse (B6C3F1 Males and Females)	13 weeks	Oral gavage at 0, 28, 56,112, 225 and 420 mg kg bw^{-1} day^{-1}	NOAEL	225 mg kg body $weight^{-1}$	NTP (1992)	Valid

Table 10.10 Continued

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect dose	Reference	Study validity
Repeat Dose Toxicity (Oral)	Syrian hamster (Males)	20 weeks	0 and 0.25 % in feed	NOAEL	250 mg kg^{-1} body $weight^{-1}$	Hirose (1986) [1]	Use with care[2]
Repeat Dose Toxicity (Inhalation)	Rats (Male and Female F344/N)	14 days	0 and 34 mg m^{-3} or 8 ppm (6 hour per day)	NOAEL	No data	Flickinger (1976)[1]	Use with care[2]
	Rabbit	14 days	0 and 34 mg m^{-3} or 8 ppm (6 hour per day)	NOAEL	No data	Flickinger (1976) [1]	Use with care[2]
	Guinea pig	14 days	0 and 34 mg m^{-3} or 8 ppm (6 hour per day)	NOAEL	No data	Flickinger (1976) [1]	Use with care[2]

[1] – Cited in IUCLID (2000), 2 – Assessment made on data in IUCLID (2000)

In 2001 the Committee on the Health Council of the Netherlands produced a Health-based reassessment of current administrative occupational exposure limits in the Netherlands (Gezondheidsraad 2001) In the summary of the draft report a chronic NOAEL of 50 mg kg body weight^{-1} day^{-1} observed in the long-term oral exposure study (NTP 1992) was considered the lowest valid value from the available dataset. The key effects were those on the central nervous system. In contrast when the European Commission Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers considered the use of resorcinol in oxidation or permanent and semi-permanent hair dyes (EC 2000 – Report by the Scientific Committee on Cosmetology) a NOAEL value of 20 mg kg^{-1} body weight^{-1} was used in the calculation based on a sub-chronic gavage study in Wistar rats (Potokar and Pittermann, 1980).

Overall it appears that there is uncertainty as to which mechanism of action is responsible for the lowest toxic effects observed in mammals and humans. This uncertainty will be reduced when the data from the multi-generational resproduction study being initiated by the Resorcinol Task Force is available.

10.5.2 Studies relevant to the assessment of general toxicity in wildlife

Table 10.11 summarises the acute and chronic toxicity data located for wildlife.

10.5.2.1 Studies on aquatic organisms

A. Fish

Acute toxicity

A series of toxicity tests with several fish species have been carried out to assess the effects of resorcinol on survival (see Table 10.11). The studies show that the 96h LC_{50} values for fathead minnows (*Pimephales promelas*) are in the range 40 – 100 mg l^{-1} and for the golden orfe (*Leuciscus idus*) are in the range 31.6 – 34.7 mg l^{-1} (with corresponding NOEC values of 25 mg l^{-1}). For rainbow trout (*Oncorhynchus mykiss*) a 96h LC_{50} value of >100 mg l^{-1} was reported by DeGraeve *et al* (1980). A number of these studies used a static exposure regime and did not measure the actual test concentrations, which raises issues regarding the validity of the data.

Chronic toxicity

Van Leeuwen *et al* (1990) have carried out early life stage studies of rainbow trout (*Oncorhynchus mykiss*) and zebrafish (*Danio rerio*) in which newly fertilised eggs of each species were exposed to resorcinol for periods of 60 and 7 days respectively (see Section 10.4.2). The LOEC and LC_{50} values for mortality in fathead minnows were 320 and 262 mg l^{-1} while for rainbow trout these values were both 320 mg l^{-1}.

B. Invertebrates

Acute toxicity

Acute toxicity data has been located for a range of invertebrate species representative of different taxonomic groups (including crustaceans, molluscs and worms). A study by Ewell *et al* (1986) investigated the effects of resorcinol on six invertebrates namely the isopod *Asellus intermedius*, the water flea *Daphnia magna*, the flatworm *Dugesia tigrina*, the amphipod

Gammarus fasciatus, the molluscs *Helisoma trivolvis* and the segmented worm *Lumbriculus variegatus.* All the species except *Daphnia magna* showed no mortalities at the highest concentrations tested (100 mg l^{-1}) after 96 hours whereas *Daphnia magna* showed an LC_{50} of 0.25 mg l^{-1}. The greater sensitivity of *Daphnia magna* to resorcinol was generally consistent with other studies in this species which have shown EC_{50} (immobilisation) and LC_{50} (lethality) values of less than 1.3 mg l^{-1} (Bringmann 1959, Herbes and Beauchamp, 1977). However studies *of Daphnia sp* by DeGraeve *et al* (1980) and Devillers *et al* (1987) have reported 24 - 96 h LC_{50} values of >100 mg l^{-1}.

Chronic toxicity

No chronic toxicity data for aquatic invertebrates following exposure to resorcinol has been located.

10.5.2.2 Studies on terrestrial organisms

The only toxicity data located for terrestrial organisms following exposure to resorcinol is for the earthworm *Eisenia foetida* in a 42 day test (Hartenstein, 1982). In the study, artificial soil was spiked with resorcinol and measurements were made of mortality, and growth inhibition (as retarded body weight gain) at the end of the test. At an exposure concentration of 40000 mg kg dry $weight^{-1}$ all the animals were dead at the end of the study whilst there was a significant effect on growth at 10000 mg kg dry $weight^{-1}$.

10.5.2.3 Studies on aerial organisms

No general toxicity data for aerial organisms following exposure to resorcinol has been located.

10.5.2.4 Comparison of data from studies assessing endocrine disrupting effects and/or general toxicity in wildlife

The limited data on potential endocrine mediated responses in wildlife species precludes comparison with general toxicity data.

Table 10.11 Summary of general toxicity data for aquatic organisms (Information from IUCLID 2000)

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study validity
Acute Fish Toxicity	Fathead minnow *(Pimephales promelas)*	24h	No data - Static (Nominal)	LC_{50}	88.6 mg l^{-1}	Curtis *et al* (1978)	Valid
		48h		LC_{50}	72.6 mg l^{-1}		
		96h		LC_{50}	56.5 mg l^{-1}		
		96h	No data - Flow-through (Measured)	LC_{50}	100 mg l^{-1}	DeGraeve *et al* (1980)[1]	Valid[2]
		96h	No data – Static (Nominal)	LC_{50}	40 mg l^{-1}	Ewell *et al* (1986)	Use with care
	Golden orfe *(Leuciscus idus)*	48h	No data -Static	LC_{50}	38.4 mg l^{-1}	Hoechst AG (1981) [1]	Use with care[2]
				NOEC	25 mg l^{-1}		
		96h	No data - Static	LC_{50}	34.7 mg l^{-1}		
				NOEC	25 mg l^{-1}		
		96h	No data - Static	LC_{50}	31.6 mg l^{-1}	Hoechst AG (1981) [1]	Valid[2]
				NOEC	25 mg l^{-1}		
	Rainbow trout (*Oncorhynchus mykiss*)	96h	No data – Flow through	LC_{50}	>100 mg l^{-1}	DeGraeve *et al* (1980) [1]	Valid[2]
Chronic Fish Toxicity	Rainbow trout (*Oncorhynchus mykiss*)	60 days	No data – Static renewal (Nominal)	LC_{50}	262 mg l^{-1}	Van Leeuven *et al* (1990)	Valid
				LOEC (Mortality)	320 mg l^{-1}		
	Zebrafish (*Danio rerio*)	7 days	No data – Static renewal (Nominal)	LC_{50}	320 mg l^{-1}		
				LOEC (Mortality)	320 mg l^{-1}		

Table 10.11 Continued

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study validity
Acute Invertebrate Toxicity	Water flea *(Daphnia magna)*	48h	No data - Static	EC_{50}	<0.8 mg l^{-1}	Bringmann (1959) [1]	Use with care[2]
		48h	No data - Static	LC_{50}	1.28 mg l^{-1}	Herbes (1977) [1]	Use with care[2]
		96h	No data – Static (Nominal)	LC_{50}	0.25 mg l^{-1}	Ewell *et al* (1986)	Use with care
		24h	No data – Static (Nominal)	EC_{50}	107.6 mg l^{-1}	Devillers *et al* (1987)	Use with care
	Water flea *(Daphnia pulicari)*	48h	No data – Static (Measured)	EC_{50}	>100 mg l^{-1}	DeGraeve *et al* (1980) [1]	Valid[2]
	Amphipod *(Gammarus fasciatus)*	96h	No data – Static (Nominal)	LC_{50}	>100 mg l^{-1}	Ewell *et al* (1986)	Use with care
	Isopod *(Ascellus intermedius)*	96h		LC_{50}	>100 mg l^{-1}		
	Mollusc *(Helisoma trivolvis)*	96h		LC_{50}	>100 mg l^{-1}		
	Flatworm *(Dugesia tigrina)*	96h		LC_{50}	>100 mg l^{-1}		
	Oligochaete *(Lumbriculus variegatus)*	96h		LC_{50}	>100 mg l^{-1}		
	Grass shrimp *(Paleomonetes pugio)*	24h	No data – Static (Measured)	LC_{50}	169.5 mg l^{-1}	Curtis *et al* (1978)	Valid
		48h		LC_{50}	78.0 mg l^{-1}		
		96h		LC_{50}	42.2 mg l^{-1}		

[1] – Cited in IUCLID (2000), 2 – Assessment made on data in IUCLID (2000)

10.6 Current classification of the substance against European Commission and national regulations

Table 10.12 summarises the current classification of the substance against Council Directives in order to assess the regulations to which resorcinol is subject.

Resorcinol is listed in several European Directives as would be expected for a chemical qualifying as a High Production Volume (HPV) chemical. However, resorcinol is not the subject of substantial restriction under these regulations. Although the major applications of resorcinol is in rubber and wood adhesive formulations they are not the subject of specific product regulation. However, the minor uses in both cosmetic and food contact are covered.

Resorcinol is regulated in the EU Cosmetics Directive (76/768/EEC, with its 6th Amendment 93/35/EEC) for its use in cosmetic applications. Currently the usage is limited to 5% (w/w on-head) in oxidation hair colouring products and 0.5% in shampoos and hair lotions. However, a recent COLIPA survey of the European Cosmetics Industry evaluated the actual usages and the use concentrations of resorcinol in oxidation hair colouring products. According to the survey, resorcinol is only used in oxidation hair colouring products. The actual on-head concentration of resorcinol in these products is limited to 1.25%, which adheres to the recommendation of the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP) that the maximum concentration of resorcinol in hair dyes should be 1.25%. The overall classification of the SCCNFP was category “A”, that is the use of resorcinol in hair dyes is not linked to any particular risk for consumers (EC 2000).

Table 10.12 Current classification of resorcinol against Council Directives

Directive	Status (listed or not)
67/548/EEC - Classification, packaging and labelling of dangerous substances	Classified: Xn, N, C R phrases: 22-36/38-50
76/768/EEC – Approximation of laws relating to cosmetic products	Listed (Maximum authorised concentration 5% in oxidation hair colouring products and 0.5% in shampoos and hair lotions)
90/128/EEC - Plastic materials and articles intended to come into contact with foodstuffs	Listed (SML = 2.4 mg kg^{-1})
91/322/EEC - Protection of the health and safety of workers from risks related to chemicals agents at work	Listed (limit values of 10 ppm or 45 mg m^{-3} based on a 8 h reference period)

Under Directive 67/548/EEC the R phrases indicate that the substance is very toxic to aquatic organisms.

No national environmental quality standards have been developed in any European country to protect aquatic or terrestrial ecosystems.

10.7 Exposure data

In order to obtain information on both emissions from end products and environmental monitoring the Resorcinol Task Force has sought to develop close liaisons with the following user groups:

- Rubber tyre industry (BLIC in Europe and RMA in the United States)
- Wood adhesives (CASCO in Sweden – part of Akzo Nobel)
- Other resorcinol resins (European Phenolic Resins Association)
- Hair Dyes (Hair Colouring Technical Secretariat)
- Topical ointments and other medicinal (EFPIA)

The on-going information collection exercise is at varying stages of completeness depending on the point in time at which the process was initiated for an end-use sector and the degree of complexity of that sector.

In 2001 the Committee on the Health Council of the Netherlands produced a Health-based reassessment of current administrative occupational exposure limits in the Netherlands (Gezondheidsraad 2001). Table 10.13 summarises the occupational exposure standards for resorcinol in various European countries which showed that the typical 8 h time weighted value was 10 ppm or 45 mg/m^3.

The Committee on Updating of Occupational Exposure Limits considered these values in the light of available data. In the summary of the draft report a chronic NOAEL of 50 mg kg body $weight^{-1}$ day^{-1} observed in the long-term oral exposure study (NTP 1992) was considered as the lowest valid value from the available dataset. A scaling factor of 4 to account for differences in calorific demands between rats and humans and an overall assessment factor of 9 to account for intra- and inter-species variation were applied. Assuming 100% absorption, an average body weight of 70 kg and a breathing volume of 10 m^3 per 8 h for the worker this resulted in a preferred value of 10 mg m^{-3}. The committee considered that a Health-Based Recommended Occupational Exposure Limit (HBROEL) of 10 mg m^{-3} (as an 8 hour time weighted average) would be sufficiently low to protect against irritation by resorcinol. It was considered that a 'skin notation' was not warranted.

Table 10.13 Occupational exposure standards for resorcinol in various countries

Country	Occupational exposure limit		Time weighted average	Type of exposure limit	Reference
	ppm	mg m^{-3}			
Denmark	10	45	8h	-	Arbejdstilsynet (1996)
Netherlands	10	45	8h	Administrative	SZW (2000)
Sweden	10	45	8h	-	NBOSH (1996)
United Kingdom	10	46	8h	Occupation exposure standard	HSE (1999)
	20	92	15 min	-	

10.7.1 Worker exposure data

Resorcinol has historically been considered to be a low risk chemical and this has led to the adoption of occupational exposure limits commensurate with this status, typical values currently being 10 ppm or 45 mg/m^3. Since there are very few scenarios that would lead to such exposures in practice, the routine monitoring of workplace environments for resorcinol is rare. Even in resorcinol production facilities where substantial epidemiological data exists it is unusual to have this linked directly to personal exposure profiles. The situation is compounded by the fact that resorcinol has a melting point of 109-111^{0}C resulting in a low vapour pressure at normal ambient temperatures. While the compound sublimes at the elevated temperatures operated in some down-stream processes, these processes are well understood and appropriate worker exposure measures are in place.

For the larger resorcinol uses such as *rubber manufacture* there has been considerable work in seeking to establish the availability of free resorcinol. Occupational exposure to resorcinol during rubber processing only occurs at the weighing, mixing and preparation areas. Measurements by tyre producers suggest that typical air-borne concentrations are less than 0.1 mg/m^3 and remain at levels below 5 mg/m^3 (8-h TWA) even under exceptional conditions.

INDSPEC has carried out its own survey of available information and has also sought to fill data gaps. The main themes arising from the work to date are:

- That resorcinol tends to be reacted into its end-products by way of irreversible reactions leading to the observation that only very low levels of free resorcinol remain (often below detection limits)

- That end-users have considered resorcinol a fairly low-risk chemical historically because of its relatively low toxicity and high biodegradability and have therefore not focused on resorcinol for routine monitoring.

Most of the available published data relating to rubber manufacture (DiPico *et al* 1975, Gamble *et al* 1976) originates from the 1970s and, therefore, its relevance to current manufacturing/production processes and practices has to be questioned.

The production of *hair colouring dyes* containing resorcinol is carried out in a closed process, under vacuum, with simultaneous absorption of volatile compounds in water. Thus, no volatile substances or dyestuffs (due to their low vapour pressure) are emitted to the air. Given the use of closed systems, human occupational exposure should not occur.

Overall there is no strong evidence from occupational epidemiological studies that exposure to resorcinol causes hypothyroidism (for example Roberts *et al* 1990) indicating that workers do not appear to be a vulnerable group at least not at the prevailing occupational exposure levels.

10.7.2 Consumer exposure data

For consumers the key sources of exposure to resorcinol are via the use of cosmetics (such as hair colouring products) and pharmaceuticals (such as topical ointments). In the case of the hair colouring sector, HCTS (2002) has carried out a preliminary environmental risk assessment. In the case of the use of resorcinol in non-prescribed topical ointments, even the mapping of the use patterns is a problem.

10.7.2.1 Cosmetics

In 1993 the European Commission Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers considered the use of resorcinol in oxidation or permanent and semi-permanent hair dyes (EC 2000 – Report by the Scientific Committee on Cosmetology). Table 10.14 summarises the calculations for the margins of safety in the report using the data applied at the time. For oxidation or permanent hair dyes a usage volume of 100 ml was assumed containing a maximum 1.25% of resorcinol, whereas for semi-permanent hair dyes a usage volume of 35 ml was assumed containing at maximum 0.5% of resorcinol. A NOAEL value of 20 mg kg^{-1} body $weight^{-1}$ is used in the calculation based on a subchronic gavage study in Wistar rats (Potokar and Pittermann, 1980).

Table 10.14 Summary of the calculation of margins of safety (MOS) for oxidation or permanent or semi-permanent hair dyes (from EC 2000)

Parameter	Oxidation or permanent hair dyes	Semi permanent hair dyes
Maximum amount of ingredient applied (I)	1250 mg	(35 x 500)/100 =175 mg
Typical body weight of humans (W)	60 kg	60 kg
Maximum absorption through the skin (A)	0.076%	0.076%
Dermal absorption per treatment (I x A)	(1250 x 0.076) x 100 = 0.95 mg	(175 x 0.076) x 100 = 0.133 mg
Systemic exposure dose (SED) [(Ix A)/W]	(0.95 /60) = 0.0158 mg kg bw^{-1}	(0.133/60) = 0.0022 mg kg bw^{-1}
No observed adverse effect level (NOAEL)	20 mg kg bw^{-1} (Rat, oral 90 days)	20 mg kg bw^{-1} (Rat, oral 90 days)
Margin of safety (NOAEL/SED)	20/0.0158 = 1266	20/0.0022 = 9091

A review of the use of resorcinol in the cosmetics industry the Hair Colouring Technical Secretariat (HCTS 2002) indicated that due to the fact that resorcinol is only used in oxidative hair dye products, the exposure scenarios are limited to the following;

- Hair dyeing at home (consumer exposure)
- Hair dyeing at the hairdressers (consumer and worker exposure)

For these scenarios expected consumer contact is via the dermal route through the scalp given:

1. The stringent EU precautionary labelling and the inclusion of protective gloves in marketed hair dye kits;
2. The absence of inhalation exposure taking into account that resorcinol is non-volatile and hair colouring products are marketed in cream formulations.

The HCTS (2002) has argued that in the calculation of the margin of safety for oxidative or permanent hair dyes (see Table 10.14) by the SCCNFP a worst case scenario was used: Hair dyes are not applied daily, but are generally applied intermittently, that is once per 4 to 6 weeks. On this basis the HCTS have argued that the resulting MOS for oxidative and permanent hair dyes would be >10000 if the minimal peak exposure resulting from the 30 minutes and once per month hair dyeing process is converted to an average dose per day over time. Such MOS values would indicate no or negligible risk to consumers.

10.7.2.2 Pharmaceuticals

Consumers may be exposed to resorcinol through the use of topical ointments, though the amounts involved will depend on the proportion of resorcinol in the product and the application procedure (the amount applied and the frequency of application). Lederer (1982) estimated that under reasonable maximum use, topical application of resorcinol-containing ointments to treat acne would result in exposures up to 1.2 mg kg body weight^{-1} day^{-1}. This is based on the application of 600 mg of resorcinol (present at 2% in a 30 g product) every 7 days by a person with a body weight of 70 kg. For these dermal calculations Lederer (1982) assumed that all the resorcinol in the ointment would be absorbed and did not include factors for the short usage time frame. Even with these conservative assumptions, potential exposures to resorcinol through pharmaceutical use are well below levels that could be associated with adverse thyroid effects (that is a NOAEL of greater than 10 mg kg body weight^{-1} day^{-1}). If the dermal penetration rate of 0.37 μg cm^{-2} hour^{-1} associated with the use of a 2% resorcinol ointment as reported in human volunteers by Yeung *et al* (1983) is used to estimate dermal exposure, even lower exposures are capable for normal to exaggerated use conditions. For example assuming that a person applied ointment to 1000 cm^2 of their skin (more than 5% of the body surface area) and that the calculated dermal penetration rate was constant for 24 hours, daily exposures would be of the order of 0.1 mg kg body weight^{-1} day^{-1}. This would result in a margin of safety of > 100 based on a NOAEL of >10 mg kg body weight^{-1} day^{-1}.

10.7.3 Environmental exposure data

10.7.3.1 Data on emissions of resorcinol

As part of the evaluation of the levels of resorcinol to which target groups of humans and/or wildlife a summary of the available information on anthropogenic emissions of resorcinol to the environment has been provided by the Resorcinol Task Force.

Resorcinol Production

There are no production facilities for resorcinol in Europe, and therefore no emissions will result.

Rubber Products

Resorcinol is used as a ‘methylene group acceptor’ and is used to improve adhesion of rubber stocks to steel and textile interfaces. As a methylene group acceptor, it can also react with formaldehyde and hexamethylene tetramine (Hexamine) to produce resins. It is also used in Resorcinol-Formaldehyde-Latex (RFL) tyre cord dipping systems.

Emissions during tyre production

A typical extraction unit in a factory draws approximately 425 m^3 per minute, which implies a flow of 51 g of resorcinol per hour. A typical tyre plant will produce 30,000 to 40,000 tyres per day on a three-shift (24 hr) system. A typical tyre weighs 11 to 11.5 kg of which 21% is a resorcinol-containing constituent. Typical resorcinol usage levels are around 1.6% within the resorcinol-containing constituent.

This data means that approximately 38 g of resorcinol are used per tyre leading to the conclusion that between 1134 kg and 1512 kg are used per 24 hours. Since the 24-hour

extraction losses are 1224 g, this implies that the percentage loss of resorcinol is around 0.1% of usage.

Most of the resorcinol lost in the processing of tyres is removed from the extraction air by water-based scrubbers (resorcinol is highly soluble) and then treated off-site at wastewater treatment plants. Assuming that the scrubbers are at least 80% effective, the total amount of resorcinol reaching European wastewater plants from this source would be around 5 tonnes annually with a further 1.5 tonnes possibly reaching the atmosphere.

Emissions from tyres in use and at end-of-life

Information from INDSPEC indicates that no free resorcinol could be detected in chopped cured rubber using proton NMR techniques. In attempts to leach out resorcinol from cured rubber samples, detection once again proved a problem. No resorcinol was observed in the leachate above the detection limit of 0.2 $\mu g\ ml^{-1}$. Further work on the extraction of resorcinol from cured rubber by heating up to 150^0C also failed to yield any positive detection.

Although work continues on this issue, it is impossible to identify any meaningful release mechanism for the emission of resorcinol from cured rubber. Accordingly, no emissions can currently be ascribed to in-use or end-of-life phases.

Wood Adhesives

Resorcinol is used in two areas related to wood adhesives. The first (and largest) is the production of phenol- resorcinol-formaldehyde (PRF) glues for the laminated beams industry. The second is for the manufacture of hardeners for melamine-urea-formaldehyde (MUF) glues. In contrast to the rubber industry, the full cross-linking of the resorcinol within the adhesives takes place further down the supply-chain providing more opportunities for losses and making these more difficult to quantify.

Emissions during glue production

Good manufacturing practice using intermediate bulk containers (IBCs) minimises the handling losses during addition of resorcinol to batches and these are believed to be typically below 0.05% in most cases (<1.35 tonnes). Since the bags themselves are routinely incinerated, this figure could be significantly lower. Vessels used to manufacture glues are washed periodically and the wastewater is passed to biologically active treatment plants where reductions in overall phenolic waste are typically better than 99.7%, thereby reducing the outflow to adjacent waters to very low levels. Even if all this remaining phenolic content were to be resorcinol (which is not the case), the total loss to the outside aqueous environment by this route would be 0.0033%, or 88 kg per year for the entire industry in Europe.

Emissions during glue use

The glues supplied to the user industries will typically contain resorcinol at various degrees of reaction. However, using a typical product mix, the anticipated free resorcinol in products shipped to the user market will be as much as 1000 tonnes. Accordingly, the safe handling of these products and the further reaction of the monomers is a critical part of the control of resorcinol emissions to the environment. Although there is very little emissions data available in this downstream sector, it might not be unreasonable to assume that process losses run at 0.5–1.0% (i.e. around 5 - 10 tonnes). This reflects the fact that the glue using industry is fairly

fragmented and may well consist of small, independent operators without substantive emission reduction capabilities (although it is known that some incineration of off-cuts takes place). It would be expected that most (possibly around 90%) of the emissions occurring would reach the water-course, with the balance being released to the air. This assumes that most processes are not conducted at significantly elevated temperatures.

Emissions from glued products in use and at end-of-life

There is no information available for emissions from glues in the 'use' and 'end-of-life' phases. However, with full cross-linking of the adhesive achieved in processing, the losses to atmosphere are expected to be below 0.01% (< 100 kg).

Flame Retardants

The use of resorcinol for the manufacture of resorcinol diphenyl phosphate (RDP) is declining rapidly and is expected to disappear completely by 2005, being replaced by the more favoured bisphenol A bis diphenyl phosphate (BDP). The decline of RDP has also been accentuated by the downturn in the communications industry where flame retardant engineering plastics have widespread use.

Emissions during manufacture of RDP

The consumption of resorcinol in the manufacture of RDP is a classical chemical intermediate use. Although no information is available on specific losses in process, an assumption of 0.05% would seem an appropriate default, leading to overall emissions of approximately 1050 kg from this source annually, albeit on a reducing basis through to 2005. All losses are expected to be into the aqueous phase.

Emissions from engineered plastic products in manufacture, use and at end-of-life

Since the resorcinol is already reacted into RDP before inclusion in any engineered thermoplastic, it is hard to postulate a breakdown method in the manufacturing and use phases. There may be more of a case to postulate a breakdown mechanism at end-of-life where recycling, incineration or landfill can all potentially take place. However, while wood adhesives could arguably have some un-reacted resorcinol still in place, this will not be the case in this instance. Therefore, a default figure between that adopted for rubber products and wood adhesives is proposed at 0.005%, leading to an assumed emission to air of $\leq$105 kg.

UV Stabilisers

Resorcinol is used as an intermediate in the manufacture of hydroxybenzophenones which themselves are used as UV stabilisers. This makes them plastics additives in the same way as RDP and they therefore demonstrate similar emissions characteristics.

Emissions during manufacture of hydroxybenzophenones

The prime area for the potential emission of resorcinol during the manufacture of hydroxybenzophenones is in the mixing phase. Here any dust is collected in a bag-house and

then sent to a wet scrubber system where any phenolics are extracted and scavenged using para-formaldehyde. Once cross-linked, the material can be disposed of as non-hazardous waste. Overall losses from this treatment route are expected to be less than 0.05% (i.e. 500 kg).

Emissions from plastic products in manufacture, use and at end-of-life

As with the flame retardant application, it is difficult to postulate a breakdown mechanism for the UV stabiliser in the plastics manufacturing phase. In the use phase and at end-of-life, there may be more of a prospect of some product breakdown, but any resorcinol freed by the reversal of a previous reaction would normally remain locked within the plastic matrix in any event. On this basis, it is hard to envisage losses greater than 0.01% (100 kg) in the form of free resorcinol.

Dyes

Resorcinol is used significantly as an intermediate in the dye industry for the production of xanthene and azo dyes amongst others.

Emissions during manufacture and use

No emissions data is available on the application of resorcinol in the manufacture and use of dyes. However, the normal processing routes for dyes would be expected to yield losses of some significance in aqueous media, even though concentration will be low. Default losses in the range of 1% could be expected, leading to an overall total of around 3 tonnes per annum from this industry sector.

Meta Amino Phenols

A more efficient and, arguably, cleaner way of producing xanthene dye intermediates is through the production and supply of meta amino phenols (MAPs). Under controlled manufacturing conditions losses of less than 0.05% of resorcinol would be expected. In the case of MAPs, all manufacture takes place in Japan and hence losses of resorcinol in Europe (e.g. through transport of MAPs) are assumed to be insignificant.

Hair Dyes

Resorcinol is used in oxidative hair dyes as a dye intermediate. Levels are limited by regulation to 5% in this application and 0.5% in shampoos and hair lotions. In practice, the hair dye industry limits the level of free resorcinol in oxidative hair dyes to 1.25%.

Emissions during production of hair dyes

Since hair dyes are manufactured in a closed process under vacuum, there are no losses to atmosphere. However, losses in aqueous wastewater resulting from batch processing can amount to 1% because of the relatively small batch sizes used. This represents 1.5 tonnes of the 150 tonnes used by the industry annually.

Emissions during use of hair dyes

Since the reaction of free resorcinol in oxidative hair dyes takes place during use, the degree of reaction in the 30-minute period of typical use can vary. However, estimates of non-reacted

resorcinol remaining at the end of the 30-minute period range from 52 to 72%. This means that 70-80 tonnes of resorcinol could enter wastewaters under normal hair dye use patterns.

Pharmaceuticals

There is little, if any, data on the environmental consequences of the use of resorcinol in pharmaceutical applications such as topical ointments. However, in the absence of any postulated reaction mechanism for resorcinol in this context, it has to be assumed that the 75 tonnes used for these applications is completely released to the environment either through discarded bandages or through the wastewater systems of Europe. For the purposes of this review, it is assumed, as a worse case, that 100% of the resorcinol reaches the wastewater stream, either directly or from the output of domestic landfills. Where bandages are disposed of through medical channels, incineration is the most likely route. This will effectively prevent some of the assumed resorcinol releases from occurring.

Others

Other applications include the use of resorcinol in the production of intermediates for herbicides and explosives. In addition, resorcinol acts as a building block for several other chemical intermediates. Although these applications and their associated chemistries are varied, all of them are expected to be conducted using good manufacturing practice, leading to resorcinol losses of around 0.05%.

Summary of Projected Emissions to Air and Water

Losses of free resorcinol to atmosphere from products in use and at end-of-life is believed to be very low and this is reflected in Figure 10.1 set out below:

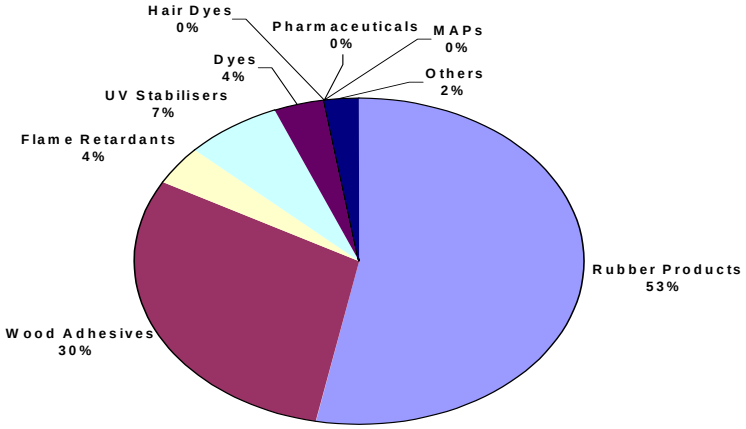


Figure 10.1 Resorcinol losses to air (Total 2.8 tonnes per annum – 0.02%)

When considering losses to atmosphere, it is important to note that there are few high temperature processes conducted where free resorcinol is available. This explains why the predominant losses are recorded in the rubber processing area despite the employment of scrubbing facilities at most (if not all) producers.

In the aqueous environment, the situation is more significant with over 150 tonnes (1.25%) of resorcinol lost to the wastewater streams from a variety of uses (see Figure 10.2). However, the fact that resorcinol undergoes rapid degradation and easily meets the OECD criteria for 'ready biodegradability' means that the burden of resorcinol to the environment is considered to be minimal. It should be noted that the current assessment makes assumptions for the aqueous emissions from the wood adhesives industry that could be substantially greater in practice.

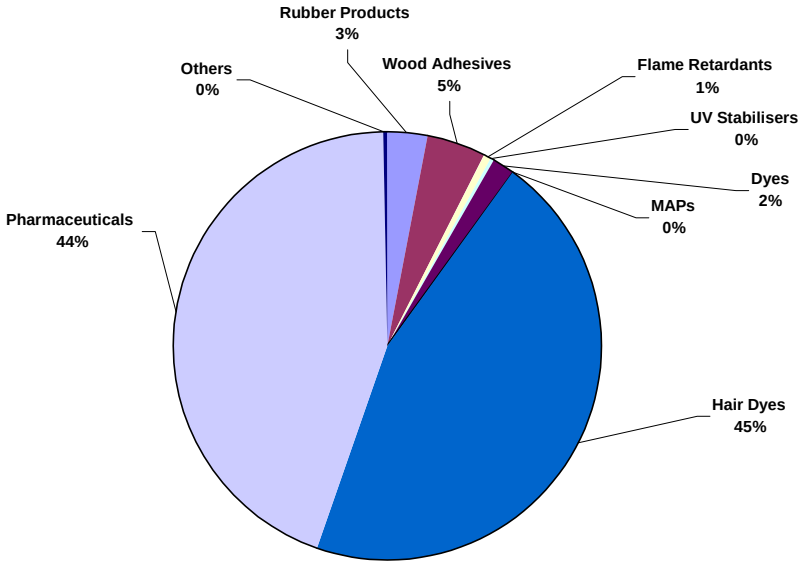


Figure 10.2 Resorcinol losses to water (total 168.7 tonnes per annum – 1.25%)

10.7.3.2 Data on environmental concentrations of resorcinol

No recent measured environmental exposure data has been obtained based on searches of the COMMPS database and literature sources.

10.8 Overall Conclusions on Resorcinol

The following conclusions have been drawn from the review of resorcinol:

10.8.1 Data from studies assessing potential endocrine disruption effects

10.8.1.1 Human related studies

- Resorcinol has a limited package of regulatory mammalian toxicology data and there is currently no multi-generational reproduction or fertility study carried out to an internationally recognised test guideline, which represents a key area of uncertainty in terms of the assessment of endocrine disruption. However this is going to be addressed by a Resorcinol Task Force which has already formulated a comprehensive test programme. This will comprise of an extensive dose range finding study and a test guideline compliant two-generation reproduction study with expanded thyroid endpoints. It will also provide additional observations on sub-chronic exposure NOEL's derived from the pre-mating dosing period.

- *In vitro* studies indicate that the anti-thyroidal activity observed following resorcinol exposure is due to inhibition of thyroid peroxidase (TPO) enzymes, as evidenced by disruption of thyroid hormone synthesis and changes in the thyroid gland consistent with goitrogenesis.

- Certain older *in vivo* laboratory animal studies (Doniach and Logothetopoulos 1953, Samuel 1995) have revealed reversible anti-thyroid activity. The thyroid effects in these studies resulted from continuous exposure to high resorcinol doses and required a vehicle (such as arachis, peanut or ground nut oil) to establish a reservoir of resorcinol and to alter the pharmacokinetics such that resorcinol was continuously bioavailable.

- Studies conducted as part of the National Toxicology Programme (NTP 1992) have shown no effects on the thyroid of rats or mice at doses of up to 520 mg kg body weight^{-1} day^{-1} in rats and 450 mg kg body weight^{-1} day^{-1} in mice for 13 weeks and 150 – 225 mg kg body weight^{-1} day^{-1} for 5 days per week over 2 years in rats and mice.

- It needs to be recognised that rodents, especially rats, have been reported to be particularly susceptible to goitrogens, primarily due to the lack of thyroid binding protein (TBP) which is the primary protein for thyroid hormone binding and transport (Dohler *et al* 1979, Curran and DeGroot 1991, Alison *et al* 1994). In the rat, the absence of TBP results in a much shorter half life of T4 and much higher levels of TSH (Dohler *et al* 1979, Capen *et al* 1991, Alison *et al* 1994). These differences suggest that the activity of the thyroid gland in rats is considerably higher than that of other species, including humans, and this increased activity correlates to a greater susceptibility to hormonally-induced thyroid effects (Alison et al 1994, McClain 1994). Given the relative insensitivity of humans to changes in the thyroid gland, it has been suggested by certain authors (Ames *et al* 1987, Alison *et al* 1994, McClain 1994) that high doses of substances such as resorcinol which cause hormonally-induced changes of the thyroid in rodents (particularly rats) have limited relevance to humans.

- The available data the 13-week NTP rat and mouse studies also provides evidence of effects on adrenal weights at all doses tested. However, the observed responses did not show dose-dependent relationships. Possible adrenal effects will be addressed in the test programme formulated by the Resorcinol Task Force.

- No evidence of gross teratogenicity or embryotoxicity has been recorded in studies with rats and rabbits at the highest exposure concentrations tested even though maternal toxicity was evident.

- In 2001 the Committee on the Health Council of the Netherlands produced a Health-based reassessment of current administrative occupational exposure limits in the Netherlands (Gezondheidsraad 2001**)**. The Committee on Updating of Occupational Exposure Limits considered current values in the light of available data. In the review the Committee considered that "the NTP studies were well performed and attaches more importance to these studies than to the studies of Cooksey *et al* (1985) and Seffner *et al* (1995). The Committee questions the relevance of the thyroid effects found by these authors". The Committee also concluded that "resorcinol is not embryotoxic or teratogenic.".

10.8.1.2 Wildlife studies

- The limited data for potential endocrine mediated responses in wildlife precludes general assessments of the effects of resorcinol. In a teratogenicity studies with rainbow trout and zebrafish embryos teratogenic effects were evident at exposure concentrations $\geq$ 100 mg l^{-1}. However, there is no available data as to whether the observed effects were endocrine mediated.

- Importantly no data is available on the effects of resorcinol on the reproduction of invertebrates (such as *Daphnia magna*) or fish. The data gap for *Daphnia magna* reproduction is important given that 48h EC_{50} values of $\leq$1.3 mg l^{-1} have been reported (see Section 10.5.2.1). The Resorcinol Task Force is planning to address the key data gaps in this area of study over the next 6-12 months.

10.8.2 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity

10.8.2.1 Human related studies

- The primary signs of acute resorcinol intoxication (animals and humans) have been shown to include initial stimulation of the central nervous system followed by CNS depression, renal glomerular and tubule degeneration, central hepatic necrosis, myocardial depression following systemic exposure and pruritis and reddening of the skin following topical exposure. Repeated exposure to resorcinol also results in microscopic changes in the kidneys and in the liver and changes in the function and morphology of the thyroid.

- The effects on the thyroid in mammals have been noted after oral, intravenous and sub-cutaneous administration and the lowest dose levels exerting thyroid effects were 5 mg kg body $weight^{-1}$ day^{-1} for both oral (drinking water) and sub-cutaneous exposure. However, other NTP studies using the oral gavage exposure route have shown an absence of effects on the thyroid at concentrations above 150 mg kg body $weight^{-1}$ day^{-1}. In these studies effects on the adrenals were observed at all test doses (lowest levels being 28 – 32 mg kg body $weight^{-1}$ day^{-1}), though there is currently uncertainty as to the significance of these findings.

- In 2001 the Committee on the Health Council of the Netherlands produced a Health-based reassessment of current administrative occupational exposure limits in the Netherlands (Gezondheidsraad 2001). In the summary of the draft report a chronic NOAEL of 50 mg kg

body weight^{-1} day^{-1} observed in the long-term oral exposure study (NTP 1992) was considered as the lowest valid value from the available dataset. The key effects were those on the central nervous system.

- In contrast when the European Commission Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers considered the use of resorcinol in oxidation or permanent and semi-permanent hair dyes (EC 2000 – Report by the Scientific Committee on Cosmetology) a NOAEL value of 20 mg kg^{-1} body weight^{-1} was used in the calculation based on a sub-chronic gavage study in Wistar rats (Potokar and Pittermann, 1980).

10.8.2.2 Wildlife studies

- The limited data on potential endocrine mediated responses in wildlife species precludes comparison with general systemic toxicity data.

10.8.3 Exposure data

- There is no strong evidence from occupational epidemiological studies that exposure to resorcinol causes hypothyroidism (for example Roberts *et al* 1990) indicating that workers do not appear to be a vulnerable group at prevailing exposure levels.

- For consumers using hair dye products the risk of effects appears to be minimal given that the MOS for a worst case scenario is in the region of 1200.

- For consumers using topical ointments containing resorcinol the risk of effects appears to be minimal given that the MOS for a typical scenario exceeds 100.

- No recent measured environmental exposure data has been obtained based on searches of the COMMPS database and literature sources.

10.9 Summary of the weight of evidence for endocrine disrupting effects in humans and wildlife and associated uncertainties

The summary of the weight of evidence for endocrine disrupting effects of resorcinol in humans and wildlife along with associated uncertainties are given in Table 10.15.

Table 10.15 Summary of the weight of evidence conclusion and uncertainties associated with the assessment of the endocrine disrupting effects of resorcinol

	Target group	
	Humans	Wildlife
Weight of evidence	*In vitro* studies indicate that the anti-thyroidal activity observed following resorcinol exposure is due to inhibition of thyroid peroxidase (TPO) enzymes, as evidenced by disruption of thyroid hormone synthesis and changes in the thyroid gland consistent with goitrogenesis. Certain older *in vivo* laboratory animal studies have revealed reversible anti-thyroid activity. The thyroid effects in these studies resulted from continuous exposure to high doses and required a vehicle (such as peanut oil) to establish a reservoir of resorcinol and to alter the pharmacokinetics such that resorcinol was continuously bioavailable. Studies conducted as part of the National Toxicology Programme have shown no effects on the thyroid of rats or mice at doses of up to 520 mg kg body weight^{-1} day^{-1} in rats and 450 mg kg body weight^{-1} day^{-1} in mice for 13 weeks and 150 – 225 mg kg body weight^{-1} day^{-1} for 5 days per week over 2 years in rats and mice. There is evidence of effects on adrenal weights at all doses tested in NTP rat and mouse 13-week studies. However, the observed responses did not show dose-dependent relationships. Currently available data indicates that resorcinol is not embryotoxic or teratogenic. The available exposure data indicate that resorcinol does not represent a risk to workers or consumers based on current exposure pathways.	The available aquatic effects data from teratogenicity studies with rainbow trout and zebrafish embryos shows teratogenic effects are evident at exposure concentrations $\geq$ 100 mg l^{-1}. However, there is no available data as to whether the observed effects were endocrine mediated.
Uncertainties	There are uncertainties with regard to the evaluation of potential adverse effects of resorcinol on reproductive and developmental endpoints since data is not available from a definitive multi-generation study. These issues will be addressed in study being initiated by the Resorcinol Task Force, along with the uncertainties regarding the significance of effects in the adrenals observed in certain studies.	There are uncertainties with regard to the potential adverse effects of resorcinol on reproduction and development in wildlife due to the absence of key data for aquatic organisms particularly invertebrates. This is planned to be addressed by the Resorcinol Task Force. The absence of data on terrestrial and aerial organisms is not a major uncertainty since the physico-chemical properties of resorcinol mean the potential for these organisms to be exposed is limited. There are no environmental exposure data for resorcinol in the aquatic, terrestrial and aerial compartments.

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11. REVIEW OF DATA FOR 4-*TERT* OCTYLPHENOL

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Notes:

This section contains information collected and collated in the preparation of a Targeted Risk Assessment for 4-*tert* octylphenol (by the United Kingdom) as part of the Risk Reduction Strategy for nonylphenol and its ethoxylates. This involved liaison with CEPAD, the sector arm of CEFIC dealing with alkylphenol ethoxylates. It also draws on:

- A SIDS Initial Assessment Report (SIAR) and full SIDS dossier on 4-*tert* octylphenol prepared by Switzerland in 1994;

- A Background Document for 4-*tert* octylphenol being prepared by OSPAR since it features on the Priority List of Hazardous Substances due to its perceived endocrine disrupting properties.

This review has been carried out in accordance with the evaluation framework described in Section 2. In the review the Internation Programme for Chemical Safety (IPCS) definition of an endocrine disrupter has been adopted, namely that it is "*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*".

In the context of the review it is recognised that there are various laboratory-based *in vivo* and *in vitro* methods utilising a range of (eco)toxicological endpoints that are claimed by different sources to be relevant to the assessment of endocrine disruption in humans and/or wildlife. However, since this field is still in an early stage of development there is uncertainty regarding the significance of many of the current findings.

From the numerous recent reviews of potential test methods (such as the Detailed Review Paper prepared by OECD in 1997) there is a clear consensus in terms of the hierarchy of the relevance of test methods. In this hierarchy longer-term *in vivo* studies considering effects on reproduction and/or development (and including mechanistic information) are of greater relevance than short-term *in vivo* screening tests which are of greater relevance than *in vitro* assays. The greater relevance of chronic *in vivo* tests or those assessing effects during critical windows of sensitivity is also evidenced by the fact that these are the key (eco)toxicological methods being developed in the OECD Endocrine Disruption Testing and Assessment (EDTA) Programme. This hierarchy approach to data relevance has been adopted in the review along with a weight of evidence consideration of the available data.

The review has been carried out to address three key questions:

1. Does the available data indicate there is evidence that a chemical causes endocrine disrupting effects in target groups of humans and/or wildlife?

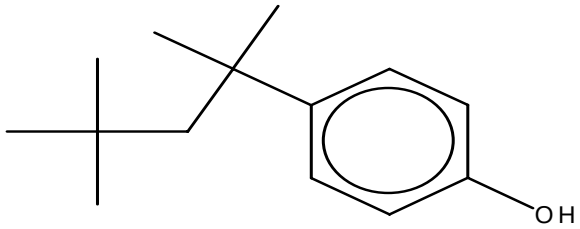
2. Do endocrine disrupting effects of the chemical in target groups of humans and/or wildlife occur at lower concentrations than those causing effects on general systemic toxicological endpoints?

3. Are particular target groups of consumers or organisms in the environment likely to be exposed to concentrations of chemicals which exceed effects thresholds due to current emission patterns?.

It should be recognised that this review is not designed to be a full Risk Assessment of a substance under the Existing Substances Regulation 793/93.

11.1 Physico-chemical data for 4-*tert* octylphenol

11.1.1 Summary details on the substance

CAS Number	140-66-9
EINECS Number	205-426-2
IUPAC Name	4-(1,1,3,3-tetramethylbutyl)-phenol
Other names	(1,1,3,3,-Tetramethyl-4-butylphenol)
Molecular weight	206.3
Chemical formulae	$C_{14}H_{22}O$
Chemical structure	

11.1.2 Physico-chemical properties and environmental fate information (from IUCLID 2000)

The data on the physico-chemical properties of 4-*tert* octylphenol and its environmental fate (see Table 11.1) indicate that the substance is inherently biodegradable. In the aquatic environment photodegradation and hydrolysis of 4-*tert* octylphenol may occur but are considered to be negligible processes.

Volatilisation may represent a removal process from the aquatic environment based on the Henry's Law Constant of 1.06 x 10^1 Pa-m^3 mol^{-1} (1.07 x 10^{-4} atm-m^3 mol^{-1}) being within a value range of 1-100 Pa-m^3 mol^{-1} which is considered to indicate volatility. However, in the air degradation of 4-*tert* octylphenol through reactions with hydroxyl radicals is rapid with a half life of 0.25 days being reported.

Table 11.1 Physico-chemical properties and environmental fate data (from IUCLID 2000)

Physico-chemical property	Value (and comments)
Physical state at ambient temperature	Solid
Water solubility	17-19 mg l^{-1} at 22 ^{o}C
Octanol-water partition coefficient (log Kow)	4.12 at 20.5 ^{o}C (By shake flask method to OECD 107)
Organic carbon water partition coefficient (Koc)	No data
Henry's Law Constant	1.06 x 10^{1} Pa-m^{3} mol^{-1} – Calculated) (1.07 x 10^{-4} atm-m^{3} mol^{-1} - Calculated)
Type of degradation	Information
Aquatic - abiotic	Photodegradation and hydrolysis of 4-*tert* octylphenol may occur but are considered to be negligible processes.
Aquatic - biotic	Biodegradation studies have indicated that 4-*tert* octylphenol is inherently biodegradable.
Terrestrial	No data
Atmospheric	Degradation of 4-tert octylphenol through reactions with hydroxyl radicals is rapid with a half life of 0.25 days reported.

A Mackay Level 1 fugacity model has shown that for a discharge of 1000 tonnes of 4-*tert* octylphenol 77% of the substance will partition into the soil with 14.7% and 6.6% respectively partitioning into the air and water (Table 11.2). Amounts present in other compartments are minimal.

Table 11.2 Summary of the results of a Mackay Level 1 fugacity model

Compartment	Volumes of different compartments	% of substance present in different compartments
Water	2 x 10^{11}	6.6
Suspended sediment	10^{6}	0.053
Bottom sediment	10^{8}	1.71
Fish	2 x 10^{5}	0.0043
Air	10^{14}	14.7
Aerosol	2000	4.3 x 10^{-4}
Soil	9 x 10^{9}	77.0

11.2 Production and Uses

11.2.1 Production Patterns

The production processes used to produce 4-*tert* octylphenol, are analogous to those used to manufacture nonylphenol (CEPAD 2002).

1. Phenol and 4-*tert* octene (di-isobutene) are reacted in the presence of an ion-exchange resin or boron trifluoride complex in a batch reactor. Neutralised/deactivated catalyst is disposed of via authorised waste facilities in accordance with regulations.

2. Phenol and 4-*tert* octene (di-isobutene) are reacted in the presence of a fixed bed ion exchange resin in a continuous process. The deactivated catalyst is discharged directly into an incineration plant.

The 4-*tert*-octene is produced by dimerisation of isobutene which ensures that the branched octene is obtained rather than the linear one. The reaction with phenol leads predominantly to substitution by t-octene in the 4 (para) position.

An important additional source for production of octylphenol is during the production of nonylphenol. Nonylphenol is produced by the reaction of a commercial nonene feedstock with phenol and the feedstock may contain up to 10% of octene, although typically it contains 3-5% (CEPAD 2002). Consequently, a similar proportion of the nonylphenol produced will be octylphenol.

Table 11.3 outlines production volumes, exports and imports of octylphenol within Europe for the five years from 1997 to 2001.

Table 11.3 European production volume, exports and imports (from CEPAD 2002)

	Amount (tonnes/year)				
	1997	1998	1999	2000	2001
Production volume	17520	18259	19626	22215	22633
Exports	234	104	6	0	150
Imports	1035	1337	1240	1308	375
Tonnage used	18051	19492	20928	23523	22858
Captive use*	14969	16074	17592	19910	20060

* use on site to produce other substances

Production is carried out at six sites in Europe at various production plants in Germany, Belgium, Switzerland, France and the UK.

11.2.2 Use Patterns

Information on the uses of 4-*tert* octylphenol has been obtained from CEPAD and these data are summarised in Table 11.4. This indicates that the majority of the 22848 tonnes used in Europe in 2001 is for the production of phenolic/formaldehyde resins (98%). A much smaller proportion (2%) of the production is used to produce octylphenol ethoxylates, and of these ethoxylates produced a percentage is used to produce octylphenol ether sulphates.

Table 11.4 Main Uses within Europe in 2001 (from CEPAD 2002)

Uses	Volume (tonnes)	Percentage (fraction of total use)
Production of octylphenol ethoxylates		
Emulsion polymerisation	220	0.96
Water-based paints	20	0.09
Pesticide formulation	40	0.18
Textiles/leathers	60	0.26
Production of octylphenol ether sulphates	60	0.26
Total	***400***	***2***
Production of Phenol / formaldehyde resins		
Rubber compounds	18458	80.8
Electrical insulating varnishes	2000	8.8
Printing inks	1000	4.4
Ethoxylated resins	200	0.87
Other uses	800	3.5
Total	***22458***	***98***
Total used in EU	**22858**	**100**

Phenol/formaldehyde resins manufacture is based almost exclusively on discontinuous batch processes using a traditional reactor or 'kettle'. Most of the 4-*tert* octylphenol in the produced resins is chemically bound and cannot be released even on subsequent chemical or biological degradation, but the resins also contain a small proportion (3-4%) of unreacted 4-*tert* octylphenol.

11.3 Toxicokinetics, metabolism and bioaccumulation

11.3.1 Toxicokinetics and metabolism

Certa *et al* (1996) assessed the toxicokinetics of 4-*tert* octlyphenol in male Wistar rats which had received either single oral (gavage) applications of 50 or 200 mg kg body weight^{-1} or a single intavenous injection of 5 mg kg body weight^{-1}. The 4-*tert* octylphenol blood concentrations (determined by GCMS) was ~ 1970 ng ml^{-1} immediately after a single intravenous application, decreased rapidly within 30 minutes and was no longer detectable 6-8 hours after application. The curve of blood concentration against time was used to calculate an elimination half-life of 310 minutes. Octylphenol was detected in blood as early as 10 minutes after gavage administration, indicating rapid uptake from the gastro-intestinal tract. Maximal blood levels reached 40 and 130 ng ml^{-1} after applications of 50 and 200 mg kg body weight^{-1} respectively. Using the area under the curve of blood concentration verses time low oral bioavailabilities of 2 and 10% were calculated for the 50 and 200 mg kg body weight^{-1} groups respectively.

Octylphenol toxicokinetics after repeated administration was investigated in male Wistar rats receiving daily gavage administrations of 50 or 200 mg kg body weight^{-1} for 14 consecutive days. Profiles of octylphenol blood concentrations verses time determined on day 1 and 14 were similar, indicating that repeated oral gavage did not lead to increased blood concentrations. Another group of rats received octylphenol via drinking water saturated with 4-

tert octylphenol (corresponding to a mean daily dose of ~ 800 μg kg^{-1}) over a period of 28 days. Octylphenol was not detected in any blood sample from animals treated via drinking water (LOD = 1-5 ng ml^{-1} of blood). Octylphenol concentrations were also analysed in tissues obtained from the repeated dose gavage (14 days) and drinking water (14 and 28 days) groups. In the 50 mg kg body $weight^{-1}$ group low octylphenol concentrations were detected in fat and liver from some animals with average concentrations of 10 and 7 ng g $tissue^{-1}$ respectively. Octylphenol was not detected in the other tissues analysed from this group. In the 200 mg kg body $weight^{-1}$ group octylphenol was found in all tissues analysed, namely fat, liver, kidney, muscle, brain and lung (but not testes) at average concentrations of 1285, 87, 71, 43, 9 and 7 ng g $tissue^{-1}$ respectively. Octylphenol was not detected in tissues of animals receiving the substance via drinking water for 14 or 28 days. Except in muscle and kidney tissue of one single animal receiving octylphenol for 14 days. Using rat liver fractions it was demonstrated that octylphenol was conjugated via glucuronidation and sulphation *in vitro*. A V_{max} of 11.24 nmol/(min * microsomal protein) and a K_m of 8.77 μmol l^{-1} were calculated for enzyme catalysed octylphenol glucorinidation. For enzyme catalysed sulphation, a V_{max} of 2.85 nmol/(min * microsomal protein) and a K_m of 11.35 μmol l^{-1} were calculated. The results indicate that octylphenol does not bioaccumulate in rats receiving low oral doses, which is in agreement with the hypothesis of a rapid first-pass elimination of octylphenol by the liver after oral ingestion, via glucorinidation and sulphation. Only if these detoxification pathways are saturated may excessive doses lead to bioaccumulation.

Upmeier *et al* (1999) subsequently investigated the toxicokinetics of 4-*tert* octylphenol in female DA/Han rats administered the substance either intravenously (5 mg kg body $weight^{-1}$) or orally by gavage (50 or 200 mg kg body $weight^{-1}$). After intra-veous administration the blood concentration-time curve of octylphenol was fitted to a tri-exponential model, resulting in a final half life of 36.1 hours. This contrasts with the more rapid elimination previously reported in male Wistar rats. The oral bioavailablity of 50 mg kg body $weight^{-1}$ was 12.3% and of 200 mg kg body $weight^{-1}$ was 8.4%. The higher dose was adsorbed slower than the higher dose probably due to the low solubility of octylphenol in aqueous media. Maximal octylphenol blood levels in female DA/Han rats receiving 50 and 200 mg kg body $weight^{-1}$ were 4.5 and 3 times higher than previously reported in male Wistar rats. The blood concentration-time curves revealed pronounced inter-individual difference indicating extensive enterohepatic circulation of octylphenol in this rat strain. In contrast to male Wistar rats, after application of high doses of octylphenol to female DA/Han rats the compound was not completely eliminated within 48 hours and under these circumstances bioaccumulation might occur.

Ferreira-Leach and Hill (2001) investigated the biotransformation, bioconcentration and tissue distribution of 4-*tert*-octylphenol in juvenile rainbow trout. The study suggested that exposure to waterborne 4-*tert*- octylphenol results in rapid conjugation and elimination of the chemical via the liver/bile route, but that high amounts of the parent substance can accumulate in a variety of other fish tissues.

11.3.2 Bioaccumulation

The estimated bioconcentration factor (BCF) for octylphenol is 2303 (SRC BCFWIN version 2.14), which would suggest that appreciable bioaccumulation could occur. There are a few tissue samples from aquatic organisms in European waters that have been collected and analysed for octylphenol to determine Bioconcentration Factors.

In order to ensure that any octylphenol used in production chemicals at offshore installations did not reach the food supply chain, a number of marine fish species (dab, haddock and herring) taken from around North Sea offshore installations were analysed for octylphenol as

part of a preliminary Food Quality Assurance monitoring programme. The results of this study are reported in CEFAS (1997) and showed that concentrations of octylphenol in fish liver and muscle were always below the limits of detection (i.e. 0.004 - <0.1 mg kg^{-1} depending on the species and tissue type tested).

Lye *et al* (1999) measured the accumulation of 4-octylphenol in juvenile and mature male flounder (*Platichthys flesus*) collected from the Tees and Tyne estuaries. Octylphenol (0.017 mg kg^{-1} wet weight) was detected in homogenised tissue of fish from the Tees Estuary but was not detected in tissue from fish from the Tyne Estuary (<0.005 mg g^{-1} wet weight). Octylphenol was not detected in water samples.

Overall the available measured data indicates that 4-*tert* octylphenol has a low to moderate potential for bioaccumulation in aquatic organisms.

11.4 Studies relevant to the assessment of potential endocrine disrupting effects

11.4.1 Studies relevant to the assessment of endocrine disrupting effects in humans

11.4.4.1In vitro studies

There is a considerable body of data available on the effects of 4-*tert* octylphenol on *in vitro* systems using mammalian cells and tissues and the following sections provide a summary of key data.

A. Receptor competitive binding assays

Nagel *et al* (1997) assessed the *in vitro* effects of 4-*tert* octylpehnol using a relative binding affinity-serum modified access (RBA-SMA) assay which was developed to determine the effect of serum on the access of xeno-oestrogens to oestrogen receptors within intact cultured MCF-7 human breast cancer cells. This is achieved by comparing the relative binding affinity (relative to oestradiol of a xeno-oestrogen in serum free medium to its RBA in 100% serum. Serum was found to have an inhibitory effect on 4-*tert* octylphenol relative to oestradiol and decreased the relative access of the substance to oestrogen receptors by 22 fold. This finding was taken as evidence that the activity of 4-*tert* octylphenol, relative to oestradiol would be overestimated in serum free assays.

B. Recombinant yeast assays

The results from YES assays with 4-*tert* octylphenol have shown an oestrogenic activity that was approximately 1500 times less than for oestradiol. In a comparison of short-term oestrogenicity tests for the identification of hormone-disrupting chemicals Andersen *et al* (1999) two laboratories carried out testing of 10μM of 4-n-octylphenol and found that the activity was similar to that measured for a 10nM concentration of 17β oestradiol. The results indicated an oestrogenic activity for 4-n-octylphenol which was approximately 1000x lower than that for oestradiol.

C. Mammalian cell growth assays

Results on the oestrogenic activity of 4-*tert* octylphenol in assays using the MCF-7 cell line have shown a potency that was approximately 500 times less than for oestradiol (White *et al* 1994) while E-screen tests with 4-*tert* octylphenol have shown that the oestrogenic activity in this cell-line was approximately 330 times less than for oestradiol (Soto *et al* 1995). In a comparison of short-term oestrogenicity tests for the identification of hormone-disrupting chemicals Andersen *et al* (1999) three laboratories carried out testing of 2.5μM of 4-n-octylphenol and found that a 2.3x increase in activity compared to controls. The increase in activity for a 10nM concentration of 17β oestradiol was 3.6x that of the controls meaning that 4-n-octylphenol had an oestrogenic potency approximately 400 times lower than for oestradiol.

Summary of *In vitro* data

Table 11.5 summarises the available *in vitro* data for 4-tert octylphenol which primarily relates to *in vitro* assays assessing oestrogenic mechanisms of action in mammalian cells and tissues. The data indicates that 4-*tert* octylphenol has a binding affinity for the human oestrogen receptor which is 1500 times lower than that for oestradiol. However, Nagel *et al* (1997) reported effects may be overestimated in serum free assays. 4-*tert* octylphenol has been reported to be oestrogenic in mammalian cell culture assays at levels 330-500 times lower than oestradiol. No data is available on the androgenic or anti-androgenic effects of 4-*tert* octylphenol on and effects on thryoid function or hormone synthesis or secretion or steroidogenesis in mammalian cells and tissues.

Table 11.5 Summary of the *in vitro* data in isolated mammalian cells and tissues relating to different mechanisms of action of 4-tert octylphenol

Mechanism of endocrine disruption	Responses observed in *in vitro* systems
Oestrogenecity/anti-oestrogenecity	4-*tert* octylphenol has a binding affinity for the human oestrogen receptor which is 1500 times lower than that for oestradiol. However, Nagel *et al* (1997) reported effects may be overestimated in serum free assays. 4-*tert* octylphenol has been reported to be oestrogenic in mammalian cell culture assays at levels 330-500 times lower than oestradiol.
Androgenecity/anti-androgenecity	No data identified
Thyroid effects	No data identified
Effects on hormone synthesis or secretion	No data identified
Effects on steroidogenesis	No data identified

11.4.4.2 In vivo studies

Tables 11.6 and 11.7 summarises the results of the *in vivo* mammalian studies described in detail in Section 11.4.4.1.

A. Effects on endocrine glands and hormone sensitive tissues

Bicknell *et al* (1995) sub-cutaneously injected young female Wistar rats with 10 mg kg body weight^{-1} of 4-*tert* octylphenol on three consecutive days, and the weight and gross pathology of the uteri were investigated. Exposure to 4-*tert* octylphenol doubled the weight of the uteri without any effect on overall body weight.

Majdic *et al* (1996) investigated the effect of maternal exposure to octylphenol on cytochrome P450 in foetal rat testis. Reduction in 17α-hydroxylase activity (accompanied by reduced expression of P450c17 mRNA) was seen on 17 day old foetuses after the pregnant rats were injected subcutaneously with 600 mg of octylphenol on day 11 and day 15 of gestation. Synthesis of testosterone is dependent on several enzymes, including P450 17α-hydroxylase, which is strictly regulated in the testis.

Milligan *et al* (1998) investigated the relative potency of xenobiotic oestrogens, including 4-*tert* octylphenol, in an *in vivo* mammalian assay in ovariectomised female Swiss albino mice. The ovariectomies were performed at least two weeks before the start of an experiments. The endpoint in the assay was the change in uterine vascular permeability 4 hours after sub-cutaneous injection of the test substance. A quantitative index of the permeability of the uterine vasculature was obtained from the leakage from the circulation of radiolabelled albumin (injected into the jugular vein of anaethetised rats) 3.5 hours after the injection of the test substance). The study also assessed whether:

1. administration of the anti-oestrogen ICI 182,780 inhibited oestrogen-induced increases in uterine permeability;

2. low doses of 4-*tert* octylphenol (below the level required to induce an oestrogenic response in the assay) could act as anti-oestrogens.

In the study 4-*tert* octylphenol was found to have an ED_{50} (median effective dose to increase uterine vascular permeability by 50%, relative to controls) of approximately 20.6 mg l^{-1} (10^{-4} mole). The potency of 4-*tert* octylphenol was about 100000 x lower than that for oestradiol.

When ovariectomised mice were subjected to daily injections of a potent anti-oestrogen (ICI 182,780) for 4 days before a single injection of 10^{-4} M 4-*tert* octylphenol (1hour after the last injection of the anti-oestrogen) the anti-oestrogen suppressed the vascular responses. When ovariectomised mice were injected sub-cutaneously with oestrogen (10^{-10} M) together with 4-*tert* octylphenol (10^{-6} M) there was no evidence that the test substance produced any inhibitory effect on the oestradiol stimulated increase in vascular permeability. The mean index of vascular permeability ($\pm$ Standard Error) was 12.9 $\pm$ 2.5 µl mg^{-1} for oestradiol at 10^{-10} M, while for the combination of oestradiol and 4-*tert* octylphenol (10^{-6} M) the value was 10.3 $\pm$ 1.5 µl mg^{-1}. The results were taken to indicate that even short-term exposure to 4-*tert* octylphenol can induce typical oestrogenic effects *in vivo*, however, the potency of 4-*tert* octylphenol is very weak even when assessed in an acute response.

Wenzel *et al* (2001) evaluated the effects of 4-*tert* octylphenol on the gonads of two strains of rats (Wistar and Sprague-Dawley) using short-term screening tests. The animals used were ovariectomised and after 14 days of endogenous hormonal decline the animals were treated 3 days post operation by gastric tube. Animals were randomly allocated to control and three treatment groups (5, 50 and 200 mg kg^{-1} body weight^{-1}). The substance was given in total volume of 1ml/animal. Animals were sacrificed by decapitation after light anaesthesia with CO_2 inhalation. Uterus wet weight was determined, and the histological analysis of the uteri of individuals was performed. Uteri were sliced and an Azan stain was performed. The thickness of the uterine epithelium was examined using an image analysis system. Vaginas were also

sliced and a Scott stain was performed before the thickness of the vaginal epithelium was examined using an image analysis system.

In both strains of rats exposure to 4-*tert* octylphenol resulted in a dose-dependent stimulation of the uterine wet weight and increase in the thickness of the uterine epithelium. For both parameters statistically significant increases (relative to the controls) occurred at 200 mg kg body weight^{-1} day^{-1}. There was also a dose-dependent increase in the thickness of the vaginal epithelium in both strains of rats. However, while the increase was statistically significant at 200 mg kg body weight^{-1} day^{-1} in Wistar rats, it was statistically significant at 50 mg kg body weight^{-1} day^{-1} in Sprague-Dawley rats.

B. Reproduction and fertility studies

Blake and Ashiru (1997) investigated the effects of 4-*tert* octylphenol on reproduction in female caesarian derived (CD) (Sprague-Dawley) rats (see table below for details). The study evaluated whether:

1. exposure of neonatal rats interfered with the onset of vaginal opening or their ability to have regular oestrous cycles as adults[1];

2. exposure of adult rats interfered with oestrous cyclicity;

3. exposure of adult rats interfered with ovulation.

Type of study in Blake and Ashiru (1997)	Methodology
Effects of exposure of neonates	I day old pups were injected sub-cutaneously with 10 mg ml^{-1} of 4-

[1] Changes in the onset of vaginal opening and persistent oestrus are responses to exposure to oestrogenic substances

on vaginal opening and oestrous cyclicity	*tert* octylphenol in corn oil. After weaning, female rats were checked daily for vaginal opening. At 40 days of age, 9-11 rats were selected at random in each group to be kept for monitoring of vaginal oestrous cyclicity starting at 3 months after birth. At that time, vaginal smears were prepared and examined daily for 3 weeks.
Effects of exposure of adults on oestrous cyclicity	Starting at 10 weeks of age (2 weeks after arrival) vaginal smears were prepared and examined daily to monitor the oestrous cycle. Rats showing at least two regular 4 day oestrous cycles were injected with 20 or 40 mg 4-*tert* octylphenol in corn oil. The first injection was given without regard to the day of the oestrous cycle. Injections were given three times weekly on Monday, Wednesday and Friday for 2 weeks. Vaginal smears were prepared and examined daily throughout the study. Testing on rats showing persistent oestrous continued for a further three weeks.
Effects of exposure of adults on ovulation	Starting at 10 weeks of age (2 weeks after arrival) vaginal smears were prepared and examined daily to monitor the oestrous cycle. Rats showing at least two regular 4 day oestrous cycles were used on pro-oestrous. One group of five control rats was injected intra-peritoneally with sodium pentobarbital (35 mg kg body weight^{-1}) at 14.00 h and ovulation was blocked in all the rats indicating that sufficient lutenising hormone was not released prior to 14.00 h to induce ovulation. Other rats were injected sub-cutaneously with 0.2 ml of corn oil or 40 mg 4-*tert* octylphenol in corn oil at 13.00, 14.30 and 16.00 h. The remaining rats were injected subcutaneously with 100 mg 4-*tert* octylphenol in 0.1 ml of 30% ethanol at 13.00, 14.30 and 16.00 h. Multiple injections of 4-*tert* octylphenol were given, and administered in the two different vehicles to assess any acute effect on ovulation. The occurrence of ovulation was determined microscopically on excised tissue between 08.00 and 10.00 h the next morning.

Injection of 10 mg ml^{-1} 4-*tert* octylphenol on the day after birth did not affect the day of vaginal opening. However, 9 of the 11 treated rats (81%) were in persistent vaginal oestrous when examined at three months after birth compared with 0 of 9 control (0%), which cycled regularly. Sub-cutaneous injection of 20 or 40 mg 4-*tert* octylphenol three times weekly into previously untreated adult cyclic rats caused persistent oestrous in 2 of 6 (33%) and 16 of 21 (78%) rats respectively. When injections were continued for three more weeks , 5 of the 16 rats rendered persistent oestrous by the 40 mg 4-*tert* octylphenol treatment the rats remained in permanent oestrous for that period. The other 11 persistent oestrous rats in the 40 mg 4-*tert* octylphenol treatment group started to cycle regularly within 5-7 days after the last injection. Injection of 4-*tert* octylphenol into cyclic rats during the afternoon of pro-oestrous did not block ovulation (unlike pentabarbital). These results were taken to provide strong evidence that 4-*tert* octylphenol acts in a similar manner to oestrogen *in vivo* in both neonatal and adult female rats to exert effects that block reproductive cyclicity.

In a series of studies the effects of 4-*tert* octylphenol on sex organs and hormone levels were investigated in male Fischer 344 rats (Blake and Boockfor, 1997; Boockfor and Blake, 1997). The groups of rats were given subcutaneous injections with 4-*tert* octylphenol three times a week for up to two months. The test substance was dissolved in corn oil and the doses were 20 or 80 mg/rat. The dosing equates to about 30 and 160 mg kg^{-1} day^{-1}. Oestradiol valerate was used as a positive control.

The result of each study are summarised in the table below and indicate that two months of exposure to the high-dose of 4-*tert* octylphenol induced a clear depression in final body weight, while the low-dose induced a minor effect (less than 9 %). The rats in the high-dose group had increased relative spleen weights, decreased relative kidney, testis, epididymis, seminal vesicle, and ventral prostate weights. There was no effect on the weight of the pituitary gland. There was no effect on the relative weights in the low-dose group. Weights of other organs were not recorded.

Endpoints in Blake and Boockfor (1997) and Boockfor and Blake (1997)	Percent of control	
	Low-dose (~ 30 mg kg^{-1} day^{-1})	High-dose (~160 mg kg^{-1} day^{-1})
Relative testis weight	101	***22 (P<0.05)***
Relative epididymis weight	95	***27 (P<0.05)***
Relative seminal vesicle (full) weight	90	***10 (P<0.05)***
Relative seminal vesicle (empty) weight	96	***21 (P<0.05)***
Relative ventral prostate weight	97	***53 (P<0.05)***
Luteinizing hormone serum concentration	64[a]	***7 (P<0.05)[a]***
Follicular stimulating hormone serum concentration	95[a]	***50 (P<0.05)[a]***
Prolactin, serum concentration	120[a]	***340 (P<0.05)[a]***
Testosterone, serum concentration	100[a]	***15 (P<0.05)[a]***
Luteinizing hormone, concentration in the pituitary gland	64	***14 (P<0.05)***
Follicular stimulating hormone, concentration in the pituitary gland	76	***22 (P<0.05)***
Prolactin, concentration in the pituitary gland	***182 (P<0.05)***	***349 (P<0.05)***
Sperm heads per gram testis	***75 (P<0.05)[a]***	***5 (P<0.05)[a]***
Sperm heads per gram epididymis	100[a]	**<5 (P<0.05)[a]**
Sperm head abnormalities	140[a]	-
Sperm tail abnormalities	***350 (P<0.05)[a]***	-

[a] estimated from graph

The serum levels of luteinizing hormone, follicular stimulating hormone and testosterone were decreased and the serum prolactin level was increased in the high-dose group[2]. In the low-dose group the luteinizing hormone level was somewhat decreased compared to control but the change was not statistically significant. The number of sperms in the testis decreased in the low and high dose groups in a dose related manner.

The number of sperms in the epididymis was also decreased among the animals in the high-dose group. There were marked histological changes in the testis, epididymis and ventral prostate among the rats in the high-dose group. The testes were reduced in size and showed disruption of normal epithelial organization. No mature spermatozoa or developing spermatids were seen in the high-dose group. Luminal areas were reduced in the epididymis and the epithelium was thicker than normal and appeared stratified with some vacuolation. Few mature germ cells were seen. The ventral prostate showed collapsed glandular lumen or a

[2] In male mammals gonadotropin releasing hormone (GnRH) from the hypothalmic-pituitary axis stimulates pituitary gonadotrophs to secrete follicle stimulating hormone (FSH) and lutenising hormone (LH) . FSH acts on the Sertoli cells to produce androgen-binding globulin. Inhibin has a negative feedback effect on FSH. LH activates Leydig cells to produce testosterone via the cAMP second messenger pathway. Feedback inhibition of GnRH by testosterone regulates this pathway. Prolactin (PRL) has an inhibitory effect on testosterone production if present at high levels.

reduced area. The seminal vesicles had shortened mucosal folds. The qualitative effects seen after 4-*tert* octylphenol exposure was similar to that of the positive control, oestradiol valerate.

RTI (1999) described a two generation reproductive toxicity study (one litter per generation) of 4-*tert* octylphenol administered in feed to CD (Sprague-Dawley) rats. Thirty male and female CD® (Sprague-Dawley) weanling rats (the F_0 generation) per dose were administered 4-*tert* octylphenol in the feed *ad libitum* at 0, 0.2, 20, 200 and 2000 ppm for ten weeks (with the doses used approximating to 0, 0.015, 1.5, 15 and 150 mg kg^{-1} day^{-1}). This study was conducted according to the OPPTS Draft Testing Guidelines (870.3800; 1996 with additions). The selection of the unusual dose range for the study was based on the need to evaluate the potential reproductive toxicity of 4-*tert* octylphenol under standard EPA test guidelines, as well as attempt to replicate published low dose effects (Sharpe *et al* 1995) of 4-*tert* octylphenol in rats. Therefore, additional assessments were added (beyond the guideline requirements) for this study, including dorsal prostate weights in all adult males, and epididymal sperm and testicular spermatid measurements in the F_2 males.

In the study, body weights and feed consumption were recorded weekly, and clinical signs were recorded at least once daily. Vaginal cytology was evaluated for the last three weeks of the prebreed period. Animals were then randomly mated within treatment groups for a two-week mating period to produce the F_1 generation, with exposure continued. F_0 males were necropsied after the delivery period, with histologic evaluation of reproductive and other organs and andrological assessments (reproductive organ weights, epididymal sperm number, mortility and morphology, testicular homogenization-resistant spermatid head counts, daily sperm production, and efficiency of daily sperm production). F_1 litters were culled to ten pups on postnatal day (pnd) 4 and weaned on pnd 21. At weaning, up to three weanlings/sex/litter were necropsied, and 30/sex/dose were selected as F_1 parents of the F_2 generation. F_0 females were then necropsied with organ weights, stage of oestrus at necropsy, enumeration of ovarian follicles, and histopathology of reproductive and other selected organs. Selected F_1 weanlings (30/sex/dose) were administered octylphenol in the diet for a ten-week prebreed exposure period, with acquisition of vaginal patency in females and preputial separation in males assessed, and vaginal cytology for oestrous cyclicity evaluated during the last three weeks. They were then mated for a two-week mating period as described above. At weaning of the F_2 litters, up to three weanlings/sex/litter were necropsied, and 30/sex/dose were selected for retention, with dietary exposure continued through acquisition of vaginal patency for F_2 females. At this time they were terminated without further evaluation, and through acquisition of preputial separation for F_2 males, until age 111$\pm$5 days when F_2 males were subjected to necropsy, histopathology, and andrological assessments (as described above). F_1 males were necropsied after the delivery period, with histopathology and andrological assessments (as described above). After weaning of the F_2 litters, parental F_1 females were then necropsied with histopathology as described above.

Adult systematic toxicity was evident for F_0 and F_1 parental animals and F_2 retained animals at 2000 ppm, expressed as consistent and persistent reductions in body weights and weight gains, during the prebreed (F_0 and F_1) and postwean (F_2) exposure periods. Feed consumption in g day^{-1} and g kg^{-1} day^{-1} and food efficiency were variable. There was no treatment- or dose-related clinical signs of toxicity in either sex in any of the generations. Body weights during gestation were unaffected and were reduced during lactation in F_0 and F_1 females at 2000 ppm. There were no effects of treatment in F_0 or F_1 females on mating, fertility, pregnancy, or gestational indices. There was also no effects of treatment in F_0 or F_1 males on mating or fertility indices. Oestrous cycle length and stage of oestrus at necropsy were equivalent across all groups for F_0 and F_1 females. Gestational length in days was equivalent across all groups for F_0 females and slightly but significantly prolonged at 0.2 ppm

(by 0.3 days), but not at 20, 200, or 2000 ppm for F_1 females. At necropsy, F_0 and F_1 parental and retained F_2 male absolute organ weights were almost uniformly unaffected for the liver, kidneys, adrenal glands, spleen and brain. Relative organ weights exhibited only occasional increases, almost exclusively at 2000 ppm, most likely due to the reduced body weights at this dietary concentration. There was no treatment-related gross or microscopic findings on these organs. Absolute and relative weights of F_1 female reproductive organs (ovaries and uterus) were unaffected by treatment; F_0 absolute and relative uterine weights were significantly reduced at 2000 ppm; F_0 ovarian weights were unaffected. There were no effects of treatment on any adult male reproductive organs, including no effects on absolute or relative weights of testes, epididymides, prostate, dorsal prostate, seminal vesicles with coagulating glands, and no gross or microscopic effects of treatment on these organs. There were also no effects of treatment on epididymal sperm concentration, percent motile or progressively motile sperm, testicular homogenization-resistant spermatid head counts, daily sperm production, efficiency of daily sperm production, or percent abnormal sperm for F_0 and F_1 parental males and F_2 retained adult males.

For F_1 and F_2 offspring, there were no effects of treatment on stillbirth or live birth indices, for number of live pups per litter on pnd 0, sex ratio (% males) throughout lactation, lactational survival index (pnd 4-21), or 4-, 7-, 14- or 21-day survival indices. Pup body weights per litter were significantly reduced at 2000 ppm for F_1 and F_2 litters on pnd 14 and 21. Reduced pup weights were observed only during the latter portion of the lactational period when the pups generally began to self-feed, and, therefore, were likely to be directly exposed to the test chemical in the diet (CD rat pups began to self-feed late in the second week of life). Acquisition of vaginal patency and preputial separation in F_1 and F_2 offspring was significantly delayed (by less than two days) at 2000 ppm, which was most likely related to the lower body weights of these pups at this dose. These minimal effects on reproductive development in the F_1 offspring required (by guideline) measurement of anogenital distance in F_2 offspring at birth (pnd 0). F_2 male pups exhibited no effect of treatment on anogenital distance while F_2 female pups exhibited statistically significantly longer mean anogenital distances at 0.2 ppm (0.79 mm), 20 ppm (0.81 mm), 200 ppm (0.85 mm), and 2000 ppm (0.79 mm) relative to the control group value (0.76 mm). These changes were not considered biologically significant.

In conclusion, dietary exposure to octylphenol for two generations, with one litter per generation, at 0, 0.2, 20, 200, and 2000 ppm, resulted in:

- Decreased body weights and weight gains at 2000 ppm in F_0 and F_1 parental animals and F_2 retained animals during the pre-breed (F_0 and F_1) and post wean (F_2) exposure period;
- Offspring toxicity (reduced body weight in F_0 and F_1 females during lactation) at 2000 ppm;
- Delayed vaginal opening and preputial separation at 2000 ppm in F_1 and F_2 offspring which was considered to be related to body weight decreases;
- No effects on reproductive parameters;
- No effects on testes weights or morphology;
- No effects on epididymal sperm counts, motility, or morphology;
- No effects on daily sperm production or efficiency of daily sperm production;
- No effects on prostate or dorsal prostate weights or histopathology;

- No oestrogen-like effects on males or females.

The NOAELs reported for systematic and postnatal toxicity were 200 ppm (15 mg kg^{-1} day^{-1}) and at or above 2000 ppm (150 mg kg^{-1} day^{-1}) for reproductive toxicity.

C. Developmental and teratogenicity studies

Sharpe *et al* (1995) carried out a series of three studies to assess whether exposure of male rats to 4-*tert* octylphenol during gestation or neonatal life had any adverse effects on testicular size and spermatogenesis when these animals reached adulthood. The design of the studies is summarised in the table below in terms of the test duration, timing of exposure, exposure route and concentrations and endpoints measured. In study 1 the postnatal period (days 1-22 after birth) of Sertoli cell proliferation was evaluated whereas in studies 2 and 3 the complete period of Sertoli cell proliferation was covered. The exposure period for studies 2 and 3 was used to assess the possible effects of bioaccumulation. In all three studies, exposure of male offspring to 4-*tert* octylphenol was largely indirect via the placenta or milk. In study 1 in which there was no prenatal exposure to the test chemical, litters were culled to eight pups on the day of birth (day 1) by culling excess females. The same procedure was carried out in study 2. In study 3, the full litter size was maintained from birth through day 22. The female offspring of test litters were not evaluated. Adult females used for mating in studies 2 and 3 were the same, when offspring of these females were weaned at the completion of study 2, the mothers were maintained on the same treatment for 2 weeks, then mated and exposed until the weaning of study 3 offspring. In study 3 the intake of 4-*tert* octylphenol based on water intake was calculated only for the group exposed to 1000 μg l^{-1} and was reported to range from 129 μg kg body $weight^{-1}$ day^{-1} in the first 2 days after birth to 367 μg kg body $weight^{-1}$ day^{-1} just before weaning.

The results of the studies are summarised in the table below and in study 1 when male offspring were treated postnatally only with 100 and 1000 μg l^{-1} 4-*tert* octylphenol on days 1-22 after birth, there was a small but statistically significant reduction in relative testis weight (that is relative to both body weight or to kidney weight)(see Table 11.5). Kidney weight was increased in animals exposed to the two concentrations of 4-*tert* octylphenol.

In studies 2 and 3 male offspring exposed to the highest exposure dose of 1000 μg l^{-1} showed small but statistically significant decreases (see Table 11.5) in absolute testis weight (by 6 - 12%), the ratio of testis-to-kidney size (by 7 -10%) and relative ventral prostate weight (by 9 - 13%). Absolute prostate weight showed a significant reduction at the lower exposure concentration (100 μg l^{-1}) in both studies, though relative prostate weight was only reduced significantly in study 2. Relative testis weight was only reduced significantly in study 2 at 100 μg l^{-1}. In study 3 testicular morphology was indistinguishable from controls and no abnormalities were seen. Further, the use of image analysis revealed no adverse effects on the cross-sectional parameters of seminiferous tubules. However, daily sperm production was significantly reduced ($P<0.05$) at the highest exposure concentration of 1000 μg l^{-1}. Overall the results were taken to indicate that maternal exposure of male rats to 4-*tert* octylphenol during gestation or neonatal life can result in reduced testicular size and sperm production in adulthood.

Study number in Sharpe *et al* (1995)	Organisms exposed	Period of exposure	Exposure concentrations	Organisms used for measurements	Endpoints measured
1	New born male Wistar rats (1 day after birth)	21 days	0, 10, 100 and 1000 μg l^{-1} 4-*tert* octyl phenol in drinking water daily	Male rats (offspring) of 90 – 95 days of age	Body weight of males at day 22 Body weight, testis weight, kidney weight, testis/kidney weight ratio, relative weights of testis and kidney in males at day 90 - 95
2	Adult female Wistar rats	8-9 weeks (From 2 weeks before mating, throughout mating and gestation and up until 22 days after giving birth)	0, 100 and 1000 μg l^{-1} 4-*tert* octyl phenol in drinking water daily	Male rats (offspring) of 90 – 95 days of age	Litter size, % males at birth, body weight of males at day 22 Body weight, testis weight, kidney weight, ventral prostate weight, testis/kidney weight ratio, relative weights of testis, kidney and ventral prostate in males at day 90 - 95
3	Adult female Wistar rats	16-18 weeks (From 2 weeks before mating throughout 2 mating and gestation periods and up until 22 days after giving birth to the second litter)	0, 100 and 1000 μg l^{-1} 4-*tert* octyl phenol in drinking water daily	Male rats (offspring) of 90 – 95 days of age	Litter size, % males at birth, body weight of males at day 22 Body weight, testis weight, kidney weight, ventral prostate weight, testis/kidney weight ratio, relative weights of testis, kidney and ventral prostate in males at day 90 – 95 Seminifierous tubule area and seminiferous epithelium area, daily sperm production

Parameters measured in Sharpe *et al* (1995)	Percent change relative to the controls (Statistical level of significance where appropriate)						
	Study 1			Study 2		Study 3	
	10 µg l^{-1}	100 µg l^{-1}	1000 µg l^{-1}	100 µg l^{-1}	1000 µg l^{-1}	100 µg l^{-1}	1000 µg l^{-1}
Litter size	No data	No data	No data	108	106	86	88
% Males at birth	No data	No data	No data	87	75	104	116
Birth weight of males at day 22	***108 (P<0.001)***	***108 (P<0.001)***	100	***125 (P<0.001)***	***115 (P<0.001)***	***108 (P<0.05)***	104
Body weight of males at day 90 - 95	101	***110 (P<0.001)***	102	***109 (P<0.001)***	***108 (P<0.001)***	***96 P<0.05)***	99
Testis weight of males at day 90 - 95	99	101	96	101	***94 (P<0.01)***	***94 (P<0.01)***	***87 (P<0.001)***
Kidney weight of males at day 90 - 95	102	***113 (P<0.001)***	***108 (P<0.001)***	102	***104 (P<0.05)***	97	***93 (P<0.01)***
Ventral prostate weight of males at day 90 - 95	No data	No data	No data	***94 (P<0.05)***	***91 (P<0.01)***	***90 (P<0.01)***	***88 (P<0.01)***
Testis/kidney weight ratio	97	***90 (P<0.001)***	***90 (P<0.001)***	99	***90 (P<0.001)***	96	***93 (P<0.01)***
Relative testis weight of males at day 90 - 95	99	***94 (P<0.01)***	***96 (P<0.01)***	***93 (P<0.01)***	***88 (P<0.001)***	98	***87 (P<0.001)***
Relative kidney weight of males at day 90 - 95	103	***104 (P<0.05)***	***107 (P<0.01)***	***94 (P<0.01)***	97	101	***93 (P<0.001)***
Relative ventral prostate weight of males at day 90 - 95	No data	No data	No data	***88 (P<0.05)***	***87 (P<0.01)***	95	***91 (P<0.05)***

In Sharpe *et al* (1998) the authors reported their inability to replicate the findings of study 3 (Sharpe *et al* 1995) at 1000 μg l^{-1}. The paper expressed continued confidence in the findings of the original publication and hypothesised that the inability to replicate the work may have been due to changed but unknown biological factors which had not been controlled. It highlighted the fact that in their laboratory testis weight in control animals had markedly changed in the period 1995 to 1998 (see table below). No clear cause for the decline in testis weight in control seen in 1996 was evident but it did follow a permanent change in water supply to the animal facility.

Test date	Mean testis weights in control animals (± Standard deviation) in Sharpe *et al* (1995, 1996)
1995 - Study 1	1968 ± 163 mg
1995 - Study 2	2014 ± 155 mg
1995 - Study 3	1954 ± 118 mg
1996	1828 ± 121 mg
1998	1956 ± 124 mg

The changes in the control over this period were of the same magnitude to those recorded in studies 1-3 (Sharpe *et al* 1995). This finding emphasises the importance of defining the normal background variability of a particular endpoint so that any chemical-induced changes can be assessed in the light of these changes. Consequently there has to be some concern regarding the significance of the data from Sharpe *et al* 1995.

Nagel *et al* (1997) studied the effect of dietary exposure to 4-*tert* octylphenol on the male offspring of pregnant mice. The study involved feeding pregnant mice 2 and 20 μg kg body weight^{-1} day^{-1} once daily on days 11-17 of gestation. This time in pregnancy was chosen to expose the foetuses to 4-*tert* octylphenol throughout the prenatal period of sexual differentiation and during the initial differentiation of the prostate. The last dose was administered on gestation day 17 to reduce the possibility of interfering with normal parturition Prostates were subsequently collected from 6 month old males. In the study, prostates from males exposed prenatally to either of the doses of 4-*tert* octylphenol (2 or 20 μg kg body weight^{-1} day^{-1}) were not significantly different in weight from the prostates from control animals. Adjusting prostate weight for body weight did not alter the results with regard to group differences in prostate weight.

Bicknell *et al* (1995) investigated the effect of 4-*tert* octylphenol on the sexually dimorphic nucleus in the preoptic area in male rats, since the development and cytoarchitecture of the sexually dimorphic nucleus in rats are dependent on perinatal oestrogen levels and high doses of oestrogenic chemicals have been shown to interfere with normal parturition (vom Saal *et al* 1995). On each of the four last days of gestation, pregnant rats received sub-cutaneous injections of 40 mg 4-*tert* octylphenol. After delivery, all pups received daily injections on postnatal day 1 to 4 (2 mg 4-*tert* octylphenol). At 60 days of age 6 male and 6 female rats were killed and the whole brains were removed. The area of the sexually dimorphic nucleus section was measured. The 4-*tert* octylphenol treatment had no effect on the sexually dimorphic nucleus area in either males or females. The absence of effects on the sexually dimorphic nucleus in the study could possibly be due to the low dose used or that 4-*tert* octylphenol does not pass the blood-brain barrier.

Table 11.5 Summary of data on potential endocrine mediated responses in laboratory mammals following oral exposure

Species	Life stage of test organism	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Rat (Wistar)	I day old males	Indirect via 10, 100 and 1000 μg l^{-1} in drinking water of females between days 1 and 22 after birth	Significant decrease (relative to the controls) in testis/kidney weight ratio at day 90-95 after 21 days exposure at 100 μg l^{-1}, with a NOEL of 10 μg l^{-1} Significant decrease (relative to the controls) in relative testis weight at day 90-95 after 21 days exposure at 100 μg l^{-1}, with a NOEL of 10 μg l^{-1}	Sharpe *et al* (1995)	Medium	Use with care
	Males at conception	Indirect via 100 and 1000 μg l^{-1} in drinking water of females from 2 weeks before mating to day 22 after birth (8-9 weeks)	Significant decrease (relative to controls) in absolute testis weight at day 90 - 95 after 8-9 weeks exposure at 1000 μg l^{-1}, with a NOEL of 100 μg l^{-1} Significant decrease (relative to the controls) in testis/kidney weight ratio at day 90 - 95 after 8-9 weeks exposure at 1000 μg l^{-1}, with a NOEL of 100 μg l^{-1} Significant decrease (relative to the controls) in relative testis weight at day 90 - 95 after 8-9 weeks exposure at 100 and 1000 μg l^{-1} Significant decrease (relative to the controls) in absolute and relative ventral prostate weight at day 90 - 95 after 8-9 weeks exposure at 100 and 1000 μg l^{-1}	Sharpe *et al* (1995)	High	Use with care
	Males at conception	Indirect via 100 and 1000 μg l^{-1} in drinking water of females from 2 weeks before mating to day 22 after birth (16-18 weeks)	Significant decrease (relative to the controls) in absolute testis weight at day 90 - 95 after 16-18 weeks exposure at 100 and 1000 μg l^{-1} Significant decrease (relative to the controls) in absolute ventral prostate weight at day 90 - 95 after 16-18 weeks exposure at 100 and 1000 μg l^{-1} Significant decrease (relative to the controls) in testis/kidney weight ratio at day 90 - 95 after 16-18 weeks exposure at 1000 μg l^{-1}, with a NOEL of 100 μg l^{-1} Significant decrease (relative to the controls) in relative testis weight at day 90 - 95 after 16-18 weeks exposure at 1000 μg l^{-1}, with a NOEL of 100 μg l^{-1}	Sharpe *et al* (1995)	High	Use with care

Table 11.5 Continued

Species	Life stage of test organism	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Rat (Wistar)	Males at conception	Indirect via 100 and 1000 μg l^{-1} in drinking water of females from 2 weeks before mating to day 22 after birth (16-18 weeks)	Significant decrease (relative to the controls) in relative ventral prostate weight at day 90 - 95 after 16-18 weeks exposure at 1000 μg l^{-1}, with a NOEL of 100 μg l^{-1} No significant effect (relative to controls) in testicular morphology or abnormalities at day 90 - 95 after 8-9 weeks exposure at 100 and 1000 μg l^{-1} Significant decrease (relative to the controls) in daily sperm production at day 90 - 95 after 16-18 weeks exposure at 1000 μg l^{-1}	Sharpe *et al* (1995)	High	Use with care
Mice	Male foetuses	Dietary exposure of pregnant mice to 0.002 and 0.02 mg kg bw^{-1} day^{-1} on gestation days 11 - 17	No significant effect (relative to the controls) in absolute or relative prostate weight after 6 days exposure at 0.002 and 0.02 mg kg bw^{-1} day^{-1}	Nagel *et al* (1996)	Medium	Use with care
Rat (CD – Sprague-Dawley)	Male and female weanlings of F_0, F_1 and F_2 generation	Dietary exposure of F_0, F_1 and F_2 organisms to 0, 0.2, 20, 200 and 2000 ppm *ad libitum* for 10 weeks	No significant effect (relative to the controls) on reproductive parameters at any test dose No significant effect (relative to the controls) on testis weight or morphology at any test dose No significant effect (relative to the controls) on epididymal sperm count, motility or morphology at any test dose No significant effect (relative to the controls) on daily sperm production testis weight or efficiency of daily sperm production at any test dose No significant effect (relative to the controls) on prostate or dorsal prostate weight or histopathology at any test dose Significant delays (relative to the controls) in vaginal opening and preputial separation at 2000 ppm, with a NOEL of 200 ppm	RTI (1999)	High	Valid

Table 11.6 Summary of the data on potential endocrine mediated responses in laboratory mammals following sub-cutaneous injection

Species	Life stage of	Exposure route and	Description of endocrine disruption measurement	Reference	Test	Study

	test organism	concentration series	parameter(s) and effect concentrations		Relevance	Validity
Rat (Wistar)	Young females	Sub-cutaneous injections of 10 mg kg^{-1} 4-*tert* octylphenol on three consecutive days	Significant increase (relative to the controls) in the weight of uteri after exposure to 10 mg kg^{-1} on three consecutive days	Bicknell *et al* 1995	Medium	Use with care
Rat	11 day old foetuses	Maternal exposure to 600 mg 4-*tert* octylphenol on day 11 and 15 of gestation	Reduction in 17α-hydroxylase activity	Majdic *et al* 1996	Medium	Use with care
Rat	I day old pups	Sub-cutaneous injection of 10 mg kg^{-1} 4-*tert* octylphenol	No significant effect (relative to controls) on the day of vaginal opening after exposure to 10 mg kg^{-1} Significant increase (relative to controls) in proportion of female rats in persistent oestrous after exposure to 10 mg kg^{-1}	Blake and Ashiru 1997	Medium	Use with care
	Adult cyclic female rats	Sub-cutaneous injections of 20 and 40 mg 4-*tert* octylphenol three times weekly for 2 weeks	Significant increase (relative to controls) in the proportion of rats in permanent oestrous	Blake and Ashiru 1997	Medium	Valid

Table 11.6 Continued

Species	Life stage of	Exposure route	Description of endocrine disruption measurement	Reference	Test	Study

	test organism	and concentration series	parameter(s) and effect concentrations		Relevance	Validity
Rat (Male Fischer 344)	Adult	Three times a week sub-cutaneous injections of 20 mg per rat (30 mg kg^{-1} day^{-1}) or 80 mg per rat (160 mg kg_{-1} day^{-1}) for 2 months	Significant decrease in relative testis weight at 160 mg kg^{-1} day^{-1} Significant decrease in relative epididymis weight at 160 mg kg^{-1} day^{-1} Significant decrease in relative seminal vesicle (full) weight at 160 mg kg^{-1} day^{-1} Significant decrease in relative seminal vesicle (empty) weight at 160 mg kg^{-1} day^{-1} Significant decrease in relative ventral prostate weight at 160 mg kg^{-1} day^{-1} Significant decrease in lutenizing hormone concentration in the serum and pituitary gland at 160 mg kg^{-1} day^{-1} Significant decrease in follicular stimulating hormone concentration in the serum and pituitary gland at 160 mg kg^{-1} day^{-1} Significant decrease in follicular stimulating hormone concentration in the serum and pituitary gland at 160 mg kg^{-1} day^{-1} Significant increase in prolactin serum concentration at 160 mg kg^{-1} day^{-1} and prolactin pituitary gland concentration at 30 mg kg^{-1} day^{-1} Significant decrease in testosterone serum concentration at 160 mg kg^{-1} day^{-1} Significant decrease in sperm head per gram testis at 30 mg kg^{-1} day^{-1} Significant decrease in sperm head per gram epididymus at 30 mg kg^{-1} day^{-1} Significant increase in sperm head abnormalities at 30 mg kg^{-1} day^{-1} Significant increase in sperm tail abnormalites at 30 mg kg^{-1} day^{-1}	Blake and Boockfor 1997, Boockfor and Blake 1997	Medium	Valid

D. Carcingenicity and oncogenicity studies

No data from carcinogenicity and oncogenicity studies has been found in SIDS (1994) and IUCLID (2000) .

E. General conclusions on the endocrine mediated responses to 4-tert octylphenol in laboratory mammals in vivo studies

A series of studies of differing quality in rats and mice have assessed the effects of 4-*tert* octylphenol on reproductive and developmental parameters following oral exposure. Other studies have also assessed the effects of 4-*tert* octylphenol on these parameters following sub-cutaneous injections.

A definitive two generation reproduction study in rats using oral exposure (see Table 11.7) provided no evidence for effects of 4-tert octylphenol on reproductive or developmental endpoints or the histology of endocrine glands or hormone sensitive organs which may be endocrine mediated. This absence of effects occurred even in the presence of high dose (2000ppm or 150 mg kg^{-1} day^{-1}) toxicity of parental animals. Exposure of pregnant rats to 4-*tert* octylphenol at doses of 0.002 and 0.02 mg kg^{-1} during the period of organogenesis does not induce any embryo or foetotoxicity or malformations.

Following sub-cutaneous injections of doses of 4-tert octylphenol of 30 mg kg^{-1} body $weight^{-1}$, histopathological effects have been found on the testis and hormone levels in male and female rats, but these were did not coincide with changes in hormone levels which were only measured at a higher dose (30 mg kg^{-1} body $weight^{-1}$). The histological changes were statistically significant but the longer-term biological consequences of these effects is not certain given the absence of such responses in the two generation study in rats.

Table 11.7 Summary of the potential endocrine mediated responses reported in valid *in vivo* studies with laboratory mammals

Type of study	Species and exposure route used	Dose series used	NOEL (mg kg body $weight^{-1}$ day^{-1})		Reference
			Potential endocrine mediated responses	Systemic toxicity	
Subchronic oral toxicity (OECD 408)	Rat (Fischer 344 males) – Sub-cutaneous injection	0, 20 and 80 mg per rat twice weekly (30 and 160 mg kg^{-1} day^{-1})	< 30 (Histopathology)	No data reported	Blake and Boockfor 1997, Boockfor and Blake (1997)
Reproduction – One generation (OECD 415)	No data		-	-	-
Reproduction – Two generation (OECD 416)	Rats (Sprague Dawley) - dietary	0, 0.2, 20, 200 and 2000 ppm (0, 0.015, 1.5, 15 and 150 mg kg^{-1} day^{-1})	150 (2000 ppm) (Reproduction and histopathology)	15 (200 ppm)	RTI (1999)
Development/ teratogenicity (OECD 414)	Mice - dietary	0, 0.002 and 0.02 mg kg^{-1}	0.02 (Foetotoxicity)	No data reported	Nagel *et al* (1996)

The lowest dose tested in the oral exposure studies were 0.0015 - 0.002 mg kg body weight^{-1} day$^{-1.}$ No effects which may be endocrine mediated were evident in mice at a dose of 0.002 mg kg body weight^{-1} ^{l} in Nagel *et al* (1996), while a low dose of 0.0015 mg kg body weight^{-1} in a two generation reproduction study resulted in no effects on reproductive or developmental parameters.

11.4.1.3 Human studies

No information on potential endocrine mediated responses of workers and consumers following exposure to 4-*tert* octylphenol have been identified.

11.4.2 Studies relevant to the assessment of potential endocrine disrupting effects in wildlife

11.4.2.1 In vitro studies

Table 11.8 summarises the results of in vitro studies on 4-tert octylphenol using cells and tissues from aquatic organisms.

Lutz and Kloas (1999) assessed the *in vitro* oestrogenic potency of 4-octylphenol as part of a programme studying the effects of endocrine disruption on the amphibian the African clawed frog *Xenopus laevis*. Competitive displacement effects for oestrogen receptor binding of 4-octylphenol in a liver cytosol fraction were investigated. The IC_{50} value for 4-octylphenol was 78 μM (16068 μg l^{-1}) compared with an IC_{50} value of 42 nM (11.4 μg l^{-1}) for 17β-oestradiol, indicating that 4-octylphenol has a relative potency (compared to 17β-oestradiol) of 0.00054.

Jobling and Sumpter (1993) measured the production of vitellogenin in superfused rainbow trout hepatocytes after exposure to a range of alkylphenolic compounds including 4-*tert* octylphenol. Vitellogenin production in the hepatocytes showed a U shaped response curve with levels increasing at concentrations from 1 μM l^{-1} (206 μg l^{-1}) to 50 μM l^{-1} (10300 μg l^{-1}) and decreasing markedly at 100 μM l^{-1} (20600 μg l^{-1}) due to cytotoxic effects. The ED_{50} for octylphenol was 2.11 μM (434 μg l^{-1}) which compared with the mean ED_{50} value of 0.00181 μM (0.492 μg l^{-1}) for 17β-oestradiol, indicating that 4-*tert* octylphenol has a relative potency *in vitro* (compared to 17β-oestradiol) of 0.0037.

Navas and Segner (2000) also found that VTG production in rainbow trout liver cells exposed to OP was significantly higher than in unexposed control cells at concentrations of 1 μM l^{-1} (206 μg l^{-1}). The data showed a traditional S shaped concentration-response curve with vitellogenin production increasing as the 4-*tert* octylphenol concentration increased.

Monteverdi and Giulio (1999) and Toomey *et al* (1999) also investigated the production of vitellogenin in hepatocytes of channel catfish (*Ictalurus punctalatus*) and brown bullhead catfish (*Americurus nebulosus*) respectively. Significant inceases in VTG production (relative to controls) were evident at 0.01 μM l^{-1} (2.06 μg l^{-1}) for channel catfish hepatocytes and 10 μM l^{-1} (2060 μg l^{-1}) for brown bullhead catfish hepatocytes. In the study with channel catfish hepatocytes a traditional S shaped concentration-response curve was observed with vitellogenin production increasing as the 4-*tert* octylphenol concentration increased. The OP induced VTG concentration (303 + 67 ng VTG ml^{-1}) was approximately 12 fold greater than for nonylphenol (25 + 8 ng VTG ml^{-1}). In contrast, in the study with brown bullhead catfish hepatocytes the concentration-response curve was U shaped with the VTG production increasing in the range 10 – 50 μM l^{-1} (2060 - 10300 μg l^{-1}) and decreasing at 100 μM l^{-1} (20600 μg l^{-1}), probably due to cytotoxic effects.

Although the implications of the induction of vitellogenin for the reproductive function of fish are not yet fully understood, it can be used as a sensitive indicator of exposure of fish to exogenous oestrogens and oestrogen mimics.

In a number of these studies oestrogen antagonists (such as tamoxifen) were added to the cell cultures along with 4-*tert* octylphenol and this combination reduced or eliminated the production of vitellogenin. These findings indicated that the effect of 4-*tert* ocytlphenol was mediated through the oestrogen receptor (White *et al* 1994, Monteverdi and Giulio 1999, Toomey *et al* 1999) and that it is an agonist.

Andreassen and Korsgaard (2000) investigated the effects of 4-*tert*-octylphenol on the estrogen binding activity in cytosolic fractions of hepatic extracts from female eelpout (*Zoarces viviparous*). The specificity of the E_2 binding sites in cytosolic extracts was examined by inhibition studies. Each ligand was tested for its ability to compete with 5 nm ^3H-E_2 for the binding sites. The binding was specific to oestrogens but 4-*tert* octylphenol only inhibitied binding at high ligand concentrations.

Recently the Chemicals Evaluation and Research Institute in Japan (CERI 2001) have developed a competitive binding assay for the medaka (*Oryzias latipes*) oestrogen receptor α and has used the assay to measure the relative binding affinity of 4-octylphenol (and other alkylphenols). Linear 4-octylphenol had a relative binding affinity of 0.077 compared to 100 for 17β-oestradiol. However, alkylphenols with branched chains exhibited relatively higher affinities, and branched 4-octyphenol had the highest RBA value (16) of the alkylphenols tested in the study.

11.4.2.2 In vivo studies

A. Studies on aquatic organisms

Information on the endocrine disrupting effects of 4-*tert* octylphenol on aquatic organisms is summarised in Table 11.9 and 11.10.

In vivo studies in amphibians

Kloas *et al* (1999) investigated the potential oestrogenic effects of a range of industrial chemicals (including 4-*tert* octylphenol) in *in vivo* (and *in vitro*) assays using the amphibian the African clawed frog *Xenopus laevis*. Larvae at developmental stage 38/40 (2-3 days after hatching) were exposed to 2.1 and 21.0 $\mu g\ l^{-1}$ 4-*tert* octylphenol (nominal concentrations) for approximately 12 weeks at which time metamorphosis was accomplished in approximately 90% of animals. Replacement of test solutions occurred three times weekly and at the end of the exposure period the extent of differentiation into male and females was established. The control groups consisted of approximately 60% males and 40% females whereas the two 4-*tert* octylphenol treatments both resulted in significant changes in the sex ratio with an increased proportion of females (approximately 35% males and 65% females in both cases). Exposure to a 17β-oestradiol concentration of 27.2 $\mu g\ l^{-1}$ resulted in the sex ratio being skewed to 5% males and 95% females while a concentration of 2.7 $\mu g\ l^{-1}$ resulted in a sex ratio of 30% males and 70% females.

Table 11.8 Summary of *in vitro* studies on 4-tert octylphenol using cells and tissues of aquatic organisms

Cell type	Exposure series	Endocrine disruption measurement parameter(s)		Potency (relative to 17β oestradiol = 100)	Reference
		Description of effect	Concentration-response type		
African clawed frog (*Xenopus laevis)* liver cytosol fraction	10^{-3} to 10^{3} μM (0.21 - 206000 μg l^{-1})	Significant decrease in competitive displacement of [^{3}H]oestradiol binding at 10 μM (2060 μg l^{-1})	Decreasing S shaped	0.00054	Lutz and Kloas (1999)
Rainbow trout (*Oncorhynchus mykiss*) hepatocytes	10^{-1} to 10^{2} μM (20.6- 20600 μg l^{-1})	Significant increase (relative to controls) in vitellogenin after 96h exposure at 10 μM (2060 μg l^{-1})	-	0.0037	Jobling and Sumpter (1993)
Rainbow trout (*Oncorhynchus mykiss*) hepatocytes	10^{-1} to 10^{2} μM (20.6- 20600 μg l^{-1})	Significant increase (relative to controls) in vitellogenin at 0.1μM (20.6 μg l^{-1})	Increasing S shaped	No data	White *et al* (1994)
Rainbow trout (*Oncorhynchus mykiss*) liver cells	10^{-3} to 10^{1} μM (0.21 - 2060 μg l^{-1})	Significant increase (relative to controls) in vitellogenin synthesis after 72h exposure at 1 μM (206 μg l^{-1}) No significant effect on basal EROD activity after 72h exposure at any exposure concentration	Increasing S shaped None	No data No data	Navas and Segner (2000)
Channel catfish (*Ictalurus punctalatus*) primary hepatocytes	10^{-2} to 10^{1} μM (2.1- 2060 μg l^{-1})	Significant increase (relative to controls) in vitellogenin synthesis after exposure at 0.01 μM (2.06 μg l^{-1})	No data given	No data	Monteverdi and Giulio (1999)
Brown bullhead catfish (*Americurus nebulosus)* hepatocytes	10, 25, 50 and 100 μM (2060, 5650, 10300 and 20600 μg l^{-1})	Significant increase (relative to controls) in vitellogenin synthesis after 24 h exposure at 10 μM (2060 μg l^{-1}) Significant increase (relative to controls) in the disruption of the membranes of hepatocytes after 24 h exposure at 100 μM (20600 μg l^{-1}) Significant increase (relative to controls) in apoptotic cell death after 24 h exposure at 100 μM (20600 μg l^{-1})	No data given No data given No data given	No data No data No data	Toomey *et al* (1999)
Eelpout (*Zoarces viviparous*) hepatic cytosolic extracts	10^{-5} to 10^{2} μM (0.0021 - 2060 μg l^{-1})	Significant decrease in binding affinity to oestrogen binding sites as concentration of competitor inhibiting specific binding of [^{3}H] oestradiol at 5.9 μM (1215 μg l^{-1})	Decreasing S shaped	0.0011	Andreassen and Korsgaard (2000)

***In vivo* studies in fish (Aqueous exposure)**

Concern has been raised with regard to the potential oestrogenic effects of alkylphenols and their ethoxylates because of a number of recent studies which have demonstrated oestrogenic responses in fish. A range of different types of *in vivo* endpoints in fish have been considered including:

- biochemical changes (for example the production of proteins)
- histopathological changes in cells and tissues
- changes in reproductive activity and sexual development (for example sex ratios of offspring)
- changes in behaviour particularly in relation to sexual reproduction.

Biochemical changes

One of the key functions of endogenous oestrogens in fish is to stimulate the induction in the liver of a large phospholipoprotein, vitellogenin (VTG) (Chen 1983) which is released into the blood stream and sequestered by developing oocytes for production of egg yolk (Wallace 1985, Tyler *et al* 1988, Tyler 1991). In maturing female fish, vitellogenin is a major constituent of the blood proteins, while in male fish it is not normally present in appreciable amounts. However, if male fish are exposed to oestrogens or oestrogen mimics, vitellogenin can be produced at similar levels to that found in maturing females.

There is a degree of uncertainty as to the potential ecological relevance of the induction of vitellogenin in fish. Evidence from laboratory, semi-field and field studies carried out on fish exposed to natural and synthetic steroids in aquatic systems in Europe (CSTEE 1999, NRC 1999, Cheek *et al* 2001) has shown that VTG induction in male fish is a biomarker for exposure to oestrogens and oestrogen mimics and that:

- induction in early life stage fish could have serious energetic consequences for the organisms;
- high levels of vitellogenin induction in fish are known to cause kidney failure and are associated with some haematological disturbances;
- a weak, but nevertheless significant correlation, has been shown between VTG induction in wild roach (*Rutilus rutilus*) and the severity of the intersex condition in fish (that is male gonads show evidence of feminisation).

In *in vivo* systems 4-*tert* octylphenol has been shown to be capable of binding to the oestrogen receptor resulting in the induction of vitellogenin. In whole organisms exposure of fish to 4-tert octylphenol in the aqueous phase (see Table 4.5) has been shown to result in the induction of vitellogenin in juveniles and adult males of a range of species including rainbow trout (*Oncorhynchus mykiss)* (Ashfield *et al* 1995, Jobling *et al* 1996, Routledge *et al* 1998, Pedersen *et al* 1999) roach (*Rutilus rutilus)* (Routledge *et al* 1998) and eelpout (*Zoarces vivaparous*).

The lowest aqueous exposure concentration of 4-tert octylphenol which have been shown to result in a statistically significant induction of vitellogenin in males (relative to controls) is 4.8 -

10 μg l^{-1} in rainbow trout (Jobling *et al* 1996, Routledge *et al* 1998), with a corresponding NOEC value of 1.6 μg l^{-1}. A higher exposure concentration of 100 μg 4-tert octylphenol l^{-1} was required to elicit VTG production in roach (Routledge *et al* 1998). The data indicate that there are inter-species differences in the sensitivity of fish in terms of the levels of 4-tert octylphenol required to elicit VTG induction.

Histopathological changes

A number of studies have investigated whether exposure to 4-*tert* octylphenol can result in changes in the structure of tissues in both male and female fish.

Jobling *et al* (1996) reported that there was significant inhibition of testicular growth (as measured by the gonadosomatic index) in rainbow trout exposed to a single measured 4-*tert* octylphenol concentration of 39 μg l^{-1} for three weeks during sexual development. Exposure of sexually maturing fish to 39 μg l^{-1} 4-*tert* octylphenol also altered the histology of testes relative to controls indicative of inhibition of spermatogenesis (Jobling *et al* 1996). However, in a concentration-response study (0.3 to 43.9 μg l^{-1}) no fish exposed to 4-*tert* octylphenol displayed any significant differences in gonadal size. No clear mechanism could be attributed to the potential inhibition of testicular growth by oestrogenic substances.

Ashfield *et al* (1998) investigated the effects of 4-*tert* octylphenol on the ovosomatic index of female rainbow trout exposed from immediately post-hatch. In the study fish were exposed to nominal concentrations of 1, 10 and 30 μg l^{-1} for 35 days before being exposed to clean water for 431 days. The ovosomatic index [100 x gonad weight/(body weight – gonad weight)] measured in the OP exposed groups of fish after 466 days was not significantly different from that measured in the controls.

Changes in reproductive success and development

Gray *et al* (1999a) investigated the effects of 4-*tert* octylphenol (and 17β-oestradiol) on the sexual differentiation and development of Japanese medaka (*Oryzias latipes*) in a series of studies:

Study 1: Medaka were exposed to a nominal concentration of 100 μg l^{-1} beginning at 1, 3, 5, 7 21 and 35 days post hatch (dph) and continuing to 100 dph

Study 2: Medaka were exposed to a nominal concentration of 100 μg l^{-1} beginning at 1 dph and continuing until 1, 2 and 3 months post hatch. All exposed fish were sacrificed at 3 months post hatch.

Study 3: Adult male medaka (approximately 11 months old) were exposed to nominal concentrations of 200 and 300 μg l^{-1} for periods of 18 and 36 days.

In each study weight, lengths and sex ratios of male and female medaka were measured and histological assessments of the gonads were made. It was established in an associated study that fish were probably exposed to only around 50-60% of the nominal 4-*tert* octylphenol concentrations. A 100 μg l^{-1} 17β-oestradiol control was used in all the studies.

In male medaka exposed to a single nominal concentration of 100 μg l^{-1} beginning at 1, 3, 7, 21 and 35 days posthatch (study 1), the incidence of testis-ova (an intersex condition) at 100 day posthatch was highest (and statistically significant) in the 3 day posthatch treatment (29%) and declined when exposures were initiated with older fry. In study 2 exposure to

100 μg l^{-1} 4-*tert* octylphenol from hatch for a period of 1 or 2 months did not induce testis-ova, but exposure for 3 months resulted in 6% of males developing this condition. In study 3 exposures of adult male medaka to 200 and 300 μg l^{-1} 4-*tert* octylphenol for either 18 or 36 days resulted in only 17% of male fish developing testis-ova in the 36 day exposure to 300 μg l^{-1} treatment. Overall, these data indicate that only prolonged exposure of male medaka to beginning around the period of gonadal differentiation resulted in statistically significant levels of testis-ova development (Gray *et al* 1999a). None of the sex ratios for the 4-*tert* octylphenol exposed groups was significantly different from the sex ratios of the control groups. No consistent concentration-response relationship was observed between exposure to 4-*tert* octylphenol and the mean weight and length of the treated fish.

Gray *et al* (1999b) attempted to investigate some aspects of reproductive success; these included courtship behaviour, fertilisation rates, development and survival of offspring (Gray *et al*, 1999b). In this study all of the male fish were exposed, however the females were divided into exposed and unexposed groups. The nominal exposure concentrations were 10, 25, 50 and 100 μg l^{-1}, although it was estimated that the fish were exposed to only around 70% of the nominal concentration. Exposure took place from 1 day post-hatch until 6 months which covered early sexual differentiation and development through to maturity.

The results of this study appeared to show some reduction in courtship intensity in the males following exposure to 4-*tert* octyphenol. Eggs produced by unexposed females from reproduction trails and solvent exposed control females from the general exposures were equally likely to be fertilised. However, male exposure to 4-*tert* octylphenol did influence fertilisation rates and within the trails eggs produced by unexposed females that were mated with males from the 10, 50 and 100 μg l^{-1} treatments (but not the 25 μg l^{-1} treatment) were signficantly less likely to be fertilised than those resulting from copulations with control males. In addition, eggs produced by exposed females mated with males from the 10, 25 and 100 μg l^{-1} treatments (but not the 50 μg l^{-1} treatment) were less likely to be fertilised than those resulting from copulations with control males. Several developmental problems were observed in the medaka embryos that failed to hatch, and to a lesser extent, in the young fry after hatch. Significant increases in the developmental problems (relative to the controls) of embryos and larvae were produced in:

- unexposed females mating with exposed males in the 10 and 25 μg l^{-1} treatments (but not the 50 and 100 μg l^{-1} treatments)

- exposed females mating with exposed males in the 10 μg l^{-1} treatments (but not the 25, 50 and 100 μg l^{-1} treatments)

Gray *et al* (1999b) concluded that "*exposure to OP during early development through to maturity negatively affected the reproductive performance of male medaka as a result of reductions in courtship intensity and fertilisation rates. Also indications were present of trans-generational effects after exposure of parents to OP*". However, as with all of the effects observed in this study, there was no real concentration-dependence in the results both with increasing level within a test group type, and also across types, and therefore there is considerable doubt in the true significance of the reported values. Some developmental abnormalities were recorded in the eggs and fry of both exposed and unexposed females which were fertilised by exposed males, however only in the lower concentrations of 4-*tert* octylphenol. There was no obvious reason why these should have occurred at low doses and not at the higher levels. Finally, it was noted with interest that one male fish with the intersex condition was able to fertilise eggs, thus indicating that intersex gonadal development may not necessarily prevent reproduction.

Gronen *et al* (1999) investigated the effects of 4-*tert* octylphenol on reproductive impairment in adult male japanese medaka (*Oryzias latipes*) and the link to induction of vitellogenin. Organisms were exposed to nominal concentrations of 20, 50, 100 and 300 μg l^{-1} for 21 days, with chemical analysis showing actual exposure concentrations of 20, 41, 74 and 230 μg l^{-1}. At the end of the exposure period measurements were made of serum vitellogenin concentrations. Exposed males from each treatment were then mated in the absence of 4-*tert* octylphenol with unexposed females and eggs were collected daily for 9 consecutive days beginning 2 days after cessation of 4-*tert* octylphenol exposure. Eggs were counted and evaluated microscopically to determine percent fertilisation. Groups of viable eggs in each treatment were transferred to hatching systems and assessed daily for abnormal development, survival and hatching success. Following final egg collection males from each treatment were tested for serum VTG before histopathology of the gonads was performed.

The serum VTG levels in male fish after 21 days exposure increased with increasing 4-*tert* octylphenol concentrations but decreased after exposure was discontinued. Breeding groups composed of exposed males and control females produced about 50% fewer eggs than control groups. Significant correlations were observed between VTG levels in exposed male fish and percent of fertilised eggs and survival of embryos, with the result that 4-*tert* octylphenol induced VTG synthesis and reproductive impairment appearing to be closely linked phenomena. Histological examination indicated that spermatogenesis in 4-*tert* ocylphenol exposed fish was inhibited, and some exposed fish had oocytes in their testis. Finally, 4-*tert* octylphenol caused a significant increase in the number of abnormally developing embryos at all test concentrations, suggesting that 4-tert octylphenol may be genotoxic as well as oestrogenic.

Toft and Baatrup (2001) assessed the effects on the sexual characteristics of adult male guppies (*Poecilia reticulata*) of short-term exposure of to 4-*tert* octylphenol (and 17β-oestradiol). In the study groups of fish were initially exposed to nominal 4-*tert* octylphenol concentrations of 100, 300 and 900 μg l^{-1} for 30 days. The actual measured concentrations were found to be within 14% of the nominal concentrations in all cases. Effects on male sperm count, body colouration index (the proportion of body area covered by characteristic orange spots) and gonopodial length were measured at the end of the exposure period. After carrying out the analyses the surviving animals from the 300 and 900 μg l^{-1} treatments were then divided into two groups:

- One group was exposed to 100 and 300 μg l^{-1} 4-*tert* octylphenol for a further 30 days when repeat measurements of male sperm count, body colouration index and gonopodial length were made.

- Animals from the 300 and 900 μg l^{-1} treatments were allowed to mate with virgin females for 24 hours after which time they were removed and the resulting offspring counted during a 4 month period after mating.

The 100 and 300 μg l^{-1} treatments (but not the 900 μg l^{-1} treatment) caused significant increases in the sperm counts after 30 days while only the 300 μg l^{-1} treatment (but not the 100 μg l^{-1} treatment) significantly increased sperm counts after 60 days. Light microscopy examination revealed no obvious effects on sperm cell morphology and motility. Colouration index was significantly decreased in the 300 and 900 μg l^{-1} treatments (but not the 100 μg l^{-1} treatment) after 30 days but only the 300 μg l^{-1} treatment (but not the 100 μg l^{-1} treatment) after 60 days. Significant increases in sperm count and decreases in coloration index were measured after 30 days exposure to a nominal concentration of 1 μg l^{-1} of 17β-oestradiol.

Preliminary results on male reproduction capability indicated that males exposed to the highest exposure concentration of 900 μg l^{-1} for 30 days produced fewer offspring (4.9 per untreated female) compared to untreated males (6.2 per untreated female). However, males exposed to 300 μg l^{-1} produced larger numbers of offspring (8.2 per untreated female). Exposure of males to a nominal concentration of 0.1 μg l^{-1} 17β-oestradiol resulted in unexposed females giving birth to an average of 1.8 young per female corresponding to only 29% of the offspring in the control group.

Wenzel *et al* (2001) have carried out a life cycle test in zebrafish (*Danio rerio*) with 4-*tert* octylphenol in flow-through-facilities. A nominal exposure concentration series of 0, 1.2, 3.7, 11.9 and 38 μg l^{-1} was used in the 185 day study and measured exposure concentrations were confirmed by chemical analysis to be 1.2, 3.2, 12 and 35 μg l^{-1}. The study consisted of three periods and was initiated with 100 fertilised eggs of unexposed zebra fish in each test set (see table below). The first 42 days of the full life cycle test corresponded to the fish early life stage toxicity test (with F_0 generation) in accordance with the OECD Guideline 210 with a reduction in surveillance dates. The fish were then exposed until they reached sexual maturity. Mortality, behavioural abnormalities, growth, time to first spawning, egg production and fertilisation capacities were recorded. The offspring (F_1 generation) were used to conduct a second fish early life stage toxicity test again in accordance with OECD Guideline 210 with a reduction in surveillance dates.

Period in Wenzel *et al* (2001)	Activity	Days after start of test	Key endpoints
1 Fish early life stage Toxicity – F_0 generation	Start with 100 fertilised eggs per vessel	0	
	Hatch	3	Hatching time and rate
		6	Survival rate
		9	
	First transfer	14	Survival rate
	End of F_0 generation study	35-42	Survival rate, length
2 Reproduction	Number of fish equated to 50 per vessel	35-42	
	Juvenile growth	75	Length development
	Sexual maturation	75 onwards	Time to first egg production
	Reproduction	91 – 120	Quantitative determination of daily egg production and fertilisation capacity
	End of exposure of F_0 generation	135	Length, weight, survival rate
3 Fish early life stage Toxicity – F_1 generation	Start with 100 fertilised eggs per vessel, transferred from the vessels of period 2	135	
	Hatch	138	Hatching time and rate
		141	Survival rate
		144	Survival rate
		149	Survival rate
	First transfer	155	
	End of F_1 generation	174	Survival rate, length, weight

In test period 1 there were no observed effects of 4-*tert* octylphenol at the highest concentration on the survival or growth (as mean fish length) of early life stages of zebrafish exposed as eggs from unexposed parental fish.

In period 2 no effects of the any test concentration on mortality was observed between days 38 and 78. However, the following effects were evident in period 2:

- Growth of the fish over the period from day 38 to 78 was significantly reduced at the highest exposure concentration of 35 μg l^{-1} (in controls mean length = 2.0 $\pm$ 0.2 cm compared to 1.9 $\pm$ 0.16 cm at 35 μg l^{-1})

- Time to first spawning was significantly delayed by 3-4 weeks at 35 μg l^{-1} (in controls time to first spawning = 104 to 116 days compared to 132 to 138 days at 35 μg l^{-1})

- The total number of eggs per test female and day was significantly reduced at 35 μg l^{-1} (in controls mean total number of eggs per female and day = 56.6 compared to 11.6 at 35 μg l^{-1})

- The fertilisation capacity (%) and cumulative number of fertilised eggs was significantly reduced at 35 μg l^{-1} (in controls fertilisation capacity = 86.7 % compared to 30.3 % at 35 μg l^{-1})

No effects on the sex ratios of resulting adult fish were evident at any exposure concentration.

In period 3, even at the highest concentration of 35 μg l^{-1}, there was no clear effect on survival and performance of early life stages of zebrafish exposed as fertilised eggs from parental fish exposed during their whole life cycle.

Van den Belt *et al* (2001) also investigated the impact of 4-*tert* octylphenol on reproduction in zebrafish (*Danio rerio*) using spawning and fertilisation success, gonadosomatic index and plasma vitellogenin (VTG) levels as endpoints. Adult male and female zebrafish were exposed under semi-static (daily renewal) conditions to 12.5, 25, 50 and 100 μg l^{-1} for three weeks and analytical confirmation of exposure levels was conducted. The experiments were performed twice with, respectively, five and seven successful breading pairs per treatment group. Five days before the end of the exposure, the males were separated from the females in all treatment groups to allow the females to mature new eggs and to synchronise spawning for the evaluation period. After these 5 days, the exposure to 4-*tert* octylphenol was stopped and individual breeding pairs were formed and maintained in clean tap water. In order to assess the effects on the male reproductive system apart from those on the females, exposed males were paired with non-exposed females and exposed females with non-exposed males. The non-exposed males and females were maintained in clean tap water for 3 weeks in the same way as the exposed fish, including the daily renewal of water and the 5 day separation of breeding males. To evaluate reproduction success after the 3 weeks of exposure, the breeding pairs were kept together for a period of 5 days and the % spawning females and the males with a post-exposure fertilisation above 70% were counted. For each treatment group the mean of % successful spawning females and the mean of % fertile males were calculated. After the 5 days evaluation period plasma was collected for VTG analysis and gonadosomatic indices (ovarian somatic index in females and testis somatic index in males) were measured. To allow improved interpretation of the gonadosomatic index data reference values were obtained from non-exposed fish of both sexes.

In the study no inhibitory effects of 4-*tert* octylphenol on spawning ability were observed at any test concentration. Mean ovarian somatic (OSI) index in females exposed to 25, 50 and 100 μg l^{-1} (but not 12.5 ug l^{-1}) were significantly lower than the reference value for non-exposed fish. However, the macroscopic structure of these ovaries was comparable to that of control females. The OSI of spawning females exposed to 4-*tert* octylphenol was not

significantly different from the reference OSI of spawning females. No significant effects of 4-*tert* octylphenol on male fertilisation success or testis somatic index were measured. No significant increase in VTG concentrations was measured in exposed male and females

Changes in behaviour

Bayley *et al* (1999) investigated the effects of 4-*tert* octylphenol (and 17β-estradiol) on the sexual behaviour of adult male guppies (*Poecilia reticulata*) as part of a study to validate a test system. The sexual display of the male guppy is strongly linked to reproductive success and this preliminary study indicated that 4-tert octylphenol causes a dramatic decrease in the rate and intensity of sexual display at a nominal concentration of 150 $\mu g\ l^{-1}$ (a predicted concentrations of 42 $\mu g\ l^{-1}$ based on a similar exposure study). A nominal concentration of 10 $\mu g\ l^{-1}$ 17β-oestradiol also caused a marked reduction in the rate and intensity of sexual display

***In vivo* studies in fish (Intra-peritoneal injections)**

In vivo data from a series of studies where different fish species were given intra-peritoneal injects of 4-tert octylphenol are available. The environmental relevance of these studies is low but they provide information on whether biochemical, anatomical or physiological changes in fish observed following aqueous exposure are seen when fish are exposed via this alternative route. In the studies intra-peritoneal injections have been shown to elicit induction of vitellogenin in certain studies (Pedersen *et al* 1999, Andreassen and Koorsgaard 2000).

***In vivo* studies in invertebrates**

Limited information is also available on the endocrine disrupting effects of 4-*tert* octylphenol on aquatic invertebrates.

A 21-day life cycle toxicity study was carried out with *Daphnia magna* according to a US EPA procedure under flow-through conditions and according to GLP (IUCLID, 2000). Exposure levels were 37, 62, 120, 230 and 510 $\mu g\ l^{-1}$ based on measured concentrations. Statistical analysis of survival for *Daphnia magna* after a 21 day exposure period indicated that adult survival was significantly different from the controls in the mean measured concentration of 510 $\mu g\ l^{-1}$. All the daphnids died in this highest exposure concentration by day 9 of the study and no reproduction or adult length data were available. Mean young produced per surviving adult per day after 21 days were significantly affected at the exposure levels of 120 and 230 $\mu g\ l^{-1}$, while no effects (relative to the controls) were evident at a NOEC of 62 $\mu g\ l^{-1}$.

In another 21-day *Daphnia magna r*eproduction study a 21-day LOEC of 100 $\mu g\ l^{-1}$ and a NOEC of 30 $\mu g\ l^{-1}$ was obtained based on the juvenile production per surviving adult endpoint (IUCLID, 2000). This study has been classified as 'use with care' because the full study could not be obtained to assess the data, and while it is known that the test was carried out under OECD test criteria and to GLP, the concentrations reported are nominal.

Zou and Fingerman (1997) investigated the effects of octylphenol on the moulting of the freshwater cladoceran *Daphnia magna*. *Daphnia magna* does not change morphology in its adult life cycle and moulting frequency was measured by visually inspecting each animal every 12 hours and recording if moulting had occurred. The test was initiated with <12h old neonates and the organisms were exposed to nominal 4-octylphenol concentrations of 10, 20 and 40 $\mu g\ l^{-1}$ until the fourth instar (day 4-7 of the experiment). 4-Octylphenol did not inhibit the moulting and development at the highest concentration tested (40 $\mu g\ l^{-1}$).

In the fiddler crab (*Uca pugilator*) Zou and Fingerman, (1999a,b) investigated the effects of 4-octylphenol on chitobiase activity after between 3 and 7 days exposure to concentrations of 2000 and 10000 μg l^{-1}. At concentrations up to 10000 μg l^{-1} no significant effects on epidermal chitobiase activity were recorded while 7 days of exposure to 10000 μg l^{-1} significantly inhibited hepatopancreatic chitobiase activity. Because chitobiase is necessary for the partial digestion of the chitinous exoskeleton as part of the moulting process, it was suggested that inhibition of this enzyme by oetsrogenic agents could account for at least some slowing of moulting that occurs when crustaceans are exposed to these agents. However, the concentrations producing these effects were significantly higher (at least an order of magnitude) than those causing effects on more traditional endpoints in other aquatic invertebrates.

Andersen *et al* (2001) investigated the effects of 4-octylphenol on the larval development of the marine copepod *Acartia tonsa*. A semi-static procedure was used covering the period from egg until the time approximately 50% of the larvae in the control had reached the copepodite stage (after approximately 5 days). The EC_{10} and EC_{50} values for the inhibition of naupliar development were 5.2 μg l^{-1} and 13 μg l^{-1} respectively. The corresponding EC_{10} and EC_{50} values for 17β-oestradiol were 370 and 720 μg l^{-1} which indicates that the effect on naupliar development is probably not oestrogenically mediated.

Oehlmann *et al* (2000) investigated the effects of suspected endocrine disrupting chemicals including octylphenol on freshwater (*Marisa cornuarietis*) and marine (*Nucella lapillus*) prosobranch snails. In a series of three studies animals were exposed to octylphenol at nominal concentration ranges between 1 and 100 μg l^{-1}. The studies were:

1. *Marisa cornuarietis* parental generation test in which adult animals of comparable age were exposed to nominal concentrations of 1, 5, 25 and 100 μg l^{-1} for 5 months including a solvent control. Thirty specimens from each group were collected for analysis at the beginning of the experiment and at monthly intervals.

2. *Marisa cornuarietis life cycle test* in which the spawning masses with eggs produced by the adult snails in the solvent control, 1 and 100 μg l^{-1} groups during the parental generation test were further exposed to these nominal concentrations over a period of 12 months until the hatched F_1 specimens were one year old. They reached sexual maturity in their 8^{th} month. Thirty specimens from each group were collected for analysis at an age of 6, 8 and 12 months. Additionally the hatching success of the F_1 generation was recorded.

Nucella lapillus test in which groups of adults were exposed to nominal concentrations of 1, 25 and 100 μg l^{-1} (along with a solvent control) for 3 months. Thirty specimens from each group were collected at the beginning of the experiment and at monthly intervals.

In both experiments with the apple snail *Marisa cornarietis*, octylphenol induced a complex syndrome of alterations in females (referred to as “superfemales”) at the lowest concentration of 1 μg l^{-1}. Affected specimens were characterised by the formation of additional female organs, an enlargement of the accessory pallial sex glands, gross malformations of the pallial oviduct section resulting in an increased female mortality, and a massive stimulation of oocyte and spawning mass production. Exposure to OP resulted in inverted U-type concentration response relationships for egg and spawning mass production. Adult *Nucella* from the field were tested for three months in the laboratory. As in *Marisa*, superfemales with enlarged accessory pallial sex glands and an enhancement of oocyte production were observed. No oviduct malformations were found probably due to species differences in the gross anatomical

structure of the pallial oviduct. A lower percentage of exposed specimens had ripe sperm stored in their vesicula seminalis and additionally male *Nucella* exhibited a reduced length of penis and prostate gland when compared to the control. Because statistically significant effects were observed at the lowest nominal test concentrations (1µg OP l^{-1}), the authors concluded that even lower concentrations may have a negative impact on the snails. The results were taken to show that prosobranchs are sensitive to endocrine disruption at environmentally relevant concentrations and that especially *M. cornuarietis* is a promisong candidate for a future organismic invertebrate model to identify endocrine-mimetic test compounds. However, it needs to be recognised that these studies were not carried out to standard regulatory test protocols and the data needs to be considered in this context.

B. Studies on terrestrial organisms

Only one study of the effects of 4-*tert* octylphenol on terrestrial species has been identified. In the study Millam *et al* (2001) investigated the short-term effects of oral post-hatch exposure of 4-*tert* octylphenol (and oestradiol benzoate) on the reproductive performance of zebrafinches. Chicks were weighed daily and dosed orally according to body mass, once per day, on days of age 5 through 11 with 1 µl g body $mass^{-1}$ of 100 mM 4-*tert* octylphenol (99% purity) dissolved in canola oil resulting in a dose of 100 nmol g body $mass^{-1}$ day^{-1} (20.6 mg kg body $weight^{-1}$ day^{-1}). A 1 µl g body $mass^{-1}$ dose of canola oil alone was used as a control. The 100 nmol g^{-1} dose was selected for testing because it was the lowest oral dose required to induce maximum masculinisation of song nuclei in female finches in an earlier study. Other chicks were exposed to 10 and 100 nmol g-1 doses of oestradiol benzoate.

Table 11.9 Summary of the data on potential endocrine mediating effects in aquatic organisms following exposure though the water column

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Amphibians						
African clawed frog (*Xenopus laevis*)	2-3 day post hatch larvae	Semi-static: 0, 2.1 and 21 µg l^{-1} (Nominal)	Significant increase (relative to the controls) in proportion of females at metamorphosis after 84 days exposure to 21 µg l^{-1} with a NOEC of 2.1 µg l^{-1}	Kloas *et al* (1999)	Medium	Use with care
Fish						
Rainbow trout (*Oncorhynchus mykiss*)	Adults - males (2 yr old)	Flow-through: 0 and 38.5 µg l^{-1} only (Measured)	Significant increase (relative to the controls) in plasma vitellogenin levels after 21 days exposure at 38.5 µg l^{-1} Significant decrease (relative to controls) in testicular growth after 21 days exposure at 38.5 µg l^{-1} Significant inhibition of spermatogenesis (relative to controls) after 21 days exposure at 38.5 µg l^{-1}	Jobling *et al* 1996	Medium	Use with care
	"	Flow-through: 0,0.3, 0.6, 1.6, 4.8 14.6 and 43.9 µg l^{-1} (Measured)	Significant increase (relative to the controls) in plasma vitellogenin levels after 21 days exposure at 4.8 µg l^{-1} with a NOEC of 1.6 µg l^{-1} No significant effect (relative to the controls)on gonadal size after 21 days exposure at any test concentration	"	Medium	Valid
	Post hatch females	Flow-through: 0, 1.0, 10 and 30 µg l^{-1} (Nominal)	No significant effect (relative to the controls) in ovosomatic index in females after 35 days exposure to any test concentration	Ashfield *et al* (1998)	Medium	Use with care
	Adult - males	Flow-through: 0, 1.0, 10 and 100 µg l^{-1} (Measured)	Significant increase (relative to the controls) in plasma vitellogenin levels after 21 days exposure at 100 µg l^{-1} with a NOEC of 10 µg l^{-1}	Routledge *et al* (1998)	Medium	Valid
	Juveniles (103 – 168 g)	Flow-through: 0 and 41 µg l^{-1} only (Measured)	Significant increase (relative to the controls) in plasma vitellogenin levels after 9 days exposure at 41µg l^{-1}	Pedersen *et al* (1999)	Medium	Use with care

Table 11.9 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Roach (*Rutilus rutilus*)	Adult females	Flow-through: 0, 1.0, 10 and 100 μg l^{-1} (Measured)	No significant effect (relative to the controls) in plasma vitellogenin levels after 21 days exposure at 100 μg l^{-1}	Routledge *et al* (1998)	Medium	Valid
	Adult males	Flow-through: 0, 1.0, 10 and 100 μg l^{-1} (Measured)	Significant increase (relative to the controls) in plasma vitellogenin levels after 21 days exposure at 100 μg l^{-1}	"	Medium	"
Guppy (*Poecilia reticulata*)	Adult males	Flow-through: 0 and 150 μg l^{-1} only (Nominal)	Significant decrease (relative to the controls) in the rate/intensity of sexual display after 28 days exposure at 150 μg l^{-} followed by 10 days in clean water	Bayley *et al* (1999)	Medium	Use with care
	Adult males	Flow-through: 0, 100 and 300 μg l^{-1} only (Measured)	Significant increase (relative to the controls) in sperm count after 30 days exposure at 100 μg l^{-1} Significant increase (relative to controls) in sperm count after 60 days exposure at 300 μg l^{-1} with a NOEC of 100 μg l^{-1}	Toft and Baatrup (2001)	Medium	Valid
	"	Flow-through: 0, 100, 300 and 900 μg l^{-1} (Measured)	Significant decrease (relative to the controls) in colouration index after 30 and 60 days exposure at 300 μg l^{-1} with a NOEC of 100 μg l^{-1} Significant decrease (relative to the controls) in gonadosomatic index after 60 days exposure at 900 μg l^{-1} with a NOEC of 100 μg l^{-1}	"	Medium	"
Japanese medaka (*Oryzias latipes*)	Embryos (1 - 35 days post hatch)	Static renewal: 0 and 100 μg l^{-1} only (Nominal)	Significant increase (relative to the controls) in 3 day post hatch males developing testis ova at 100 days post hatch at 100 μg l^{-1}. No significant effects in 1, 7, 21 and 35 days post hatch organisms at 100 μg l^{-1} No significant effect (relative to the controls) on sex ratio at any exposure scenario	Gray *et al* (1999a)	Medium	Use with care

Table 11.9 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Japanese medaka (*Oryzias latipes*	Embryos (1 day post hatch)	Static renewal: 0 and 100 μg l^{-1} only (Nominal)	No significant effect (relative to the controls) in 1 day post hatch males developing testis ova after 3 months exposure at 100 μg l^{-1} No significant effect (relative to controls) on sex ratio at any exposure scenario	Gray *et al* (1999a)	Medium	Use with care
	Adult males	Static renewal: 0, 10 25, 50 and 100 μg l^{-1} (Nominal)	Significant degeneration (relative to the controls) of testicular tissue after 18 and 36 days exposure at 100 μg l^{-1} No significant effect (relative to the controls) on sex ratio at any exposure scenario	"	Medium	"
	Embryos (1 day post hatch)	Static renewal: 0, 10, 25 and 50 μg l^{-1} (Nominal)	Significant decrease (relative to the controls) in number of approaches of males after 6 months exposure at 50 μg l^{-1} with a NOEC of 25 μg l^{-1} Significant decrease (relative to the controls) in number of circles of males after 6 months exposure at 25 μg l^{-1} with a NOEC of 10 μg l^{-1} Significant decrease (relative to controls) in number of copulations of males after 6 months exposure at 50 μg l^{-1} with a NOEC of 25 μg l^{-1} Significant decrease (relative to controls) in reproductive success (number of males that fertilised eggs as proportion of total males) after 6 months exposure at 25 μg l^{-1} with a NOEC of 10 μg l^{-1}	Gray *et al* (1999b)	Medium	Use with care

Table 11.9 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Japanese medaka (*Oryzias latipes*)	Adult males	Flow through: 20, 41, 74 and 230 μg l^{-1} (Measured)	Significant decrease (relative to controls) in egg production (eggs/day) in unexposed females mated with males exposed for 120 days at 20 μg l^{-1} followed by 9 days in clean water No significant effect (relative to the controls) in fertilisation rate of eggs or percent survival of embryos produced by unexposed females mated with males exposed for 120 days at any test concentration followed by 9 days in clean water Significant increase (relative to controls) in abnormal embryos produced by unexposed females with males exposed for 120 days at 20 μg l^{-1} followed by 9 days in clean water	Gronen *et al* (1999)	High	Valid
Zebrafish *(Danio rerio)*	Fertilised eggs (F_0 generation)	Flow through: 0, 1.2, 3.2, 12 and 35 μg l^{-1} (Measured)	No significant effect (relative to the controls) on survival or growth of fish at any test concentration	Wenzel *et al* (2001)	High	Valid
	38 day old fish	Flow through: 1.2, 3.2, 12 and 35 μg l^{-1} (Measured)	Significant reduction (relative to the controls) in growth of fish at 35 μg l^{-1}, with a NOEC of 12 μg l^{-1} Significant increase (relative to controls) in the time to first spawning of fish at 35 μg l^{-1}, with a NOEC of 12 μg l^{-1} Significant reduction (relative to the controls) in total number of eggs per female and day, fertilisation capacity and cumulative number of fertilised eggs of fish at 35 μg l^{-1}, with a NOEC of 12 μg l^{-1} No significant effect (relative to controls) on sex ratios at any test concentration	"	High	Valid
	Fertilised eggs (F_1 generation)	Flow through: 1.2, 3.2, 12 and 35 μg l^{-1} (Measured)	No significant effect (relative to the controls) on survival or growth of fish at any test concentration	"	High	Valid

Table 11.9 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Zebrafish *(Danio rerio)*	Adult females	Static renewal: 0, 12.5, 25, 50 and 100 μg l^{-1} (Measured)	No significant effect (relative to the controls) on spawning success or plasma vitellogenin concentration at any exposure concentration Significant decrease (relative to controls) in ovarian somatic index at 25 μg l^{-1} with a NOEC of 12.5 μg l^{-1}	Van den Belt (2001)	Medium	Valid
	Adult males	"	No effect (relative to the controls) on male fertilisation success, testis somatic index or plasma vitellogenin concentration at any exposure concentration	"	Medium	Valid
Invertebrates						
Water flea (*Daphnia magna*)	12h old neonates	Static renewal: 0, 10, 20 and 40 μg l^{-1} (Nominal)	No significant effect (relative to controls) on development (reaching 4th instar) at any test concentration after 4-7 days	Zou and Fingerman (1997)	Medium	Use with care
Freshwater prosobranch snail (*Marisa cornuaietis*)	Adults	Static renewal: 0, 1, 5, 25 and 100 μg l^{-1} (Nominal)	Significant increase (relative to the controls) in mortalities of animals after 5 months exposure at 1 μg l^{-1}	Oehlmann *et al* (2000	Medium	Use with care
	Egg masses	Static renewal: 0, 1 and 100 μg l^{-1} (Nominal)	No significant effect (relative to controls) on vas deferens sequence index (VDSI) after 6 and 12 months exposure at either test concentration	"	Medium	Use with care
Marine prosobranch snail (*Nucella lapillus*)	Adults	Static renewal: 0, 1, 25 and 100 μg l^{-1} (Nominal)	Significant increase (relative to controls) in relative numbers of females with oocytes after 2-3 months exposure at 1 μg l^{-1} Significant increase (relative to controls) length of capsule gland and weight of female pallial glands after 3 months exposure at 1 μg l^{-1} Significant increase (relative to controls) length of penis and the prostate gland after 3 months exposure at 1 μg l^{-1}	Oehlmann *et al* (2000)	Medium	Use with care
Copepod (*Acartia tonsa*)	Eggs	Static renewal: Concentration series not given (Measured)	Threshold concentration for effects on naupliar development of 5.2 μg l^{-1} after 5 days exposure	Andersen *et al* (2001)	Medium	Use with care

Table 11.10 Summary of the data on potential endocrine mediating effects in aquatic organisms following exposure though internal injection

Species	Life stage of the test organism	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Study Validity
Rainbow trout (*Oncorhynchus mykiss*)	Juveniles (103 – 168 g)	Intra - peritoneal injection - 50 mg OP kg^{-1}	Significant increase (relative to controls) in plasma vitellogenin levels after 12 days	Pedersen *et al* (1999)	Use with care
	Embryos - eyed (21 days post fertilisation)	Injection - 0.01, 0.1 and 1.0 mg OP kg^{-1}	No significant effect (relative to controls) on sexual development after 6 months	Carlson *et al* (2000)	Valid
Eelpout (*Zoarces vivaparous*)	Adult males	Intra - peritoneal injection - 10 mg OP kg^{-1} on days 2 and 14	Significant increase (relative to controls) in plasma vitellogenesis at 2 and 14 days	Andreassen and Korsgaard (2000)	Valid
Summer flounder (*Paralichthys dentatus*)	Sexually immature juveniles	Intra-sinus injection - 2, 20 and 200 mg OP kg^{-1}	Significant decrease (relative to controls) in gonadosomatic index in males 4, 6 and 8 weeks after injection at 200 mg OP kg^{-1} No significant effect on plasma vitellogenin levels in males 4, 6 and 8 weeks after the first injection at any exposure concentration Significant increase (relative to controls) in plasma 17β-oestradiol in males 4 weeks after first injection at 2 mg OP kg^{-1}. Levels not significantly after 6 and 8 weeks Significant decrease (relative to controls) in plasma testosterone in males 4 weeks after first injection at 200 mg OP kg^{-1}. Levels not significantly after 6 and 8 weeks	Mills *et al* (2001)	Valid

Table 11.10 Continued

Species	Life stage of the test organism	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Study Validity
Summer flounder (*Paralichthys dentatus*)	Sexually immature juveniles	Intra-sinus injection - 2, 20 and 200 mg OP kg^{-1}	No significant effect on testicular size, thickened tubule walls and eosin positive staining cells 4 weeks after first injection at any test dose Significant decrease (relative to controls) in mean testis weight 4 and 6 weeks after first injection at 200 mg OP kg^{-1} No significant effect on testis development 8 weeks after first injection at any test dose	Zarrogian *et al* (2001)	Valid

Finches between 130 and 180 days of age dosed as indicated were introduced into communal cages (five females and five males) or were force paired in individual breeding cages. Each treatments was repeated once for 10 total pairs per treatment. The treatment groups in communal cages included C-treated males and females and 100 nmol g^{-1} treated males and females. A group of C-treated male and females in individual breeding cages were included to test for effects of force pairing. Data were collected over the ensuing 7-week period. The parameters studied were:

- latency in days between the start of the trail and production of the first egg
- number of eggs laid which operationally a clutch (defined as a sequence of eggs with no more than 4 days passing between the laying of successive eggs)
- percentage of eggs candled which were fertile
- percentage of eggs discovered cracked and/or broken
- percentage of eggs that could not be found after once having been identified (missing eggs)
- percentage of dead embryos (shells intact)
- number of hatched chicks

Generally, a particular pair of finches was observed to occupy a nest box and incubate a clutch of eggs. However, because of the possible high incidence of brood parasitism (females were often observed in more than one nest box) the analysis was restricted to the top five producing nest boxes (the number of possible monogamous pairs) and to the first clutches. Top producing nest boxes were identified on the basis of the presence, number of eggs, candled fertility and hatching success.

No statistically significant adverse effects of 4-*tert* octylphenol treatment were detected on any of the test endpoints, relative to the controls. In contrast, the equimolar doses of oestradiol benzoate resulted in marked effects on a number of endpoints and profound disruption of reproduction.

C. Studies on aerial organisms

No data has been located on the potential endocrine disrupting effects of 4-*tert* octylphenol on aerial species. Although 4-tert octylphenol is considered to be volatile the rapid degradation through reaction with hydroxyl radicals means the absence of data is not a major area of uncertainty.

D. General conclusions on potential endocrine mediated responses in in vivo studies with wildlife species

When examining the various end-points from studies investigating endocrine disrupting effects in aquatic, terrestrial and aerial species it is critical to understand their ecological significance. It can be argued that any irreversible physiological or histological change following exposure to a chemical (which is outside of normal background limits) is inherently adverse and should be avoided, especially if it can be linked mechanistically with anatomical or physiological effects. On the other hand, an evidence-based approach may be more appropriate, trying to

protect against demographically relevant effects particularly those related to reproductive activity and sexual development. In terms of protecting the aquatic environment, population sustainability is the ultimate goal and thus, any adverse effect that may provoke population declines is of particular relevance. Hence a key issue must be the ability for male and female of species to breed and produce viable offspring that can develop and which then successfully reproduce themselves.

The lowest NOEC from a valid study assessing the endocrine disrupting effects in fish was 1.6 μg l^{-1} for VTG induction in adult male rainbow trout (*Oncorhynchus mykiss*) after 21 days exposure to 4-t OP (Jobling *et al* 1996). A NOEC of 10 μg l^{-1} for VTG induction in the same species and with the same exposure duration was also reported by Routledge *et al* (1998). In other species higher concentrations of 4-*tert* octylphenol have been required to elicit VTG induction. While induction of vitellogenein is recognised as a valuable biomarker of exposure of fish to oestrogenic substances its relationship with regard to reproductive output and development has not been clearly established.

Wenzel *et al* (2001) in a life cycle study with zebrafish (*Danio rerio*) reported a NOEC of 12 μg l^{-1} (based on measured 4-*tert* octylphenol concentrations) for a range of endpoints affecting the reproductive success of fish which had been exposed to 4-*tert* octylphenol from the fertilised egg stage. The endpoints were: time to first spawning, total number of eggs per female and day, fertilisation capacity and the cumulative number of fertilised eggs and all these parameters were significantly affected at an exposure concentration of 35 μg l^{-1}. A valid study by Van den Bilt (2001) in which adult male and female zebrafish were exposed to 4-*tert* octylphenol for 3 weeks showed no effects on spawning ability even at a concentration of 100 μg l^{-1}. These data whilst initially appearing contradictory indicate that there are probably differences in species sensitivity to 4-*tert* octylphenol in terms of endocrine disrupting effects and that the critical window of exposure for effects on reproduction may be during the early life stage of the fish.

In a valid study on the reproductive impairment in adult male japanese medaka (*Oryzias latipes*) Gronen *et al* (1999) found that breeding groups composed of exposed males (at 20 μg l^{-1} for 3 weeks) and control females produced about 50% fewer eggs than control groups. Significant increases in abnormal embryos produced by unexposed females mated with exposed males (at 20 μg l^{-1}) were also evident. These data indicate that as might be expected there are probably differences in species sensitivity to 4-*tert* octylphenol in terms of endocrine disrupting effects.

In the amphibians a study by Kloas *et al* (1999) with a 'use with care' status reported a NOEC of 2.1 μg l^{-1} for effects on sex ratios in *Xenopus laevis* exposed to 4-*tert* octylphenol for 84 days from 2-3 day old post hatch larvae. However, it needs to be recognised that this study was not carried out to a standard regulatory test protocol and the data needs to be considered in this context.

In the invertebrates a study by Oehlmann *et al* (2000) with a 'use by care status' showed significant effects on the reproductive anatomy of a freshwater (*Marisa cornarietis*) and marine (*Nucella lapillus*) prosobranch snails. In experiments with *Marisa cornarietis* octylphenol induced a complex syndrome of alterations in females (referred to as "superfemales") at the lowest concentration of 1 μg l^{-1}. Affected specimens were characterised by the formation of additional female organs, an enlargement of the accessory pallial sex glands, gross malformations of the pallial oviduct section resulting in an increased female mortality, and a massive stimulation of oocyte and spawning mass production. Exposure to octylphenol resulted in inverted U-type concentration response relationships for egg and spawning mass

production. Adult *Nucella* from the field were tested for three months in the laboratory. As in *Marisa*, superfemales with enlarged accessory pallial sex glands and an enhancement of oocyte production were observed. No oviduct malformations were found probably due to species differences in the gross anatomical structure of the pallial oviduct. A lower percentage of exposed specimens had ripe sperm stored in their vesicula seminalis and additionally male *Nucella* exhibited a reduced length of penis and prostate gland when compared to the control. Because statistically significant effects were observed at the lowest nominal test concentrations (1 μg OP l^{-1}), the authors concluded that even lower concentrations may have a negative impact on the snails.

Overall on the basis of the valid studies the lowest NOECs for endocrine disrupting effects in aquatic organisms would be 12 μg l^{-1} , based on the data from the Wenzel *et al* (2001) study. However, data from studies (of use with care status) in molluscs and amphibians indicate that a lower NOEC, potentially less than 1 μg l^{-1}, may be appropriate.

Table 11.11 summarises the definitive endocrine disruption data obtained for aquatic species in terms of the range of NOEC values and the lowest NOEC value identified. Limited data was identified for terrestrial organisms and no data for aerial species.

11.5 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity

11.5.1 Studies relevant to the assessment of general toxicity in humans

Table 11.12 summarises all of the general toxicity test results found for laboratory mammals exposed to 4-*tert* octylphenol in the SIDS dossier (SIDS 1994) and IUCLID (2000). The following sections focus on studies considered to be valid but discuss studies marked use with care where important issues (such as the timing and duration of exposure) are addressed.

11.5.1.1 Acute studies

A. Oral exposure

The acute oral toxicity of 4-*tert* octylphenol has been investigated in two mammalian studies cited in SIDS (1994) and IUCLID (2000). In a study with Sprague-Dawley Strain rats performed according to OECD Guideline 401 and to GLP the reported LD_{50} value was > 2000 mg kg body $weight^{-1}$. A study with mice where no details are given of the protocol used reported an LD_{50} value of 3210 mg kg body $weight^{-1}$.

Table 11.11 Summary of the potential endocrine mediated responses in wildlife

Environmental compartment	Taxonomic group	Type of study	Species and exposure route used	Concentration series used	Lowest reported NOEC	Reference
Aquatic	Amphibians	Developmental	African clawed frog (*Xenopus laevis*) larvae - aqueous	0, 2.1 and 21 µg l^{-} (Nominal)	2.1 µg l^{-1} (a)	Kloas *et al* (1999)
	Fish	Life cycle	Zebrafish (*Danio rerio*) – aqueous exposure	0, 1.2, 3.2, 12 and 35 µg l^{-1} (Measured)	12 µg l^{-1} (a)	Wenzel *et al* (2001)
	Invertebrates	Developmental	Molluscs - aqueous	1 –100 µg l^{-}(Nominal)	<1 µg l^{-1} (a)	Oehlmann *et al* (2000)
Terrestrial	Birds	No data	Zebrafinches	-	20.6 mg kg body $weight^{-1}$ day^{-1}	Millam *et al* (2001)
	Invertebrates	No data	-	-	-	-
Aerial	Invertebrates	No data	-	-	-	-

a - There is a degree of uncertainty with this data

B. Dermal exposure

In a briefly reported study cited in SIDS (1994) and IUCLID (2000), a dermal LD_{50} of 1880 mg kg body weight^{-1} was determined in groups of New Zealand white rabbits. No information on the numbers of animals used or the exposure period was given.

C. Inhalation exposure

The only available study was conducted by Rohm and Haas (cited in SIDS 1994 and IUCLID 2000) but not to GLP. The results showed that inhalation exposure of 6 adult albino rats at a concentration of 116 mg l^{-1} for one hour caused the death of all the animals within 24 hours. Gross autopsy of the animals showed evidence of pulmonary haemorrhage.

D. Other routes of exposure

In mice a median lethal dose of 25 mg kg body weight^{-1} was reported following intravenous injection (SIDS 1994).

11.5.1.2 Repeat dose studies

A. Oral exposure

In a non GLP study with albino rats (carried out by Rohm and Haas 1961, cited in IUCLID 2000) fifteen young male and female animals were placed on a daily diet of finely ground Purina Dog Chow Kibbled Meal served as the basic diet. For the treatment group a 35% aqueous solution of 4-*tert* octylphenol was incorporated into the diet in an amount calculated to achieve a 5% concentration of the active ingredient. An equivalent amount of water was added to the diet of the control rats. The animals were individually caged and were weighed once a week. Food consumption data were collected over a 3 day period during the thirteenth week. Urine collected during the last week from 5 rats of each sex at each dietary level was tested semi-quantitatively for glucose (Morris Anthrone method) and protein (Pro-Teen, sulfosalicyclic acid and Shevky and Stafford methds). Haemotologic determinations on 5 rats of each sex at each dietary level were made at the end of the test period. Organ to body weight ratios for liver, kidney, spleen, heart and testes were determined at sacrifice of the 3 month survivors. Tissues taken for histopathological study were: heart, lung, liver, spleen, gasteroenteric, bladder, bone marrow, muscle, skin, brain, thyroid, adrenal and pancreas. No effect of treatment were apparent and there were no deviations in food consumption. Urinary concentrations of glucose and protein were comparable in treated and control animals. There were no haemotologic related effects to treatment. There were no statistically related differences for organ to body weight between control and treated animals. No pathologic lesions were apparent in the treatment groups.

A non GLP study with rats (BOR/WIWS, SPF Cpb) (carried out by Bayer 1982 cited in SIDS 1994 and IUCLID 2000) used twenty rats (10 females and 10 males) for each of the control and three (30, 300 and 3000 ppm) exposure concentrations. The study was conducted prior to the issuance of the US EPA's Health Effects Testing Guidelines but the test procedures, including animal and dose selection, exposure conditions, observations, test substance administration, haematology, clinical chemistry, urine analysis and gross necropsy were in accordance with the testing guidelines. Animals were fed orally daily using p-octylphenol (purity 93.1%). Treatment effects were determined by statistical comparison of mortality, body weight changes, food and water consumption, organ weight changes, clinical chemistry, haematology and histological examination of tissues from sacrificed animals. Histologic

examination were conducted on only five animals per sex from the high dosage group as opposed to the ten animals per sex suggested in the US EPA guidelines. Throughout the study, no clinical signs of toxicity were observed. Furthermore, no significant reduction in food consumption was observed in either sex at any dosage level. A 28% increase in water consumption was noted in female rats exposed to the high dosage level. However, this effect was not observed in the male rats at any dosage level. In both sexes, mean body weight gain was significantly reduced in the high dose animals. In females exposed at the high dosage, the heart weight was decreased and the brain weight was increased. The kidney, testis and brain weight were increased in males exposed at the high dosage level. In addition, males exposed to 300 ppm of the test substance experienced an increase in brain weight. Relative brain weight was the only dose–related organ weight change that was statistically significant. Haematologic parameters in all treated male rats were unaffected by exposure to the test substance. A decrease in haemoglobin and haemocrit was observed among female rats in the high dosage group. Although thyroxin (T4) concentration in female rats at the high dosage level was increased after one month, the T4 level was not significantly elevated following three months of exposure. The increase at one month was attributed to elevated values in two female rats. Since no increase in T4 values was noted at 3 months and no histopathological findings were observed in the thyroid gland, the increased T4 values were not considered to be toxicologically significant. All other findings were either not significantly different from controls, not dose-related or were within the normal range for animals in this age group. At the no treatment-related abnormalities in histopathological observations or at the gross necropsy were observed.

A GLP study with rats (Crj: Caesarian delivered, Sprague Dawley) (reported by the Chemical Investigation Promoting Committee in 1994 and cited in SIDS 1994 and IUCLID 2000) used 12 animals (6 males and 6 females) for each of the control and three (15, 70 and 300 mg kg^{-1} day^{-1}) exposure concentrations. The test was conducted to the Japanese Guidelines for the 28 day dose toxicity test of chemicals using p-tert octylphenol (98.2 %). Animals were fed daily by oral gavage. Salivation was observed on test substance administration in the medium (70 mg kg^{-1} day^{-1}) and high (300 mg kg^{-1} day^{-1}) dose females and males. Body weight gain was reduced in the high dose males, while water intake was increased in males and females of the high dose group. No changes in food consumption and haematological parameters were observed in any treatment group. A NOEL of 15 mg kg^{-1} day^{-1} was reported by no LOEL was given.

A GLP study with albino rats (Sprague-Dawley) (carried out by Huntingdon Life Sciences i1994 and cited in SIDS 1994 and IUCLID 2000) used 10 animals (5 males and 5 females) for each of the control and three (15, 150 and 250 mg kg^{-1} day^{-1}) exposure concentrations. The test was carried out to OECD TG 407 using octylphenol (purity 98.7%). Animals were fed daily by oral gavage for the 29 day study. A dose of 150 mg kg^{-1} day^{-1} led to the following symptoms:

- slightly higher food consumption in females
- higher water consumption of females
- lower cholesterol levels in female rats
- basophilic epithelium occasionally with mitotic figures in proximal convulated tubules in male rats

A dose of 250 mg kg^{-1} day^{-1} caused the following effects:

- slightly higher food consumption in males
- markedly higher water consumption of male and female rats
- lower cholesterol levels in female rats
- significantly higher kidney and liver weights in female rats
- minimal centrilobular hepatocyte enlargement in female rats
- interstitial inflammation in kidneys of males
- basophilic epithelium occasionally with mitotic figures in proximal convulated tubules in male and female rats

A NOEL of 15 mg kg^{-1} day^{-1} and a LOEL of 150 mg kg^{-1} day^{-1} was reported.

B. Dermal exposure

No repeat dose toxicity data for laboratory mammals following dermal exposure to 4-*tert* octylphenol has been located.

C. Inhalation exposure

No repeat dose toxicity data for laboratory mammals following inhalation exposure to 4-*tert* octylphenol has been located.

D. Other routes of exposure

No repeat dose toxicity data for laboratory mammals following intravenous or sub-cutaneous injection of 4-*tert* octylphenol has been located.

Table 11.12 Summary of the general mammalian toxicity data (from IUCLID 2000)

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study validity
Acute Oral Toxicity	Rat (Spague-Dawley strain)	Not relevant	No data	Median lethal dose (LD_{50})	>2000 mg kg^{-1} body $weight^{-1}$	SIDS 1994	Valid
	Mouse	Not relevant	No data	Median lethal dose (LD_{50})	3210 mg kg^{-1} body $weight^{-1}$	SIDS 1994	Valid
Acute Dermal Toxicity	Rabbit	Not relevant	No data	Median lethal dose (LD_{50})	1880 mg kg^{-1} body $weight^{-1}$	SIDS 1994	Use with care
Acute Inhalation Toxicity	Rat	1 hour	No data	24h LC_{100}	< 116 mg l^{-1}	SIDS 1994	Valid
Acute Toxicity (Intra-peritoneal injection)	Mouse	No data		Median lethal dose (LD_{50})	25 mg kg^{-1} body $weight^{-1}$	SIDS 1994	Valid
Repeated Dose Toxicity (Oral)	Male and female rats (Wistar)	90 days	0, 30, 300 and 3000 ppm day^{-1}	NOEL LOEL	30 ppm 300 ppm	SIDS 1994	Valid
	Male and female rats (CD: Sprague-Dawley strain)	28 days	0, 15, 70 and 300 mg kg bw^{-1} day^{-1}	NOEL LOEL	15 mg kg^{-1} bw day^{-1} Not given	SIDS 1994	Valid
	Male and female albino rats (Sprague-Dawley strain)	29 days	0, 15, 150 and 250 mg kg bw^{-1} day^{-1}	NOEL LOEL	15 mg kg^{-1} bw day^{-1} 150 mg kg^{-1} bw day^{-1}	SIDS 1994	Valid

11.5.1.3 Comparison of data from studies assessing potential endocrine mediated responses and/or general toxicity in laboratory mammals

The indications from the available data on potential endocrine mediated responses in laboratory mammals indicate that no effects on reproductive parameters or the histology of endocrine glands or hormone sensitive organs were evident at 150 mg kg^{-1} day^{-1} in a two year reproduction study in rats. This absence of effects occurred even in the presence of high dose toxicity of parental animals. Exposure of pregnant rats to 4-*tert* octylphenol at doses of 0.002 and 0.02 mg kg^{-1} during the period of organogenesis does not induce any embryo or foetotoxicity or malformations. Following sub-cutaneous injections of doses of 4-tert octylphenol of 30 mg kg^{-1} body $weight^{-1}$, histopathological effects have been found on the testis and hormone levels in male and female rats. The changes were statistically significant but the longer-term biological consequences of the histological effects is not certain given the absence of such responses in the two generation study in rats.

NO(A)ELs of 15-30 mg kg body $weight^{-1}$ day^{-1} have been reported for general systemic effects in mammals following exposure to 4-tert octylphenol. As a result it appears that endocrine mediated responses may be among a number of mechanisms responsible for the most toxic effects observed.

11.5.2 Studies relevant to the assessment of general toxicity in wildlife

11.5.2.1 Studies on aquatic organisms

Table 11.13 summarises all of the toxicity test results found for aquatic organisms exposed to 4-*tert* octylphenol. The following sections focus on studies considered to be valid but discuss studies marked use with care where important issues (such as the timing and duration of exposure) are addressed.

A. Fish

Acute toxicity

An acute 96-hour LC_{50} value of 250 µg l^{-1} has been reported for fathead minnow (*Pimephales promelas*) in a flow-through test (temperature 22 ^{o}C, pH 8-8.2) in which concentrations were measured and the study was carried out according to GLP. A 96-hour NOEC of 77 µg l^{-1} for lethality was also obtained (IUCLID, 2000).

A similar 96-hour LC_{50} value of 260 µg l^{-1} was calculated for golden orfe (*Leuciscus idus*) in a semi-static test, in which exposure concentrations were measured and the study was carried out to GLP (IUCLID 2000). In the same study an LC_0 of 210 µg l^{-1} and an LC_{100} of 390 µg l^{-1} were also obtained. A higher 48-hour LC_{50} of 600 µg l^{-1} was found for the same species in another study (use with care) based on nominal concentrations and not carried out to GLP (IUCLID, 2000).

Wenger *et al* (2001) reported a 96 hour LC_{50} value of 370 µg l^{-1} for zebrafish (*Danio rerio*) exposed to 4-*tert* octylphenol in a test in which exposure concentrations were measured.

A 14-day LC_{50} of 120 µg l^{-1} and NOEC of 84 µg l^{-1} (for lethality) were found in rainbow trout *(Oncorhynchus mykiss*) when tested under flow-through conditions. The concentrations were measured and the test was carried out at 12 ± 1 ^{o}C, pH 8-8.2 and to GLP. A 6-day LC_{50} of 170 µg l^{-1} was also obtained in this study (IUCLID 2000). However, it is not clear whether the fish were fed in this period and, therefore, how comparable the result is with 96-hour LC_{50} values.

Kelly and Di Giulio (2000) investigated the toxicity of 4-*tert* octylphenol to embryos and larvae of the estuarine killifish or mummichog *Fundulus heteroclitus.* Exposures occurred with embryos and larvae at three different ages, 0, 2 or 4 weeks post hatch. During the 96 hour exposure period to nominal concentrations of 2.1, 5.2, 10.5 and 21.0 mg 4-t OP l^{-1} larvae were monitored daily for mortalities, structural abnormalities (such as craniofacial, cardiac, torso/abdominal, tail effects) and behavioural abnormalities (lethargy, lack of feeding). The 96 h LC_{50} for the mortality of embryos was 3900 µg l^{-1}. For the newly hatched larvae, 2 week old and 4 week old larvae the 96h LC_{50} values were 290, 280 and 340 µg 4-t OP $l^{-1.}$ The results of these studies were taken to suggest that 4-*tert* octylphenol has the potential to cause developmental toxicity in fish. Since the addition of tamoxifen (an oestrogen receptor antagonist) reduced the effects of 4-tert octylphenol on embryos it was postulated that developmental toxicity may occur, at least in part, through the disruption of oestrogen-based signals (see Section 8.1.5).

Chronic toxicity

A 60-day post-hatch early life stage toxicity study with rainbow trout (*Oncorhynchus mykiss*) has also been carried out to an ASTM procedure under flow-through and according to GLP (Analytical Bio-chemistry Laboratory, 1986 cited in IUCLID 2000). The test concentrations were 6.1, 11, 22, 51 and 91 µg l^{-1} based on measured concentrations. The NOEC and LOEC values for the growth of fry (as measured by wet weight after 60 days exposure) were 6.1 µg l^{-1} and 11 µg l^{-1} respectively. Hatchability of the rainbow trout eggs after 20 days of continuous exposure to 4-*tert* octylphenol was not significantly affected when compared to the controls. Survival of the fry between hatch and 60 days of exposure was significantly reduced compared to the controls at 4-t OP concentrations > 22 µg l^{-1}.

Ashfield *et al (*1998) investigated the effects of exposure of 4-*tert* octylphenol on the growth of female juvenile rainbow trout. Two flow though exposure regimes were used for the critical window of sensitivity between hatching and the swim up fry stage:

1. Groups of fish immediately following hatching were exposed to nominal concentrations of 1, 10 and 50 µg l^{-1} for a period of 22 days before being exposed to clean water for a further 86 days. Fish were sampled on day 108 at the end of the study.

2. Groups of fish immediately following hatching were exposed to nominal concentrations of 1, 10 and 30 µg l^{-1} for a period of 35 days before being exposed to clean water for a further 431 days. Fish were sampled 24, 55, 84, 108, 144, 220, 300 and 466 days after the start of exposure.

At the sampling times the weight and fork length of fish were measured. There was no analytical confirmation of the nominal exposure concentrations.

Fish exposed to all concentrations of 4-*tert* octylphenol for 22 days in the first study showed a significant reduction in body weight on day 108, relative to the controls. The reduction in weight was most marked at a nominal concentration of 1 µg l^{-1} and became less marked with increasing 4-*tert* octylphenol concentration. In the second study a significant modification in growth was not observed until day 84. The most marked effect was on the weight of the fish, with the two highest concentrations of (10 and 30 µg l^{-1}) causing a significant reduction in weight, relative to control fish. Fish exposed to 1 µg l^{-1} displayed body weights significantly higher than the controls at this time. Fish exposed to 10 µg l^{-1} continued to show significantly lower weights and lengths than control fish at day 144. At day 466 the body weight of fish exposed to 10 and 30 µg l^{-1} was no longer significantly different from control values. However,

the body weight of fish exposed to 1 µg l^{-1} was still significantly lower than that of the controls. Length was significantly reduced in fish exposed to 10 µg l^{-1} at day 144, but at no other time. The absence of a concentration-response relationship for body weight complicates the interpretation of the data and means that the significance of the effect at 1 µg l^{-1} is uncertain. No clear mechanism for the effects of 4-*tert* octylphenol was advanced in the study but the possibility that growth may be inhibited via a direct oestrogenic effect was raised as one potential hypothesis.

Embryo-toxicity tests were carried out by Gray and Metcalfe (1999) on the Japanese medaka (*Oryzias latipes*). Fish were exposed from day 0 (fertilisation) to day 17 (swim-up) using a static non-renewal procedure. However, LC_{50} values (following 17 days exposure) for the three replicates had a significantly wide range with calculated values of 450, 830 and 940 µg l^{-1} (based on nominal concentrations) reported. Developmental abnormalities observed in embryos and larvae ranged from circulatory problems to failure to inflate swim bladders. However, the mean duration to hatch was not affected by exposure to 4-*tert* octylphenol.

B. Invertebrates

Table 11.13 summarises all of the toxicity test results found for 4-*tert* octylphenol to aquatic invertebrates. The following sections focus on studies considered to be valid but discuss studies marked use with care where important issues (such as the timing and duration of exposure) are addressed.

Acute toxicity

Only limited toxicity data are available for freshwater invertebrates, mostly for the water flea *Daphnia magna.* Similar 24- and 48-hour LC_{50} values of 260 and 270 µg l^{-1} were reported for *Daphnia magna* in a flow-through test carried out to GLP. The test was performed at 20 ± 2 ^{o}C, pH 8.3-8.4 and values were based on measured concentrations. The corresponding NOEC was 110 µg l^{-1} (IUCLID, 2000).

In two other studies using the same species, a 24-hour EC_{50} (immobilisation) of 170 µg l^{-1} and a 48-hour LC_{50} of 90 µg l^{-1} have been reported (IUCLID (2000) and Zou and Fingerman (1997) respectively). However, the first was not carried out to GLP and had no analytical monitoring, while no information on study conditions was provided in the second paper. Both studies should ,therefore, be used with care.

First and second instar nymphs of the freshwater shrimp *Gammarus pulex* were more sensitive with a 96-hour EC_{50} (immobilisation) of 13.3 µg l^{-1} and a corresponding 96-hour LC_{50} of 19.6 µg l^{-1} being reported (Sims and Whitehouse, 1998). The experiment involved a semi-static design with analytical confirmation of the test concentration series: 0, 5.6, 10, 18, 32 and 56 µg l^{-1}. This definitive test followed a range finding test at 0, 0.01, 0.1, 10 and 100 µg l^{-1}. There was also a clear time-dependence of toxicity in the definitive test, although toxicity appeared to begin to plateau by 96 hours.

For saltwater species, a series of acute toxicity data for the mysid (*Mysidopsis bahia*[3]) were located, providing 96 hour LC_{50} values ranging from 53.4-113.1 µg l^{-1} (Cripe *et al*. 1989). However, these data were taken from a study investigating the effects of different feeding regimes on the acute toxicity of 4 substances to *Mysidopsis* and were not carried out to any

[3] *Mysidopsis bahia* is now *Americamysis bahia*

standard guidelines. There were thus a number of different variables which could influence the results, such as nutritional deficiency (direct physiological effect on susceptibility to toxicants) or surplus food in the test environment (possible effects on dissolved oxygen in a static test, again inducing physiological stress). While the procedures themselves appeared to have been adequately carried out for the endpoints desired, and well reported, there were obvious difficulties in using these data for direct comparison with other standard invertebrate toxicity data, such as the lethality data obtained for the freshwater amphipod *Gammarus pulex*. However, it was noted that the range of LC_{50} values observed were broadly similar to those of the freshwater amphipod.

Andersen *et al* (2001) investigated the effects of 4 octylphenol on the survival of adults of the marine copepod *Acartia tonsa*. The static study was carried out according to the ISO Method 14669 and involved exposure of 10-12 day old adults to a range of 4-t OP concentrations for a period of 48 hours. The exposure concentrations were confirmed analytically. The 48 hour LC_{10} and LC_{50} values for 4-t OP were 230 and 420 µg l^{-1} respectively.

The other data located for a saltwater species were for the fiddler crab (*Uca pugilator*) where after between 3 and 7 days exposure to 4-*tert* octylphenol at a concentration of 10000 µg l^{-1} enzymic changes were recorded (Zou and Fingerman, 1999a). The changes recorded in this study may potentially be due to endocrine disruption induced by the octylphenol, and are outlined in more detail in Section 4.1.5 on endocrine effects. The concentrations producing these effects were significantly higher (at least an order of magnitude) than those causing effects on more traditional endpoints in other aquatic invertebrates**.**

Chronic toxicity

A 21-day life cycle toxicity study was carried out with *Daphnia magna* according to a US EPA procedure under flow-through conditions and according to GLP (IUCLID, 2000). Exposure levels were 37, 62, 120, 230 and 510 µg l^{-1} based on measured concentrations. Statistical analysis of survival for *Daphnia magna* after a 21 day exposure period indicated that adult survival was significantly different from the controls in the mean measured concentration of 510 µg l^{-1}. All the daphnids died in this highest exposure concentration by day 9 of the study and no reproduction or adult length data were available. A 21 day EC_{50} value for lethality was calculated to be 340 µg l^{-1}. The mean adult lengths at concentrations of 62, 120 and 230 µg l^{-1} were significantly different from the controls. However, since the mean adult length of the 62 µg l^{-1} exposure concentration was only 2.6% less than the control, the statistical difference indicated may not be biologically significant. As a result the actual NOEC for effects on adult length could be regarded as 62 µg l^{-1} which was the same as the NOEC for reproduction.

Table 11.13 Summary of general toxicity data for aquatic organisms (from IUCLID 2000)

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study validity
Acute Fish Toxicity	Fathead minnows (*Pimephales promelas)*	24, 48, 72 and 96h	0, 41, 77, 150, 340 and 630 μg l^{-1} (Measured)	Lethality - 24h LC_{50} 48h LC_{50} 72h LC_{50} 96h LC_{50} 96h NOEC	290 μg l^{-1} 250 μg l^{-1} 250 μg l^{-1} 250 μg l^{-1} 77 μg l^{-1}	Cited in IUCLID (2000)	Valid
		24h	No data	Lethality - 24h NOEC	150 μg l^{-1}	Cited in Servos (1999)	Use with care
		96h	No data	Lethality - 96h LC_{50}	250 μg l^{-1}	Cited in Bennie (1999)	Use with care
	Golden orfe *(Leuciscus idus*)	96h	No data	Lethality - 96h LC_{50}	260 μg l^{-1}	Cited in IUCLID (2000)	Valid
		48h	No data	Lethality - 48h LC_{50}	600 μg l^{-1}	Cited in IUCLID (2000)	Use with care
		96h	No data	Lethality - 96h LC_{50} 96h NOEC	1500 - 2000 μg l^{-1} 1000 μg l^{-1}	Cited in IUCLID (2000)	Use with care
	Rainbow trout *(Oncorhynchus mykiss)*	24h	No data	Lethality - 24h LC_{50} 96h NOEC	450 μg l^{-1} 170 μg l^{-1}	Cited in Servos (1999)	Use with care
	Zebrafish (*Danio rerio)*	96h	No data	Lethality 96h LC_{50}	370 μg l^{-1}	Wenzel *et al* (2001)	Use with care
	Mummichog (*Fundulus heteroclitus*) - embryos - newly hatched larvae - 2 week old larvae - 4 week old larvae	96h	No data	Lethality 96h LC_{50} 96h LC_{50} 96h LC_{50} 96h LC_{50}	3900 μg l^{-1} 290 μg l^{-1} 280 μg l^{-1} 340 μg l^{-1}	Kelly and Di Giulio (2000)	Use with care

Table 11.13 Continued

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study reliability
Chronic Fish Toxicity	Japanese medaka (*Oryzias latipes*)	17 days	0, 50, 100, 250, 500, 750 and 1000 µg l^{-1} (Nominal)	Embryo-larval (fertilisation to swim up stage) lethality 17 day LC_{50}	450 - 940 µg l^{-1}	Gray and Metcalfe (1999)	Use with care
	Rainbow trout *(Oncorhynchus mykiss)*	14 days	No data	Lethality - 14 day LC_{50} 14 day NOEC	120 µg l^{-1} 84 µg l^{-1}	Cited in SIDS (1994)	Valid
		60 days	0, 6.1, 11, 22, 51 and 91 µg l^{-1} (Measured)	Growth of fry - 60 day NOEC 60 day LOEC	6.1 µg l^{-1} 11 µg l^{-1}	Cited in SIDS (1994)	Valid
		108 days 466 days	0, 1, 10 and 50 µg l^{-1} (Nominal) 0, 1, 10 and 30 µg l^{-1} (Nominal)	Growth of fry 108 day NOEC Growth of fry 466 day NOEC	1 µg l^{-1} 30 µg l^{-1}	Ashfield *et al* (1998)	Use with care
Acute Invertebrate Toxicity	Water flea *(Daphnia magna*)	24h	No data	Immobilisation - 24h EC_{50}	170 µg l^{-1}	Cited in IUCLID (2000)	Use with care
		48h	0, 63, 110, 190, 320 and 940 µg l^{-1} (Measured)	Lethality - 24h LC_{50} 48h LC_{50}	260 µg l^{-1} 270 µg l^{-1}	Cited in SIAR (1994)	Valid
		48h	No data	Lethality - 48h LC_{50}	90 µg l^{-1}	Zou and Fingerman (1997)	Use with care
	Freshwater shrimp (*Gammarus pulex*) - first and second instar nymphs	96h	0, 5, 8.9, 10.9, 15.5, 27.2 and 50.7 µg l^{-1} (Measured)	Immobilisation - 96h EC_{50} Lethality - 96h LC_{50}	13.3 µg l^{-1} 19.6 µg l^{-1}	Sims and Whitehouse (1998)	Valid
	Mysid (*Mysidopsis bahia*) -< 24 h old neonates	96h	No data	Growth - 96h EC_{50}	53.4 µg l^{-1}	Cripe *et al* (1989)	Use with care

	Copepod (*Acartia tonsa*) – 10-12 day old adults	48h	No data	Lethality 48h LC_{50}	420 µg l^{-1}	Andersen *et al* (2001)	Use with care

Table 11.13 Continued

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study reliability
Acute Invertebrate Toxicity	Fiddler crab (*Uca pugilator*)	3 and 7 days	2000 and 10000µg l^{-1}	EC_{50} (enzymic changes)	10000µg l^{-1}	Zou and Fingerman (1999a,b)	Use with care, (Important study details lacking)
Chronic Invertebrate Toxicity	Water flea (*Daphnia magna*)	21 days	0, 37, 62, 120, 230 and 510 µg l^{-1} (Measured)	NOEC (growth) LOEC (growth) EC_{50} (Immobilisation)	37 µg l^{-1} 62 µg l^{-1} 340 µg l^{-1}	Cited in SIDS (1994)	Valid

11.5.2.2 Studies on terrestrial organisms

No general toxicity data for terrestrial organisms following exposure to 4-*tert* octylphenol has been located.

11.5.2.3 Studies on aerial organisms

No general toxicity data for aerial organisms following exposure to 4-*tert* octylphenol has been located.

11.5.2.4 Comparison of data from studies on potential endocrine mediated responses and/or general toxicity in wildlife

From the available toxicity data for 4-*tert* octylphenol it was noted that the most sensitive trophic levels based on freshwater data would appear to be aquatic invertebrates and fish. The lowest valid NOEC value was 6.1 µg l^{-1} for inhibition of growth in rainbow trout (*Oncorhynchus mykiss*) fry after 60 days exposure, with a corresponding LOEC value of 11 µg l^{-1}. This compares with NOEC values of 1-12 µg l^{-1} for potentially endocrine mediated responses in aquatic organisms indicating that on the basis of the available data a range of mechanisms may be responsible for the most toxic effects observed toxicity in these taxonomic groups.

11.6 Current classification of the substance against European Commission and national regulations

Table 11.14 summarises the current classification of the substance against Council Directives in order to assess the regulations to which 4-*tert* octylphenol is subject.

Table 11.14 Current classification of 4-*tert* octylphenol against Council Directives

Directive	Status (listed or not)
67/548/EEC - Classification, packaging and labelling of dangerous substances	Not classified (proposed)
93/793/EEC - Regulation of the risks of existing substances	Targeted RAR being produced

In the United Kingdom tentative environmental quality standards for 4-*tert* octylphenol in freshwaters and saltwater have been derived. The proposed Maximum Allowable Concentrations was 2.5 µg l^{-1} while the proposed annual average was 1 µg l^{-1}.

11.7 Exposure data

11.7.1 Worker exposure data

Occupational exposure may occur during manufacture of octylphenol and during its use as a chemical intermediate. This is most likely to be dermal exposure occurring as a result of spillage, although this should be minimised by the use of Personal Protective Equipment

(PPE). CEPAD have indicated that no monitoring information during the production of octylphenol is available.

11.7.2 Consumer exposure data

Given that octylphenol is used as an intermediate and not used as such in products potential consumer exposure should under normal circumstances be minimal. The only exposure route is via residual monomers in products however these concentrations in products such as resins are considered by CEPAD to be low.

11.7.3 Environmental exposure data

11.7.3.1 Aquatic environment

Treatment plant discharges and sewage sludges

A compilation of measured concentrations of octylphenol in wastewater discharges from industrial and municipal sewage treatment plants in European countries is given in Table 11.15. It should be noted that all values are below the analytical detection limit.

Table 11.15 Summary of the measured octylphenol concentrations in wastewaters from industrial and municipal sewage treatment works

Effluent type and location	Year	Octylphenol concentration ($\mu g\ l^{-1}$)	Reference
Sewage treatment works receiving wool processing waste (UK)	1994	$\leq$0.36	Warhurst (1995)
Sewage treatment works (UK)	1997	$\leq$3.3	SEPA (1997)
Sewage treatment works (UK)	1999	N.d.*	Lye *et al* (1999)
Textile wastewater plant (Portugal)	2000	<9.0	Pristine (2000)
Wastewater treatment plant (Portugal)	2000	<9.0	Pristine (2000)
Wastewater treatment plants (Spain)	2000	<9.0	Pristine (2000)

*N.d. not detected; authors did not quote the detection limit

B. Surface waters and sediments

A compilation of measured concentrations of octylphenol in river and estuarine waters from the United Kingdom and other European countries is provided in Table 11.16. The available data show that water concentrations of octylphenol are typically below 1 $\mu g\ l^{-1}$.

Table 11.16 Summary of the measured octylphenol concentrations in river and estuarine surface waters

Location	Year	Octylphenol concentration (μg l^{-1})	Reference
Rivers			
River Elbe and tributaries (Germany)	1998	0.0008-0.002	Working Group for the Cleanliness of the Elbe (2000)
River Lea (UK)	1994	0.4	Blackburn and Waldock (1995)
River Thames and tributaries (UK)	1994	<0.02-0.43	Britnell (1995)
River Dart (UK)	1994	<0.02-0.12	Warhurst (1995)
Manchester Ship Canal (UK)	1999	<0.2	Environment Agency (2001)
Estuaries			
Elbe Estuary (Germany)	1998-1999	0.0013-0.018	Working Group for the Cleanliness of the Elbe (2000)
Tees Estuary (UK)	1994	13	Blackburn and Waldock (1995)
Tees and Tyne Estuaries (UK)	1997	N.d.*	Lye *et al* 1999
Wyre Estuary (UK)	1999	<0.2	Environment Agency (2001)
Coastal waters			
German Bight	1998-1999	0.0001-0.016	Working Group for the Cleanliness of the Elbe (2000)

*N.d. not detected; authors did not quote the detection limit.

CEPAD, the sector arm of CEFIC dealing with alkylphenol ethoxylates, has stated that "in all likelihood the octylphenol and its derivatives measured in surface waters originate from the octylphenol present as an impurity in commercial grade nonylphenol and not from the use of octylphenol and derivatives as such, because the fraction of octylphenol derivatives is mostly constant at about 8-10% of the corresponding nonylphenol derivatives". Consistent with this assertion are data from the River Elbe and its tributaries and the German Bight on the concentrations of 4-*tert* octylphenol and its ethoxylates (OP1EO and OP2 EO) and nonylphenol and its ethoxylates (NP1EO and NP2EO) (Working Group for the Cleanliness of the Elbe). These data are presented in Table 11.17. The proportion of octylphenol to nonylphenol measured at the 13 river and estuarine locations on the River Elbe ranged from 1.7 to 14.3% with a mean value of 8%, in keeping with the statement from CEPAD. However, no site-specific data have yet been located which would support nonylphenol production as being the main source of octylphenol in waters. However, it should be noted that the risk assessment of nonylphenol carried out under the EU Existing Substances Regulation does not mention octylphenol as an impurity of nonylphenol and there is no definitive documented evidence to support this link .

Table 11.17 Concentrations of 4-*tert* octylphenol (and its ethoxylates) and nonylphenol (and its ethoxylates) measured in the water column of the River Elbe and the German Bight

Type of water sample	Water column concentrations measured in the River Elbe (ng l^{-1})					
	4-t OP	OP1EO	OP2EO	NP	NP1EO	NP2EO
Riverine	0.8-2.0	0.9-6.3	0.6-1.5	7.2-52	13-205	4.3-84
Estuarine	0.8-1.3	0.9-1.3	0.6-1.1	9.5-13	10-14	3.6-4.6
Coastal	0.1-16	0.1-11	0.1-19	0.3-63	0.7-29	0.2-10

Data on measured concentrations of 4-*tert* octylphenol in sediments taken from a series of locations from the Tees and Tyne estuaries in the UK were found to be in the range 0.03 - 0.34 mg kg^{-1} dry weight (for the Tees) and 0.002-0.02 mg kg^{-1} dry weight (for the Tyne) (Lye *et al* 1999). The octylphenol concentrations increased in the sediments in the vicinity of industrial and sewage works treatment discharges. Sediment samples taken from a number of European countries as part of the Pristine Project all showed octylphenol concentrations below the limit of detection (Pristine 2000).

Table 11.18 provides data on a study of 4-*tert* octylphenol and octylphenol exthoxylates concentrations in sediments from the River Elbe and its tributaries. Data are also given for the nonylphenol and nonylphenol ethoxylates (Working Group for the Cleanliness of the Elbe 2000). The levels of octylphenol in the River Elbe were in the same range (0.021-0.086 mg kg^{-1} dry weight) as for the Tees and Tyne estuaries. At the 11 river and estuarine sites studied the proportion of octylphenol (OP) to nonylphenol (NP) ranged from 4.7 to 8.7%. Based on the mean value for the ratio of OP to NP in the raw materials, the expected sediment ratio estimated from the different partition coefficients would be 7.3%, which is very close to the mean measured ratio of 6.7%.

Table 11.18 Concentrations of octylphenol (and its ethoxylates) and nonylphenol (and its ethoxylates) measured in the sediments of the River Elbe

Type of water sample	Concentrations measured in the River Elbe (ng l^{-1})					
	4-t OP	OP1EO	OP2EO	NP	NP1EO	NP2EO
River	21-86	30-93	45-113	387-1378	323-967	546-1611
Estuarine	32-66	78-113	100-140	367-852	712-886	972-1434

11.7.3.2 Terrestrial environments

No data are available on terrestrial levels of 4-*tert* octylphenol.

11.7.3.3 Aerial environment

No data are available on atmospheric levels of octylphenol but considering its low vapour pressure and tendency to adsorb to soils and sediments it can be expected that atmospheric concentrations will be extremely low. Any octylphenol released to the atmosphere is likely to be degraded rapidly by reaction with hydroxyl radicals (estimated half-life in air is ca. 3 hours) and deposition of the substance from the atmosphere is likely to be negligible with resulting rainwater concentrations being low. As the lifetime of octylphenol in the atmosphere is very short, it is unlikely to be transported a long distance from its point of emission and therefore concentrations due to atmospheric washout by precipitation from the atmosphere are likely to be greatest near the point of emission.

11.7.3.4 Comparison of environmental monitoring data and exposure concentration causing endocrine mediated responses

The limited data on the concentrations of 4-*tert* octylphenol in European surface waters (see Section 11.7.3.1) indicates that typical levels are < 0.1 μg l^{-1}, though most values are probably lower than this value. The lowest data on potentially endocrine mediated responses in aquatic organisms occur in the range 1-12 μg l^{-1}. The use of a margin of safety (MOS)[4] approach

[4] Margin of safety (MOS) = (Lowest NOEC for endocrine mediated responses)/Environmental concentration

would result in values of 10-120. On the basis that an MOS of 100 should be required for the risk to be acceptable then 4-*tert* octylphenol may present a risk to aquatic organisms in terms of potential endocrine disrupting effects.

11.8 Overall Conclusions on 4-*tert* octylphenol

The following conclusions have been drawn from the review of 4-*tert* octylphenol:

11.8.1 Data from studies assessing potential endocrine disruption effects

11.8.1.1 Human related studies

- A definitive two generation reproduction study in rats using oral exposure (see Table 11.7) provided no evidence for effects of 4-tert octylphenol on reproductive or developmental endpoints or the histology of endocrine glands or hormone sensitive organs which may be endocrine mediated. This absence of effects occurred even in the presence of high dose (2000ppm or 150 mg kg^{-1} day^{-1}) toxicity of parental animals.

- Exposure of pregnant rats to 4-*tert* octylphenol at doses of 0.002 and 0.02 mg kg^{-1} during the period of organogenesis does not induce any embryo or foetotoxicity or malformations.

- Following sub-cutaneous injections of doses of 4-tert octylphenol of 30 mg kg^{-1} body $weight^{-1}$, histopathological effects have been found on the testis of male rats (whereas no changes in hormone levels were evident). The changes were statistically significant but the longer-term biological consequences of the histological effects is not certain given the absence of such responses in the two generation study in rats.

- *In vitro* studies have indicates that 4-*tert* octylphenol has a binding affinity for the human oestrogen receptor which is 1500 times lower than that for oestradiol. However, Nagel *et al* (1997) reported effects may be overestimated in serum free assays. 4-*tert* octylphenol has been reported to be oestrogenic in mammalian cell culture assays at levels 330-500 times lower than oestradiol.

- No data is available on the androgenic or anti-androgenic effects of 4-*tert* octylphenol on and effects on thryoid function or hormone synthesis or secretion or steroidogenesis in mammalian cells and tissues.

11.8.1.2 Wildlife studies

- Overall on the basis of the valid studies the lowest NOECs for endocrine disrupting effects in aquatic organisms would be 12 μg l^{-1} , based on the data from the Wenzel *et al* (2001) study. However, data from studies (of use with care status) in molluscs and amphibians indicate that a lower NOEC, potentially less than 1 μg l^{-1}, may be appropriate.

11.8.2 Comparison of data from studies assessing potential endocrine disrupting effects and general toxicity

11.8.2.1 Human related studies

- The indications from the available data on potential endocrine mediated responses in laboratory mammals indicate that no effects on reproductive parameters or the histology of endocrine glands or hormone sensitive organs were evident at 150 mg kg^{-1} day^{-1} in a two year reproduction study in rats. This absence of effects occurred even in the presence of high dose toxicity of parental animals. Exposure of pregnant rats to 4-*tert* octylphenol at doses of 0.002 and 0.02 mg kg^{-1} during the period of organogenesis does not induce any embryo or foetotoxicity or malformations. Following sub-cutaneous injections of doses of 4-tert octylphenol of 30 mg kg^{-1} body $weight^{-1}$, histopathological effects have been found on the testis and hormone levels in male and female rats. The changes were statistically significant but the longer-term biological consequences of the histological effects is not certain given the absence of such responses in the two generation study in rats. NO(A)ELs of 15-30 mg kg body $weight^{-1}$ day^{-1} have been reported for general systemic effects in mammals following exposure to 4-tert octylphenol. As a result it appears that endocrine mediated responses via oestrogen-like effects may be among a number of mechanisms responsible for the most toxic effects observed.

11.8.2.2 Wildlife related studies

- From the available toxicity data for 4-*tert* octylphenol it was noted that the most sensitive trophic levels based on freshwater data would appear to be aquatic invertebrates and fish. The lowest valid NOEC value was 6.1 µg l^{-1} for inhibition of growth in rainbow trout (*Oncorhynchus mykiss*) fry after 60 days exposure, with a corresponding LOEC value of 11 µg l^{-1}. This compares with NOEC values of 1-12 µg l^{-1} for potentially endocrine mediated responses in aquatic organisms indicating that on the basis of the available data a range of mechanisms may be responsible for the most toxic effects observed toxicity in these taxonomic groups.

11.8.3 Exposure data

11.8.3.1 Worker exposure

- Occupational exposure may occur during manufacture of octylphenol and during its use as a chemical intermediate. This is most likely to be dermal exposure occurring as a result of spillage, although this should be minimised by the use of Personal Protective Equipment (PPE). CEPAD have indicated that no monitoring information during the production of octylphenol is available.

11.8.3.2 Consumer exposure

- Given that octylphenol is used as an intermediate and not used as such in products potential consumer exposure should under normal circumstances be minimal. The only exposure route is via residual monomers in products however these concentrations in products such as resins are considered by CEPAD to be low.

11.8.3.3 Environment

- The limited data on the concentrations of 4-*tert* octylphenol in European surface waters (see Section 11.7.3.1) indicates that typical levels are < 0.1 μg l^{-1}, though most values are probably lower than this value. The lowest data on potentially endocrine mediated responses in aquatic organisms occur in the range 1-12 μg l^{-1}. The use of a margin of safety (MOS)[5] approach would result in values of 10-120. On the basis that an MOS of 100 should be required for the risk to be acceptable then 4-*tert* octylphenol may present a risk to aquatic organisms in terms of potential endocrine disrupting effects, particularly at discharge 'hot spots'.

11.9 Summary of the weight of evidence for endocrine disrupting effescts in humans and/or wildlife and associated uncertainties

The summary of the weight of evidence for endocrine disrupting effects of 4-*tert* octylphenol in humans and wildlife along with associated uncertainties are given in Table 11.19.

[5] Margin of safety (MOS) = (Lowest NOEC for endocrine mediated responses)/Environmental concentration

Table 11.19 Summary of the weight of evidence conclusion and uncertainties associated with the assessment of the endocrine disrupting effects of 4-*tert* octylphenol

	Target group	
	Humans	Wildlife
Weight of evidence	The available data from *in vivo* studies in laboratory mammals (using oral or dermal exposure routes) indicates that 4-*tert* octylphenol does not cause adverse effects on reproductive and developmental endpoints (which may be endocrine mediated) at exposure levels where general systemic toxic effects are observed. The lowest NOEL in the *in vivo* studies was 150 mg kg body weight $^{-1}$ day^{-1} for effects on reproductive and developmental parameters. No effects on reproductive or developmental parameters in laboratory mammals are evident at low exposure doses (0.0015 – 0.002 mg kg^{-1}) The available data indicates that current exposure patterns to 4-*tert* octylphenol do not represent a risk to workers or consumers (including children).	The available data shows the threshold exposure concentrations of 4-*tert* octylphenol above which reproduction and development in aquatic organisms (fish, amphibians and invertebrates) are affected (NOECs = 1-12 μg l^{-1}) are similar to the threshold levels for general toxic effects (i.e. growth and lethality). The effects may be oestrogen mediated. The available exposure data indicate that 4-t*ert* octylphenol may represent a risk to aquatic organisms, particularly at discharge 'hotspots'.
Uncertainties	There are no major uncertainties with regard to the evaluation of potential adverse effects of 4-*tert* octylphenol on reproductive and developmental endpoints since data is available from a definitive multi-generation study as well as supporting reproduction and developmental studies. Mechanistic uncertainties exist because most of the available studies provide no direct measurement of changes in endocrine function (for example changes in hormone levels).	There are uncertainties with regard to the potential adverse effects of 4-*tert* octylphenol on reproduction and development in wildlife due to the absence of key data for terrestrial organisms. The absence of data on aerial organisms is not a major uncertainty since although 4-*tert* octylphenol is volatile it is rapidly degraded and the potential for these organisms to be exposed is limited. There are no environmental exposure data for 4-*tert* octylphenol in the terrestrial and aerial compartments, though levels in the aerial compartment are not expected to be high.

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12. REVIEW OF DATA FOR 2,2'4,4-TETRABROMINATED DIPHENYL ETHER (TETRA BDE)

In the project "Study on the Scientific Evaluation of 12 Substances in the context of Endocrine Disrupter Priority List of Actions" 2,2,4,4'-Tetrabrominated diphenyl ether was identified as a candidate for an in depth review. Discussions with the European Brominated Flame Retardant Industry Panel (EBFRIP), the CEFIC Sector Group responsible for these substances, have indicated that:

- The substance is not produced commercially but is present as a by product (at around levels of 34%) in the commercial material Pentabromo diphenyl ether (Penta BDE). As such no data from regulatory studies is likely to be available;

- Penta BDE is currently being reviewed as part of the formal Risk Assessment Process for Existing Substances (under Directive 793/93EEC) and a Risk Assessment Report has been completed which includes a consideration of endocrine disruption effects.

On the basis that control of Tetra BDE will result from the control of Penta BDE (particularly given that Tetra BDE is not produced commercially) it has not been considered appropriate to review this substance in further detail. This was discussed at the Stakeholder Forum held in Brussels in March 2001.

13. REVIEW OF DATA FOR OESTRONE

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Notes:

This section contains information on the effects of oestrone on wildlife collected and collated from a range of sources including published papers, reports of studies conducted by industrial companies and research organisations, data compilations and a review of natural and synthetic steroid oestrogens carried out by the United Kingdom Environment Agency (EA 2002).

This review has been carried out in accordance with the evaluation framework described in Section 2. In the review the International Programme for Chemical Safety (IPCS) definition of an endocrine disrupter has been adopted, namely that it is "*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*".

In the context of the review it is recognised that there are various laboratory-based *in vivo* and *in vitro* methods utilising a range of (eco)toxicological endpoints that are claimed by different sources to be relevant to the assessment of endocrine disruption in humans and wildlife. However, since this field is still in an early stage of development there is uncertainty regarding the significance of many of the current findings.

From the numerous recent reviews of potential test methods (such as the Detailed Review Paper prepared by OECD in 1997) there is a clear consensus in terms of the hierarchy of the relevance of test methods. In this hierarchy longer-term *in vivo* studies considering effects on reproduction and/or development (and including mechanistic information) are of greater relevance than short-term *in vivo* screening tests which are of greater relevance than *in vitro* assays. The greater relevance of chronic *in vivo* tests or those assessing effects during critical windows of sensitivity is also evidenced by the fact that these are the key (eco)toxicological methods being developed in the OECD Endocrine Disruption Testing and Assessment (EDTA) Programme. This hierarchy approach to data relevance has been adopted in the review along with a weight of evidence consideration of the available data.

The review has been carried out to address three key questions:

1. Does the available data indicate there is evidence that a chemical causes endocrine disrupting effects in target groups of wildlife?

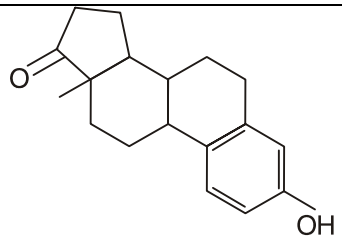
2. Do endocrine disrupting effects of the chemical in target groups of wildlife occur at lower concentrations than those causing effects on general systemic toxicological endpoints?

3. Are particular target groups of organisms in the environment likely to be exposed to concentrations of chemicals which exceed effects thresholds due to current emission patterns.

It should be recognised that this review is not designed to be a full Risk Assessment of a substance under the Existing Substances Regulation 793/93.

13.1 Physico-chemical data for oestrone

13.1.1 Summary details on the substance

CAS Number	53-16-7
EINECS Number	-
IUPAC Name	3-hyroxyestra-1,3,5(10)-trien-17-one
Other names	Oestrone, Estrone, E1
Molecular weight	270.4
Chemical formula	$C_{18}H_{22}O_2$
Chemical structure	

13.1.2 Physico-chemical properties and environmental fate information (from EA 2002)

The data on the physico-chemical properties of oestrone and its environmental fate (see Table 13.1) indicate that the substance is readily biodegradable (100% loss after 14-28 days) in a series of tests under aerobic conditions (Stumm-Zollinger and Fair 1965, Tabak *et al* 1981, Schweinfurth *et al* 1996). Sorption is considered to be an important removal process for oestrone but photolysis is considered to be of lower importance and volatilisation is considered to be negligible (Williams *et al* 2001).

Adsorption to organic carbon is an important process resulting in the partitioning of oestrone onto sediments and soils.

Table 13.1 Physico-chemical properties and environmental fate data (from EA 2002)

Physico-chemical property	Value (and comments)
Physical state at ambient temperature	Solid
Water solubility	30 mg l^{-1}at 20°C (IARC 1979, Merck 1996)
Octanol-water partition coefficient (log Kow)	3.13 - 3.43 (Lai *et al* 2000)
Organic carbon water partition coefficient (log Koc)	3.1 - 3.5 (Meylen and Howard 1995, Lai *et al* 2000)
Henry's Law Constant	No data
Type of degradation	
Aquatic - abiotic	Sorption is considered to be an important removal process for oestrone but photolysis is considered to be of lower importance and volatilisation is considered to be negligible (Williams *et al* 2001)
Aquatic - biotic	Oestrone has been reported as readily biodegradable (100% loss after 14-28 days) in a series of tests under aerobic conditions (Stumm-Zollinger and Fair 1965, Tabak *et al* 1981, Schweinfurth *et al* 1996)
Terrestrial	No data
Atmospheric	No data

13.2 Production and Uses

Natural steroid oestrogens (including oestrone) are used in human medicine, particularly in hormone replacement therapy (HRT) and for the treatment of other gynaecological disorders. They are also used in the treatment of prostate and breast cancer in men and breast cancer in post-menopausal women (Martingdale 1993).

13.3 Toxicokinetics, metabolism and bioaccumulation

13.3.1 Toxicokinetics and metabolism

The major site of metabolism for natural oestrogens is the liver, with the two major metabolic pathways being 2-hydroxylation and 16α-hydroxylation. These pathways result in a number of metabolites, conjugated with glucuronide and/or sulphate, which are considered to be biologically inactive and largely excreted in the urine (Aldercreutz *et al.* 1994, IARC 1979). The main urinary metabolites are conjugates of oestriol, 2-hydroxyoestrone, oestrone, 16α-hydroxyoestrone and oestradiol, in order of decreasing quantitative importance (IARC 1979).

Much smaller amounts of natural oestrogens are excreted in the faeces (Aldercreutz *et al* 1982). The metabolites tend to be excreted in an unconjugated form as opposed to urinary metabolites which are conjugated (Aldercruetz *et al* 1994). Aldercreutz and Järvenpää (1982) suggest that faecal excretion represents about 5-10% of total excretion in the urine and faeces.

13.3.2. Bioaccumulation

No data has been located on the bioaccumulation of oestrone in aquatic organisms, but the low octanol water partition coefficent (log Kow) of 3.13 – 3.43 indicates that the substance is unlikely to bioaccumulate in biota.

A related study was conducted by Larsson *et al* (1999) in which caged juvenile rainbow trout of both sexes were placed upstream and downstream of a domestic Swedish STW serving approximately 3500 people. After 2 to 4 weeks of exposure, bile was collected from the fish. Effluent water was analysed for oestrone (and 17β-oestradiol and 17α-ethinyloestradiol) which was found at ng l^{-1} concentrations. The bile of fish caged downstream of the STW contained oestrone (and 17β-oestradiol and 17α-ethinyloestradiol) at concentrations that were 10^4-10^6 times higher than the water concentrations (up to 2.5 μg g^{-1} bile).

13.4 Studies relevant to the assessment of potential endocrine disrupting effects

13.4.1 Studies relevant to the assessment of potential endocrine disrupting effects in humans

The effects on oestrone on human health have not been considered in this review. Oestrone is a natural vertebrate steroid that is produced in humans and mammals by conversion from the androgenic precursor androsetendione. As such homeostatic control mechanisms exist in vertebrates which operate to control the levels of oestrone within acceptable bounds.

Natural steroid oestrogens (including oestrone) are used in human medicine, particularly in hormone replacement therapy (HRT) and for the treatment of other gynaecological disorders. They are also used in the treatment of prostate and breast cancer in men and breast cancer in post-menopausal women. The doses prescribed for these conditions are designed to operate in a specific manner where the potential adverse effects on consumers are minimised.

13.4.2 Studies relevant to the assessment of potential endocrine disrupting effects in wildlife

13.4.2.1 In vitro studies

Available data on the responses of oestrone in *in vitro* systems using cells and tissues from wildlife species indicates that the mechanism of action of oestrone is via the oestrogenic receptor. However, it is evident from the data that oestrone is of lower potency than 17β-oestradiol in these *in vitro* systems.

13.4.2.2 In vivo studies

A. Studies on aquatic organisms

Table 13.2 summarises the data on the potential endocrine disrupting effects of oestrone on aquatic organisms.

In vivo studies in amphibians

No data has been located on the effects of oestrone on amphibians.

In vivo studies in fish

Biochemical changes

One of the key functions of endogenous oestrogens in fish is to stimulate the induction in the liver of a large phospholipoprotein vitellogenin (VTG) (Chen 1983) which is released into the blood stream and sequestered by developing oocytes for production of egg yolk (Wallace 1985, Tyler *et al* 1988, Tyler 1991). In maturing female fish, vitellogenin is a major constituent of the blood proteins, while in male fish it is not normally present in appreciable amounts. However, if male fish are exposed to oestrogens or oestrogen mimics, vitellogenin can be produced at similar levels to that found in maturing females.

There is a degree of uncertainty as to the potential ecological relevance of the induction of vitellogenin in fish. Evidence from laboratory, semi-field and field studies carried out on fish exposed to natural and synthetic steroids in aquatic systems in Europe (CSTEE 1999, NRC 1999, Cheek *et al* 2001) has shown that VTG induction in male fish is a biomarker for exposure to oestrogens and oestrogen mimics and that:

- induction in early life stage fish could have serious energetic consequences for the organisms;
- high levels of vitellogenin induction in fish are known to cause kidney failure and are associated with some haematological disturbances;
- a weak, but nevertheless significant correlation, has been shown between VTG induction in wild roach and the severity of the intersex condition in fish (that is male gonads show evidence of feminisation).

Routledge *et al* (1998) exposed adult male rainbow trout (*Oncorhynchus mykiss*) for 21 days to nominal oestrone concentrations of 6.25, 12.5, 25, 50 and 100 ng l^{-1} and determined the effect on plasma vitellogenin levels. Analysis confirmed that in nearly all cases, the actual concentrations were close to nominal concentrations, other than the 100 ng l^{-1} level in which the measured concentration was only 44 ng l^{-1} at the start of the test. Only the highest test concentration produced a statistically significant increase in plasma vitellogenin compared to control fish, with a NOEC of 50 ng l^{-1}. However, when the pre- and post-exposure samples of each fish were compared within each group, vitellogenin levels in the 50 ng l^{-1} post-exposure group were also significantly elevated. It was concluded that the threshold for plasma vitellogenin induction in rainbow trout for oestrone was between 25 and 50 ng l^{-1}.

Thorpe *et al* (2001) exposed juvenile female rainbow trout to six measured concentrations of oestrone (0, 1, 3.2, 10, 32, 100 or 320 ng l^{-1}) for 14 days. Plasma vitellogenin levels increased in a dose-dependent manner with NOEC, LOEC and EC_{50} values of 1.0, 3.2 and 70 ng l^{-1} respectively being reported.

Panter *et al* (1998) exposed male fathead minnows (*Pimephalas promelas*) to nominal concentrations of oestrone at 9.9, 31.8, 99.3, 318 and 993 ng l^{-1} for 21 days and determined the effect on plasma vitellogenin levels. No analysis was undertaken to confirm test concentrations. A statistically significant increase in plasma vitellogenin level (relative to the controls) was seen at concentration ≥ 31.8 ng l^{-1}, resulting in a NOEC of 9.9 ng l^{-1}.

Allner *et al* (1999) reported that a measured concentration of 66 ng l^{-1} caused a significant increase (relative to controls) in serum vitellogenin induction in golden orfe (*Leuciscus idus*) after 7 days exposure.

Van den Belt *et al* (2001a) exposed female zebrafish to oestrone for 21 days and measured effects on vitellogenin induction. No further details are currently available other than the relative potency of oestrone (0.8) was lower compared to 17β-oestradiol (1.0) and 17α-ethinyloestradiol (34.5).

Histopathology

Panter *et al* (1998) exposed male fathead minnows (*Pimephalas promelas*) to nominal concentrations of oestrone at 0, 9.9, 31.8, 99.3, 318 and 993 ng l^{-1} for 21 days and determined the effects on gonad weight (gonadosomatic index, GSI). No chemical analysis was undertaken to confirm test concentrations. The GSI value was significantly reduced (relative to the controls) at a concentration of 318 ng l^{-1} although this was not observed at the highest test concentration of 993 ng l^{-1}.

Van den Belt *et al* (2001a) exposed female zebrafish to oestrone for 21 days and measured effects on ovarian somatic index (OSI). No further details are currently available other than that the relative potency of oestrone was lower (0.8) compared to 17β-oestradiol (1.0) and 17α-ethinyloestradiol (30.8).

Changes in reproductive success and development

Metcalfe *et al* (2001) exposed Japanese medaka (*Oryzias latipes*) (1 day post hatch) to nominal oestrone concentrations of 0, 10, 100, 1000 and 10,000 ng l^{-1} until the organisms reached approximately 1.5 cm in length (which occurred at 85-110 days post hatch). Test concentrations were renewed every 48 hours and the end-points monitored included survival, growth, general condition, sex ratios and the development of testis-ova.

At the end of the study nearly all of the fish exposed to 1000 and 10,000 ng l^{-1} were female, with sex ratios differing significantly from the controls. Among the small number of males identified, well-defined testis-ova (intersex) were present in all of the males. In the lower treatment groups, intersex was observed but in a much smaller proportion, namely 2 of 38 and 3 of 42 males showing testis-ova at doses of 10 and 100 ng l^{-1}, respectively. However, given that the natural occurrence of intersex has not been clearly established in this species, it is difficult to determine whether the small observed incidence of intersex is a consequence of exposure. For some unknown reason, a higher proportion of males than females occurred in the 10 and 100 ng l^{-1} treatment groups, the result being significant for the 100 ng l^{-1} concentration. The authors concluded that the LOEC was 10 ng l^{-1} based on induction of testis-ova. A separate study was conducted to establish the degradation of oestrone under the conditions used in the bioassays. Following analysis by LCMS, concentrations of 1000 and 10,000 ng l^{-1} were found on average to be 77.8% of the nominal concentrations over a period of 0, 24 and 48 hours. Although this may give some indication of the relative stability of oestrone, the authors attempt to report all of the bioassay results as 'actuals' on the basis of these findings is considered inappropriate. It follows that the effects observed may have arisen as a result of lower concentrations than those reported (that is toxicity was underestimated).

A multi-generational study assessing the effects of oestrone on the reproduction and development of fathead minnows is currently being carried out by the University of Exeter and

Brixham Environmental Laboratory of Astra-Zeneca. The test has been initiated with the paired breeding assay (Harries *et al* 2000). The eggs produced from the 21 day breeding period when exposed with and without toxicants are then being assessed for hatchability (%) and developmental abnormalities. The test is due for completion in late 2002.

Changes in behaviour

No data has been identified on the effects of oestrone on the sexual behaviour of fish.

***In vivo* studies in invertebrates**

The only study investigating endocrine disrupting effects of oestrone in invertebrates was a life-cycle study assessing the effects of oestrone on *Tibse battagliai* (Crustacea, Copepoda) (Hutchinson *et al* (1999). Newly released (<24 hrs old) animals were exposed to nominal concentrations of 0, 0.1, 1, 10 and 100 μg l^{-1} over 21 days and effects monitored in terms of survival, development, sex ratio and fecundity. None of the concentrations had an adverse effect on these life-cycle parameters and, thus, a 21-day of NOEC of ≥100 μg l^{-1} was reported.

Information is not available on the potential endocrine mediated responses of oestrone to a wide range of invertebrate taxa.

B. Studies on terrestrial organisms

No information has been located on the potential endocrine disrupting effects of oestrone on terrestrial species. Given that sorption to organic carbon is an important process resulting in the partitioning of oestrone onto soils the absence of data on potential endocrine mediated responses in terrestrial organisms is a key area of uncertainty. However, it should be recognised that there are currently no internationally agreed methods specifically developed to assess endocrine disrupting effects in terrestrial organisms.

C. Studies on aerial organisms

No information has been located on the potential endocrine disrupting effects of oestrone on aerial species. Given that oestrone is not considered to be volatile the absence of data on potential endocrine mediated responses in aerial organisms is not a key area of uncertainty. It should be recognised that there are currently no internationally agreed methods specifically developed to assess endocrine disrupting effects in aerial organisms.

D. Summary of potential endocrine disrupting effects in wildlife

The data on potentially endocrine mediated responses of oestrone in wildlife is limited and restricted to aquatic organisms (invertebrates and fish). An assessment of aquatic toxicity indicates that, of the taxa for which data are available, fish are the most sensitive to the adverse effects of oestrone. However, relatively few aquatic vertebrate taxa (where steroid oestrogens play a central role in reproduction), notably amphibians have been studied in detail in this regard and so this remains an area of uncertainty.

Laboratory studies have measured a variety of end-points in fish, although the ecological significance of some has not yet been fully established (e.g. plasma vitellogenin induction and effect on gonadosomatic index). Nevertheless, recent research in wild roach populations indicates that marked histological effects on the gonads, such as a severe intersex condition, can result in a reduced reproductive capacity. Alteration of the timing of maturation is also

important in terms of reproductive success, as it may result in gametes being released outside the optimal breeding season and subsequently reduced recruitment. However, there can be no doubt that irreversible effects on reproductive parameters such as the production of markedly skewed sex ratios or single sex generations, marked reductions in egg production, significant increases in egg mortality and reduced fertilisation success are clearly of ecological significance, and have been shown to be sensitive end-points for this group of compounds.

The key data is from a chronic study investigating effects on growth, reproduction and survival of the Japanese medaka (*Oryzias latipes*) (Metcalfe *et al* 2001). In the study a LOEC of 10 ng l^{-1} was reported based on induction of testis-ova in 2 of 38 of male fish, with no incidences being recorded in the control males. However, the natural incidence of this condition in this species under control conditions has not been established. In addition it is difficult to ascertain whether the small incidence of testis ova were concentration related since only 3 of 42 males showed testis-ova at doses of 100 ng l^{-1} whereas at 1000 and 10000 ng l^{-1} nearly all the offspring were female and the few males present all had testis-ova.

A number of other studies have investigated the effects of oestrone exposure on the induction of the egg shell protein vitellogenin. The lowest aqueous exposure concentration of oestrone which has been shown to result in a statistically significant induction of vitellogenin in females (relative to controls) is 3.2 ng l^{-1} in rainbow trout (Thorpe *et al*. 2001), with a corresponding NOEC value of 1 ng l^{-1}. A higher exposure concentration of 31.8 ng l^{-1} was required to elicit VTG production in male fathead minnows (Panter *et al* 1998). The data indicate that there are inter-species differences in the sensitivity of fish in terms of the levels of oestrogenic substances required to elicit VTG induction.

A multi-generational study assessing the effects of oestrone on the reproduction and development of fathead minnows is currently being carried out by the University of Exeter and Brixham Environmental Laboratory of Astra-Zeneca and the results of this study will considerably reduce the uncertainty associated with endocrine mediated responses of fish to oestrone.

In contrast to fish, no effects of oestrone on the reproduction of the copepod *Tisbe battagliai* were evident at exposure concentrations up to 100 ng l^{-1}, indicating that the processes of reproduction and development in certain invertebrate taxa are evidently not affected by exposure to vertebrate steroids.

Table 13.2 Summary of potential endocrine disrupting effects in aquatic organisms involving exposure though the water column

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Fish						
Fathead minnow (*Pimephales promelas)*	Adult male	Flow-through; 0, 9.9, 31.8, 99.3, 318 and 993 ng l^{-1} (Nominal)	Significant increase (relative to controls) in plasma vitellogenin after 21 days exposure to 31.8 ng l^{-1} with a NOEC of 9.9 ng l^{-1} Significant decrease (relative to controls) in gonadosomatic index after 21 days exposure at 318 ng l^{-1}; however not observed at highest test concentration	Panter *et al* (1998)	Medium	Use with care
Golden orfe (*Leuciscus idus)*	Juvenile	Flow-through; 0 and 66 ng l^{-1} only (Measured)	Significant increase (relative to controls) in serum vitellogenin induction after 7 days exposure to 66 ng l^{-1}	Allner *et al* (1999)	Medium	Valid
Japanese medaka (*Oryzias lapites*)	Embryos (1 day post hatch – 1.5 cm length fish)	Semi-static; 0, 10, 100, 1000 and 10,000 ng l^{-1} (Nominal)	Significant effect (relative to controls) on sex ratio after ~ 85-110 days exposure at 100 ng l^{-1} (biased to males) with a NOEC of 10 ng l^{-1} Small incidence of testis-ova observed in males (2/38) after ~ 85-110 days exposure at 10 ng l^{-1} which was reported as the LOEC. However, the significance of this observation is unknown given the natural occurrence of intersex has not been clearly established in this species	Metcalfe *et al* (2001)	High	Use with care
Rainbow trout (*Oncorhyncus mykiss*)	Adult males	Flow-through; 0, 6.25, 12.5, 25, 50 and 100 ng l^{-1} (Nominal);	Significant increase (relative to controls) in plasma vitellogenin levels after 21 days exposure at 100 ng l^{-1} (measured concentration 44 ng l^{-1}) with a NOEC of 50 ng l^{-1} Significant increase (based on pre- and post-exposure samples in individual fish) in plasma vitellogenin levels after 21 days exposure at 50 ng l^{-1} with a NOEC of	Routledge *et al* (1998)	Medium	Use with care
	Juvenile females	Flow-through; 0, 1, 3.2, 10, 32, 100 or 320 ng l^{-1} (Measured)	Significant increase (relative to controls) in plasma vitellogenin levels after 14 days exposure at 3.2 ng l^{-1} with a NOEC of 1 ng l^{-1}. 14-day EC_{50} for plasma vitellogenin induction reported as 70 ng l^{-1}	Thorpe *et al* (2001)	Medium	Valid

Table 13.2 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Invertebrates						
Tisbe battagliai (Copepod)	Life cycle	Static renewal; 0, 0.1, 1, 10 and 100 $\mu g\ l^{-1}$ (Nominal)	No effect on survival, development, sex ratio and fecundity; thus NOEC $\geq$100 $\mu g\ l^{-1}$	Hutchinson *et al* (1999)	High	Use with care

13.5 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity

13.5.1 Studies relevant to the assessment of general toxicity in humans

Information on general toxicity effects on human health has not been considered in this review (see Section 13.4.1).

13.5.2 Studies relevant to the assessment of general toxicity in wildlife

13.5.2.1 Studies on aquatic organisms

A. Fish

Acute toxicity

At present no data on the acute toxicity of oestrone to fish has been located.

Chronic toxicity

Japanese medaka (*Oryzias latipes*) (1 day post hatch) were exposed to nominal oestrone concentrations of 0, 10, 100, 1000 and 10,000 ng l^{-1} until the medaka reached approximately 1.5 cm in length (which occurred at 85-110 days post hatch) (Metcalfe *et al* 2001). Test concentrations were renewed every 48 hours and the end-points monitored included survival, growth, general condition, sex ratios and the development of testis-ova. Fish exposed to the highest test concentration of 10,000 ng l^{-1} showed accumulation of eosinophilic fluid in all major organs (heart, kidney and liver) and the body cavity, and mean length and weight were also significantly lower than in other treatments. Eosinophilic fluid was also observed in organs and the body cavity in fish exposed to 1000 ng l^{-1}.

B. Invertebrates

Acute toxicity

Anderson *et al* (2001) investigated the acute toxicity of oestrone to *Acartia tonsa* and its inhibitory effect on larval development. Oestrone demonstrated no lethal effects at concentrations up to 1000 μg l^{-1} following 48 hour exposure. In the 5 day larval development test, EC_{10} and EC_{50} values of 250 and 410 μg l^{-1} were reported.

Chronic toxicity

Hutchinson *et al* (1999) conducted life-cycle studies investigating the effects of oestrone to *Tibse battagliai* (Crustacea, Copepoda). Newly released (<24 hrs old) animals were exposed to nominal concentrations 0.1, 1, 10 and 100 μg l^{-1} over 21 days and effects monitored in terms of survival, development, sex ratio and fecundity. None of the concentrations had an adverse effect on these life-cycle parameters and, thus, a 21-day of NOEC of ≥100 μg l^{-1} was reported.

13.5.2.2 Studies on terrestrial organisms

No general toxicity data for terrestrial organisms following exposure to oestrone have been located.

13.5.2.3 Studies on aerial organisms

No general toxicity data for aerial organisms following exposure to oestrone have been located.

13.5.2.4Comparison of data from studies assessing potential endocrine disrupting effects and/or and general toxicity in wildlife

The limited data on potential endocrine mediated responses in the aquatic invertebrates and fish and corresponding acute and chronic data for these taxonomic groups precludes extensive comparisons. However, in fish it appears that potential endocrine mediated effects occur at markedly lower concentrations than those causing general systemic toxicity. The uncertainty associated with relative threshold levels for different types of responses in fish will be reduced when the multi-generational study on the effects of oestrone on fathead minnows is completed (see Section 13.4.2.2). The relative insensitivity of the reproductive and developmental endpoints in the invertebrate group (crustaceans) exposed to oestrone means this taxonomic group are evidently not affected by exposure to vertebrate steroids.

13.6 Current classification of Oestrone against European Commission and national regulations

As a natural steroid oestrone is not listed or classified under any of the major Council Directives.

In the United Kingdom consideration has been given to the derivation of Predicted No Effect Concentrations (PNEC) for the natural vertebrate steroids oestrone and oestradiol and the synthetic steroid ethinyloestradiol as part of the national strategy for "Endocrine disrupting substances in the environment" (EA 2000). No PNEC value was derived for oestrone due to the limited dataset but a provisional range of 3-5 ng l^{-1} was identified.

13.7 Exposure data

13.7.1 Worker exposure data

The exposure of workers to oestrone has not been considered in this review since no large scale production facilities are found in Europe.

13.7.2 Consumer exposure data

Natural steroid oestrogens are used in human medicine (Martingdale 1993), particularly;

- in hormone replacement therapy (HRT);
- for the treatment of other gynaecological disorders;
- in the treatment of prostate cancer in men;
- in the treatment of breast cancer in men;

- in the treatment of breast cancer in post-menopausal women

However, the levels administered for these specific purposes are designated by medical staff are designed to minimise adverse side-effects.

13.7.3 Environmental exposure data

13.7.3.1Aquatic environment

The route of entry of natural steroid oestrogens into the aquatic environment will be primarily through human and animal excretion (see Section 13.3.1) and subsequent transport through sewage treatment works. The amounts of oestrone excreted in the urine or faeces from any individual human will depend on a number of factors such as sex, race, hormonal status (e.g. pre- *vs*. post-menopausal), smoking, stage of menstruation, use of oral contraceptives and pregnancy. There are characteristic changes in the concentrations of oestrogens in urine during the menstrual cycle and pregnancy. A summary of urinary and faecal excretion rates is given in Table 13.3 which shows that pregnant women excrete the largest amount of oestrone, followed by pre-menopausal women, post-menopausal women and finally men.

Table 13.3 Summary of oestrone levels released in urine or faeces by different human groups

Group	Urine oestrone concentrations (µg)	Faecal oestrone concentration (µg)
Pre-menopausal women	2.7[d] (Aldercruetz *et al* 1994) 7.8[c] (Aldercruetz *et al* 1994) 7[b] (Key *et al* 1996) 4-23[a] (Eastham 1978)	0.78 (IARC 1979, Brown *et al* 1968) 0.62 (Shore *et al* 1993) 0.31 (Shore *et al* 1993)
Post-menopausal women	1.39[b] (Key *et al* 1996)	0.13 (IARC 1979, Brown *et al* 1968)
Pregnancy	209-1460[f] (Altman 1961) 695-2585[g] (Fostis 1987) 670[h] (Aldercruetz and Luukkainen 1970) 1600[e] (Brown 1957)	98[d] (Eastham 1978)
Men	-	0.062 (IARC 1979, Brown *et al* 1968)
Children <age 8 age 8-12	-	

Key: a - Depending on stage of menstruation, b – Geometric mean adjusted for age, stage of menstruation for smokers, c – Caucasian women, d – Oriental women, e – Last week of pregnancy, f – depending on stage of pregnancy (months 2 – 8), g – depending on stage of pregnancy (weeks 27 – 37), h – weeks 36-40 of pregnancy

Excretion by farm animals can also contribute to the concentrations (and loads) of natural steroids in the environment (Shore *et al* 1993, Archand-Hoy *et al* 1998, Blok and Wösten 2000). Certainly the amount of oestrogens excreted can be significant (Knight 1980, Turan 1995,). Shore *et al* (1993) compared natural steroid oestrogen concentrations in treated sewage effluent from municipal STWs and small farm units based on data obtained using immunoassay techniques. Depending on time of year, effluent concentrations were 40-120 and 150-350 ng l^{-1}, respectively, indicating that farm units can be a source of natural steroid oestrogens to the environment.

Recently, Dutch researchers from the Association of River Waterworks reviewed the magnitude of natural steroid oestrogen excretion by domestic animals in the Netherlands

(Blok and Wösten 2000). The authors focussed specifically on cattle, pigs, chickens and horses, since contributions by other groups of animals, such as sheep and goats, were considered to be relatively low in comparison.

The studies included measurements of the concentrations of natural steroid oestrogens in manure and urine of domestic animals. Where measurements were lacking for an excretion pathway, assumptions of suspected concentrations were made. The concentrations reported relate to total natural steroid oestrogens rather than specific compounds. The authors concluded that the highest average oestrogen contributions per animal were from pregnant cows and breeding female pigs, which excreted 37 and 7 mg day^{-1}, respectively. Contributions from other animals such as non-pregnant cows, female store pigs and chickens were expected to be much lower, with excretion concentrations of 1, 0.032 and 0.028 mg $animal^{-1}$ day^{-1}, respectively. Expected contributions from pregnant horses at the end of the gestation period were shown to be 100 mg $animal^{-1}$ day^{-1}. However, this was the only value available in the literature and no other reports could be cited to confirm this high concentration.

The average daily values per animal were used to calculate the total steroid oestrogen excretion by all domestic animals and humans in the Netherlands. These results indicate that pregnant cows and breeding female pigs contribute the greatest volume of natural steroid oestrogens in the Netherlands (22 and 10.6 kg day^{-1}, respectively). It should also be noted that the total contribution of natural steroid oestrogens by Dutch livestock is estimated to be 10 times greater than contributed by the human population.

As a result an important environmental source of these hormones may be from diffuse sources which directly enter the aquatic environment.

Treatment works effluents and sewage sludges

Table 13.4 summarises the data on the concentrations of oestrone in effluent discharges from treatment works and the levels recorded in sewage sludges at the treatment plants. Monitoring studies indicate that oestrone concentrations in sewage treatment works effluents range from 0.35 to 220 ng l^{-1} and are typically in the range 5 – 20 ng l^{-1}. Further information on the studies in different countries are given in the following sections.

Table 13.4 Summary of the measured oestrone concentrations in European treatment plant discharges

Location	Location	Oestrone concentration (ng l^{-1})	Reference
Germany	10 STW final effluents	≤ 70	Ternes *et al* (1999)
	STW final effluents	≤ 80	Wegener *et al* (1999)
	3 STW final effluents	0.35 - 18	Kuch and Ballschmiter (2001
Italy	6 Activated sludge STW final effluents	2.5 – 82.1	Baronti *et al* (2000)
	3 STW final effluents	< 0.5 - 54	Johnson *et al* (2000)
Netherlands	5 STW final effluents	≤ 47	Belfroid *et al* (1999)
	3 STW final effluents	<0.4 - 47	Johnson *et al* (2000)
United Kingdom	5 STW final effluents	≤ 15	Desbrow et al (1998)
	Chelmsford STW final effluent	15 – 220	Rodgers-Gray *et al* (2000)
	3 STWs on rivers Lea and Nene	<1 – 12.2	Kanda *et al* (2001)
	Harpenden and Deephams STW final effluents	≤3	Niven *et al* (2001)

Germany

Ternes *et al* (1999) using GCMS/MS analysis, concentrations of oestrone were measured in treated effluents from 16 German STWs. Oestrone was detected in nearly all the samples with concentrations up to 70 ng l^{-1}

Similarly, Wegener *et al* (1999) detected a maximum of 80 ng l^{-1} of oestrone in German effluents but in 8 of 13 effluent samples, concentrations were below the LOD of 1 ng l^{-1}.

More recently, Kuch and Ballschmiter (2001) conducted monitoring of three German activated sludge STWs, sampled over 5 months. Oestrone was analysed by GCMS with LOD values of 0.1 being reported. Respective concentrations ranged from 0.35-18 ng l^{-1}.

Italy

Johnson *et al* (2000) measured oestrone in effluents of five Italian activated sludge STWs. Based on LCMS/MS analysis, oestrone was present in most of the samples analysed and ranged from <0.5-54 ng l^{-1}. The authors also measured steroid concentrations in effluents from three Dutch STWs but using GCMS/MS analysis. Concentrations ranged from <0.4-47 ng l^{-1} for oestrone. Similar results were reported by Baronti *et al*. (2000) who measured oestrone in 6 Italian activated sludge STWs receiving domestic inputs. Analysis was by LCMS/MS and concentrations ranged from 2.5-82.1 (median 9.3) ng l^{-1}, oestrone being detected in all of the 30 samples analysed.

Netherlands

Belfroid *et al* (1999) measured oestrone and 17β-oestradiol in 5 STWs (3 receiving domestic waste, 2 receiving industrial waste) in the Netherlands using GCMS/MS analysis. In domestic effluents, reported concentrations were up to 47 and 12 ng l^{-1}, respectively, which were higher than those found in the industrial effluents.

United Kingdom

The Environment Agency funded work to identify oestrogenic substances in effluents from STWs (Desbrow *et al* 1998). The method development focused on three STWs receiving mainly domestic sewage and used fractionation techniques followed by GCMS analysis. The initial results from final effluents demonstrated that oestrogenic activity (as measured by the Yeast Estrogen Screen (YES) bioassay) was largely concentrated in only one fraction and indicated a polar organic structure for the causal agents. Subsequently, 21 effluent samples from seven STWs receiving mainly domestic waste were assessed. All were found to contain detectable concentrations of oestrone in the fraction with high oestrogenic activity Concentrations ranged between 1.4-76 ng l^{-1} for oestrone. For five STWs, effluents contained <15 ng l^{-1} of oestrone.

Rodgers-Gray *et al* (2000) monitored Chelmsford STW effluent during two trials (November 1997-March 1998; July 1998-December 1998) using GCMS analysis. Five-day composite samples of effluent were taken each month, and oestrone was consistently detected throughout both the test periods. During the first trial the oestrone concentration varied from 15 to 220 ng l^{-1}. By comparison, the concentrations of oestrone in the second trial ranged from 27-56 ng l^{-1}, respectively. However, it should be noted that the results from the first trial (which utilised GCMS analysis) are higher compared to monitoring data reported by other researchers.

Niven *et al*. (2001) reported on monitoring results of effluent samples from two UK sewage treatment plants (Harpenden and Deephams). Trace concentrations of oestrone and were detected at concentrations up to ~ 3 ng l^{-1}, respectively.

The most recent UK study involved monitoring oestrone in effluent from one STW on the River Nene daily over 4 weeks (29 samples) and two STWs on the River Lea daily over 2 weeks (14 samples/works) (Kanda *et al* 2001, Williams *et al* 2001). Analysis was conducted using GCMS/MS and was performance tested, the LOD being 1 ng l^{-1}. For the STW on the River Nene results indicated oestrone was positively identified in all samples. It was also detected at concentrations up to ~ 11 ng l^{-1} in the dissolved fraction. Concentrations were <1 ng l^{-1} for all particulate fraction samples. For the two STWs on the River Lea, the maximum concentrations for oestrone was 12.2 ng l^{-1}. It is of note that concentrations for oestrone were highly variable changing by an order of magnitude over a few days.

Surface waters and sediments

Table 13.5 summarises the data on the concentrations of oestrone in European surface waters. Surface water oestrone concentrations typically ranged from < 0.5 to 14 ng l^{-1}. Further information on the studies in different countries are given in the following sections.

Table 13.5 Summary of the measured oestrone concentrations in European surface waters

Location	Location	Oestrone concentration (ng l^{-1})	Reference
Germany	Surface waters	<0.1	Stumpf *et al* (1996)
	15 rivers and streams	<0.5-1.6 (LOD = 0.5 ng l^{-1})	Ternes *et al* (1999)
	Lake	4	Wegener *et al* (1999)
	Surface waters	<1-14	Wegener *et al* (2001)
	Rivers Danube, Nau, Blau and Illner	<0.1-4.1	Kuch and Ballschmiter (2001)
Italy	River Tiber	<0.008-1.5	Baronti *et al* (2000)
Netherlands	Surface waters	0.3-3.4 (LOD = <0.5 ng l^{-1})	Belfroid *et al* (1999)
	Meuse and Rhine basins	$\leq$ 4	RIWA (2000)
United Kingdom	Severn Trent	0.8-7.1 (LOD = 0.3 ng l^{-1})	Fawell *et al* (2001)
	River Nene and Lea	<0.4-12.2 (LOD = 0.4 ng l^{-1})	Kanda *et al* (2001)

Germany

Ternes *et al* (1999) reported on monitoring data for oestrone in 15 German rivers and streams. Oestrone was detected at trace concentrations of 0.7-1.6 ng l^{-1} in only three of the rivers (the reported LOD being 0.5 ng l^{-1}). Other Germany studies also have supported these findings (Stumpf *et al* 1996, Wegener *et al* 1999).

More recently, Kuch and Ballschmiter (2001) analysed river and creek samples of the Danube (n=13), Nau (n=4) and Blau (n = 4) approximately 1 km downstream of STWs effluent sites. The Illner River (n=4) and three creeks (n=2 each) were analysed. All samples were analysed by GCMS and oestrone, concentrations were <0.1-4.1 ng l^{-1} (positively identified in 29 of 31 samples).

Italy

Baronti *et al* (2000) sampled two sites of the Tiber river; one was situated downstream of an activated sludge STW and the second was located 1 km before the mouth of the Tiber. After leaving Rome, the Tiber covers about 20 km and receives effluents of mechanical STWs located in small towns and raw sewage. At the first site, no significant amounts of oestrone were detected. However, analysis of the second site revealed concentrations of 1.5 ng l^{-1} of, oestrone. The authors suggest that this finding supports the hypothesis that oestrogens in environmental waters come primarily from untreated wastewater rather than from activated sludge STW effluents.

Netherlands

Only trace concentrations (generally below the LODs up to about 0.5 ng l^{-1}) of oestrone were detected in seven of eleven surface water sites in the Netherlands (Belfroid *et al* 1999) with a maximum value of 3.4 ng l^{-1} being reported. In a subsequent study of

endocrine disrupting compounds in the Rhine and Meuse basins (RIWA 2000) oestrone was detected at levels up to 4 ng l^{-1} in the Meuse and at levels up to 2.2 ng l^{-1} in the Rhine.

United Kingdom

The most comprehensive monitoring survey on UK surface waters involved GCMS/MS analysis of two rivers for oestrone (Kanda *et al* 2001, Williams *et al* 2001). Samples were taken at numerous sites downstream of sewage treatment works (5 sites from the River Nene daily over a period of 4 weeks; 5 sites from the River Lea daily over 2 weeks). The analytical method was performance tested, the LOD being 0.4 ng l^{-1} for oestrone. Oestrone concentrations ranged from <0.4 to 12.2 ng l^{-1}, although most samples were below 5 ng l^{-1}. The study demonstrated the variations in steroid concentrations that can occur in surface water.

Another recent study conducted GCMS/MS analysis of oestrone in five surface waters in the Severn Trent region of England (LOD 0.3 ng l^{-1} for all three steroids) (Fawell *et al*. 2001). Oestrone was detected at all five sites with concentrations ranging from 0.8-7.1 ng l^{-1}.

Kanda *et al* (2001) conducted a survey of sediment contamination by oestrone at 10 sites downstream of three STWs in two UK rivers (Rivers Nene and Lea). Analysis was conducted using GCMS/MS techniques and oestrone was positively identified in 15 of 29 samples at concentrations that ranged from <0.1-0.39 μg $kg^{-1.}$

COMPREHEND Programme

In the EU funded COMPREHEND programme chemical analysis of industrial and domestic effluents was undertaken by two of the partners in the Netherlands and Switzerland using samples taken from across Europe. Analysis included the natural steroids oestrone, oestradiol and oestriol and ethinyloestradiol. Oestrone measurements were the most consistent in terms of recovery and a good correlation was obtained in a comparison of the techniques of the two laboratories for measurements of the same set of wastewater samples. There was poor agreement with oestradiol measurement and both laboratories experienced very poor recoveries with oestriol and low sensitivity with ethinylestradiol. Oestrone measurements in STW effluents showed a good degree of correlation with estrogenic activity (as measured with *in vitro* assays) and oestrone and oestradiol were generally in the 0 to 10 ng l^{-1} range. Ethinyloestradiol however, was often at or below the limit of detection (approximately 1 ng l^{-1}). Oestrogenic steroids were generally below the limits of detection for most industrial waste waters (unless there was a significant component of the effluent originating from domestic/human sources within the industrial plant).

Toxicity Identification and Evaluations

In the COMPREHEND programme Toxicity Identification and Evaluations (TIE) identified oestradiol, oestrone and ethinyloestradiol as the principal estrogenic components of domestic raw sewage, with ethinyloestradiol and oestrone dominating the oestrogenic activity of the final effluent. Taking into consideration the potencies of the various oestrogenic compounds measured in municipal STW effluents, it was concluded that natural and synthetic steroids, of human origin, are by far the most important estrogenic components and are responsible for most of the estrogenic effects seen *in vivo* and *in vitro*. Ethinyloestradiol may be particularly important in this respect but the limitations of the analytical techniques used in the programme were a major constraint to confirming the importance of this component of the contraceptive pill. TIE also provided evidence of 'cooperative' effects between the different steroids, making

the measured oestrogenic activity (in the yeast based YES assay) approximately three times greater than the sum of the activity of the individual components.

These results are generally consistent with those of Desbrow *et al* (1998) which used a fractionation system combined with a yeast based *in vitro* assay to isolate and identify the major oestrogenic chemicals present in seven sewage treatment works effluents receiving primarily domestic effluent. In all the effluents tested the most active fraction (>80% total activity in domestic effluent) was found to contain low levels of natural and synthetic steroidal oestrogens. The results obtained indicated that the concentrations of ethinyloestradiol, detected in the samples were generally too low to fully account for magnitude of the vitellogenin response observed when male fish were exposed to the effluent (Sheahan *et al* 1994).

13.7.3.2 Terrestrial environments

Blok and Wösten (2000) also investigated the environmental fate of natural steroid oestrogens applied to land in the form of animal slurry. Two scenarios were tested:

a) A small-scale area with a surrounding ditch that had been intensively fertilised with breeding female pig or milk cow manure for three months.

b) A large-scale total catchment area scenario for the Rhine and Meuse rivers using the total numbers of animals within each catchment to estimate the volume of natural oestrogens released.

Results from the first scenario estimated that the concentration of natural oestrogens in water ditches surrounding the fields could rise to 150 ng l^{-1} if the highest volume of slurry permitted in a year was applied to the land over a period of three months. In the second scenario it was estimated that concentrations of natural oestrogens in the rivers could rise to 75 and 140 ng l^{-1} in the Rhine and Meuse, respectively, over a fertilising period of three months. Regular spreading of manure over a six-month period in summer was estimated to lead to water concentrations of 40 and 90 ng l^{-1}, respectively. These calculations were based on the assumption that 3% of the excreted oestrogens in animal slurry would eventually enter surface waters. Finlay-Moore *et al* (2000) have also investigated 17β-oestradiol and concentrations in soil and run-off water from soil to which broiler litter had been applied. Run-off concentrations ranged between 20 and 2530 ng l^{-1} and it was concluded that there was a significant risk of edge-of field losses.

13.7.3.3 Aerial environments

No data has been located on the aerial environmental concentrations of oestrone.

13.7.3.4 Comparison of environmental monitoring data and exposure concentrations causing potential endocrine mediated responses

The data on the concentrations of oestrone in European surface waters (see Section 13.7.3.1) indicates that typical levels are in the range < 0.5 to 14 ng l^{-1}, though most values are usually at the lower end of the range and below 5 ng l^{-1}. The key data on endocrine mediated responses of oestrone in aquatic organisms is from a chronic study investigating effects on growth, reproduction and survival of the Japanese medaka (*Oryzias latipes*) (Metcalfe *et al* 2001). In the study a LOEC of 10 ng l^{-1} was reported based on induction of testis-ova in 2 of 38 of male fish, with no incidences being recorded in the control males. However, the natural

incidence of this condition in this species under control conditions has not been established and as such difficult to interpret the significance of this result.

A number of other studies have investigated the effects of oestrone exposure on the induction of the egg shell protein vitellogenin. The lowest aqueous exposure concentration of oestrone which has been shown to result in a statistically significant induction of vitellogenin in females (relative to controls) is 3.2 ng l^{-1} in rainbow trout (Thorpe *et al*. 2001), with a corresponding NOEC value of 1 ng l^{-1}. A higher exposure concentration of 31.8 ng l^{-1} was required to elicit VTG production in male fathead minnow (Panter *et al* 1998).

If a margin of safety (MOS)[1] approach is used to compare the threshold level above which endocrine mediated responses are observed (1-10 ng l^{-1}) with environmental concentrations (0.5-14 ng l^{-1}) then MOS values of 0.07 - >20 would result in the aquatic compartment. On the basis that an MOS of 100 should be required for the risk to be acceptable then oestrone apparently presents a risk to fish (and other aquatic vertebrates) in terms of endocrine disrupting effects. This conclusion is consistent with the results of field surveys carried out in a number of European countries (for example the COMPREHEND programme) which have identified evidence of adverse effects on the development and reproductive capability of wild fish which are exposed to natural (and synthetic) steroids discharged from sewage treatment works (for review EA 2002).

13.8 Overall Conclusions on Oestrone

The following conclusions have been drawn from the review of the effects on oestrone on wildlife:

13.8.1 Data from studies assessing potential endocrine disrupting effects in wildlife

- The data on endocrine mediated responses of oestrone in wildlife is limited and restricted to aquatic organisms (invertebrates and fish) The key data is from a chronic study investigating effects on growth, reproduction and survival of the Japanese medaka (*Oryzias latipes*) (Metcalfe *et al* 2001). In the study a LOEC of 10 ng l^{-1} was reported based on induction of testis-ova in 2 of 38 of male fish, with no incidences being recorded in the control males. However, the natural incidence of this condition in this species under control conditions has not been established. In addition it is difficult to ascertain whether the small incidence of testis ova were concentration related since only 3 of 42 males showed testis-ova at doses of 100 ng l^{-1} whereas at 1000 and 10000 ng l^{-1} nearly all the offspring were female and the few males present all had testis-ova.

- A number of other studies have investigated the effects of oestrone exposure on the induction of the egg shell protein vitellogenin in fish. The lowest aqueous exposure concentration of oestrone which has been shown to result in a statistically significant induction of vitellogenin in females (relative to controls) is 3.2 ng l^{-1} in rainbow trout (Thorpe *et al*. 2001), with a corresponding NOEC value of 1 ng l^{-1}. A higher exposure concentration of 31.8 ng l^{-1} was required to elicit VTG production in male fathead minnow (Panter *et al*. 1998). The data indicate that there are inter-species differences in the

[1] Margin of safety (MOS) = (Lowest NOEC for endocrine mediated responses)/Environmental concentrations

sensitivity of fish in terms of the levels of oestrogenic substances required to elicit VTG induction.

- A multi-generational study assessing the effects of oestrone on the reproduction and development of fathead minnows is currently being carried out by the University of Exeter and Brixham Environmental Laboratory of Astra-Zeneca. The results of this study will considerably reduce the uncertainty associated with the extent of endocrine mediated responses of fish following exposure to oestrone and threshold response concentrations.

- In contrast to fish, no effects of oestrone on the reproduction of the copepod *Tisbe battagliai* were evident up to a concentration of 100 ng l^{-1}, indicating that the processes of development and reproduction in certain invertebrate taxa are evidently not affected by exposure to vertebrate steroids. Information is not available on the potential endocrine mediated responses of oestrone for a wide range of invertebrate taxa.

13.8.2 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity in wildlife

- The limited data on endocrine mediated responses in the aquatic invertebrates and fish and corresponding acute and chronic data for these taxonomic groups precludes extensive comparisons. However, in fish it appears that potential endocrine mediated effects on reproduction and development occur at markedly lower concentrations than those causing general systemic toxicity. The uncertainty associated with relative threshold levels for different types of responses in fish will be reduced when the multi-generational study on the effects of oestrone on fathead minnows is completed.

13.8.3 Exposure data

- The data on the concentrations of oestrone in European surface waters indicates that typical levels are in the range < 0.5 to 14 ng l^{-1}, though most values are usually at the lower end of the range and below 5 ng l^{-1}.

- If a margin of safety (MOS) approach is used to compare the threshold level above which endocrine mediated responses are observed (1 - 10 ng l^{-1}) with environmental concentrations (0.5 - 14 ng l^{-1}) then MOS values of 0.07 - >20 would result in the aquatic compartment. On the basis that an MOS of 100 should be required for the risk to be acceptable then oestrone apparently presents a risk to fish (and other aquatic vertebrates) in terms of endocrine disrupting effects. This conclusion is consistent with the results of field surveys carried out in a number of European countries (for example the COMPREHEND programme) which have identified evidence of adverse effects on the development and reproductive capability of wild fish which are exposed to natural (and synthetic) steroids discharged from sewage treatment works (for review EA 2002).

- No information was located on terrestrial or aerial concentrations of oestrone.

13.9 Summary of the weight of evidence for endocrine disrupting effects in wildlife and associated uncertainties

The summary of the weight of evidence for endocrine disrupting effects of oestrone in wildlife along with associated uncertainties are given in Table 13.6.

Table 13.6 Summary of the weight of evidence conclusion and uncertainties associated with the assessment of the endocrine disrupting effects of oestrone

	Target group	
	Humans	Wildlife
Weight of evidence	Not considered in the review	In fish it appears that the effects of oestrone on reproduction and development which are considered to be endocrine mediated occur at markedly lower (and environmentally relevant) concentrations (> 1-10 ng l^{-1}) than those causing general toxicity. No effects of oestrone on the reproduction of the aquatic invertebrate copepod *Tisbe battagliai* were evident at the highest exposure concentration (100 ng l^{-1}), indicating that the processes reproduction and development in certain invertebrate taxa (crustaceans) are evidently not affected by exposure to vertebrate steroids at typical environmental levels. However this may not be the case for other invertebrate taxa. The available aquatic exposure data (showing typical concentrations of < 5 ng l^{-1}) indicates that oestrone presents a risk to fish (and other aquatic vertebrates) in terms of endocrine disrupting effects. This is consistent with data from field surveys of fish populations exposed to natural and synthetic steroids discharged from sewage treatment works.
Uncertainties	Not considered in the review	The data on oestrone induced and endocrine mediated effects on reproduction and development in wildlife is limited and restricted to aquatic organisms (invertebrates and fish). The results of an on-going multi-generational study will considerably reduce the uncertainty associated with the extent of endocrine mediated responses of fish. No data are available on potential endocrine mediated effects in terrestrial and aerial organisms. Given that sorption to organic carbon is an important process resulting in the partitioning of oestrone onto soils the absence of data on potential endocrine mediated responses in terrestrial organisms is a key area of uncertainty.

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14. REVIEW OF DATA FOR 17β-OESTRADIOL

Table of Contents

Notes:

This section contains information on the effects of oestradiol on wildlife collected and collated from a range of sources including published papers, reports of studies conducted by industrial companies or research organisations, data compliations and a review of natural and synthetic steroid oestrogens carried out by the United Kingdom Environment Agency (EA 2002).

This review has been carried out in accordance with the evaluation framework described in Section 2. In the review the International Programme for Chemical Safety (IPCS) definition of an endocrine disrupter has been adopted, namely that it is "*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*".

In the context of the review it is recognised that there are various laboratory-based *in vivo* and *in vitro* methods utilising a range of (eco)toxicological endpoints that are claimed by different sources to be relevant to the assessment of endocrine disruption in humans and wildlife. However, since this field is still in an early stage of development there is uncertainty regarding the significance of many of the current findings.

From the numerous recent reviews of potential test methods (such as the Detailed Review Paper prepared by OECD in 1997) there is a clear consensus in terms of the hierarchy of the relevance of test methods. In this hierarchy longer-term *in vivo* studies considering effects on reproduction and/or development (and including mechanistic information) are of greater relevance than short-term *in vivo* screening tests which are of greater relevance than *in vitro* assays. The greater relevance of chronic *in vivo* tests or those assessing effects during critical windows of sensitivity is also evidenced by the fact that these are the key (eco)toxicological methods being developed in the OECD Endocrine Disruption Testing and Assessment (EDTA) Programme. This hierarchy approach to data relevance has been adopted in the review along with a weight of evidence consideration of the available data.

The review has been carried out to address three key questions:

1. Does the available data indicate there is evidence that a chemical causes endocrine disrupting effects in target groups of wildlife?

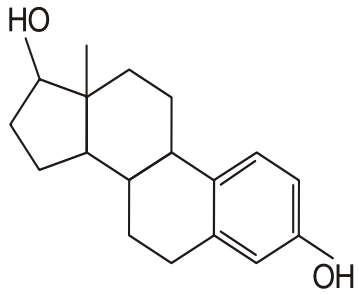
2. Do endocrine disrupting effects of the chemical in target groups of wildlife occur at lower concentrations than those causing effects on general systemic toxicological endpoints?

3. Are particular target groups of organisms in the environment likely to be exposed to concentrations of chemicals which exceed effects thresholds due to current emission patterns.

It should be recognised that this review is not designed to be a full Risk Assessment of a substance under the Existing Substances Regulation 793/93.

14.1 Physico-chemical data for 17β-oestradiol

14.1.1 Summary details on the substance

CAS Number	50-28-2
EINECS Number	-
IUPAC Name	17β-estra-1,3,5(10)-trien-3,17-diol
Other names	Oestradiol, Estradiol, 17β-Oestrone, E2
Molecular weight	272.4
Chemical formula	$C_{18}H_{24}O_2$
Chemical structure	

14.1.2 Physico-chemical properties and environmental fate information (from EA 2002)

The data on the physico-chemical properties of oestradiol and its environmental fate (see Table 14.1 indicates that 17β-Oestradiol readily undergoes biodegradation with complete loss within 14 – 28 days. Sorption is the major removal process with photolysis being of lower importance and volatilisation being negligible.

No data are available on the persistance of 17β-Oestradiol in soil, though it is likely that adsorption to organic carbon is an important process resulting in the partitioning of oestradiol onto soils.

Table 14.1 Physico-chemical properties and environmental fate data (from EA 2002)

Physico-chemical property	Value (and comments)
Physical state at ambient temperature	Solid
Water solubility	13 mg l^{-1} at 20°C
Octanol-water partition coefficient (log Kow)	2.69 - 4.0
Organic carbon water partition coefficient (log Koc)	2.78 - 3.4
Henry's Law Constant	No data
Type of degradation	
Aquatic - abiotic	Sorption is the major removal process with photolysis being of lower importance and volatilisation being negligible
Aquatic - biotic	A number of laboratory studies have indicated that 17β-Oestradiol readily undergoes biodegradation with complete loss within 14 – 28 days
Terrestrial	No data are available on the persistance of 17β-Oestradiol in soil, though it is likely that adsorption to soil is a major removal process.
Atmospheric	No data

14.2 Production and Uses

Natural steroid oestrogens (including 17β-oestradiol) are used in human medicine, particularly in hormone replacement therapy (HRT) and for the treatment of other gynaecological disorders. They are also used in the treatment of prostate and breast cancer in men and breast cancer in post-menopausal women (Martingdale 1993). 17β-Oestradiol is also used as a growth enhancer in veterinary medicine (Archand-Hoy *et al* 1998). In the USA, annual use for conjugated oestrogens (in hormone replacement therapy) and 17β-oestradiol (as a growth-enhancing hormone in agriculture) has been estimated at ~ 1.69 and 0.58 tonnes, respectively (Archand-Hoy *et al* 1998).

14.3 Toxicokinetics, metabolism and bioaccumulation

14.3.1 Toxicokinetics and metabolism

The major site of metabolism for natural oestrogens is the liver, with the two major metabolic pathways being 2-hydroxylation and 16α-hydroxylation. These pathways result in a number of metabolites, conjugated with glucuronide and/or sulphate, which are considered to be biologically inactive and largely excreted in the urine (Aldercreutz *et al* 1994, IARC 1979). The main urinary metabolites are conjugates of oestriol, 2-hydroxyoestrone, oestrone, 16α-hydroxyoestrone and oestradiol, in decreasing order of quantitative importance (IARC 1979).

Much smaller amounts of natural oestrogens are excreted in the faeces (Aldercreutz *et al* 1982). The metabolites tend to be excreted in an unconjugated form as opposed to urinary metabolites which are conjugated (Aldercruetz *et al* 1994). Aldercreutz and Järvenpää (1982)

suggest that faecal excretion represents about 5-10% of total excretion in the urine and faeces.

14.3.2 Bioaccumulation

No data has been located on the bioaccumulation of oestradiol in aquatic organisms, but this is expected to be low based on the low octanol water partition coeffcient (log Kow = 2.69 – 4.0). A related study was conducted by Larsson *et al* (1999) in which caged juvenile rainbow trout of both sexes were placed upstream and downstream of a domestic Swedish STW serving approximately 3500 people. After 2 to 4 weeks of exposure, bile was collected from the fish. Effluent water was analysed for 17β-oestradiol (and oestrone and 17α-ethinyloestradiol), all of which were found at ng l^{-1} concentrations. The bile of fish caged downstream of the STW contained all three steroid oestrogens at concentrations that were 10^4-10^6 times higher than the water concentrations (up to 2.5 μg g^{-1} bile).

14.4 Studies relevant to the assessment of potential endocrine disrupting effects

14.4.1 Studies relevant to the assessment of potential endocrine disrupting effects in humans

The effects of oestradiol on human health have not been considered in this review. This stems from the fact that oestradiol is a natural vertebrate steroid that is produced in humans and mammals from the androgenic precursor androstenedione. As such, homoestatic control mechamisms exist which normally operate to modify the levels of oestradiol within acceptable bounds.

Natural steroid oestrogens (including 17β-oestradiol) are used in human medicine, particularly in hormone replacement therapy (HRT) and for the treatment of other gynaecological disorders. They are also used in the treatment of prostate and breast cancer in men and breast cancer in post-menopausal women. The doses prescribed for these conditions are designed to operate in a specific manner where the potential adverse effects on consumers are minimised.

14.4.2 Studies relevant to the assessment of potential endocrine disrupting effects in wildlife

14.4.2.1 In vitro studies

No data on the responses of oestradiol in *in vitro* systems involving the cells and tissues from wildlife species, and assessing oestrogenicity is presented since 17β-oestradiol is generally used as the positive control in such assays and responses of other substances are calibrated against 17β-oestradiol.

14.4.2.2 In vivo studies

Table 14.2 summarises the data on the potentail endocrine disrupting effects of 17β-oestradiol on aquatic organisms.

A. Studies on aquatic organisms

In vivo studies in amphibians

Only two toxicity studies were located which have investigated the effects of waterborne 17β-Oestradiol in amphibians, (Nishimura *et al* 1997, Kloas *et al* 1999).

In the study by Nishimura *et al* (1997), African clawed toad embryos were exposed to 10^{-4} - 10 µM of 17β-oestradiol. At the highest concentration tested (2724 µg l^{-1}), survival rates decreased significantly (by stage 27) and all embryos were dead by stage 42. In addition, malformations of the head and abdomen, and suppressed organogenesis were observed at this concentration.

Kloas *et al* (1999) exposed African clawed toad (*Xenopus laevis*) tadpoles to 17β-oestradiol at nominal concentrations of 0.01 – 0.1 µM (2.74 and 27.4 µg l^{-1}, respectively) for 12 weeks. No significant differences in growth, development or survival rate were seen between the treated groups and controls. However, there was a significant increase in the percentage of females at both doses, and the higher dose resulted in almost complete feminisation.

In vivo studies in fish (Aqueous exposure)

The taxon that has received most study with regard to adverse effects of 17β-oestradiol is freshwater fish, with aqueous exposure being the route of administration.

Biochemical changes

One of the key functions of endogenous oestrogens in fish is to stimulate the induction in the liver of a large phospholipoprotein vitellogenin (Chen 1983) which is released into the blood stream and sequestered by developing oocytes for production of egg yolk (Wallace 1985, Tyler *et al* 1988, Tyler 1991). In maturing female fish, vitellogenin is a major constituent of the blood proteins, while in male fish it is not normally present in appreciable amounts. However, if male fish are exposed to oestrogens or oestrogen mimics, vitellogenin can be produced at similar levels to that found in maturing females.

There is a degree of uncertainty as to the potential ecological relevance of the induction of vitellogenin in fish. Evidence from laboratory, semi-field and field studies carried out on fish exposed to natural and synthetic steroids in aquatic systems in Europe (CSTEE 1999, NRC 1999, Cheek *et al* 2001) has shown that VTG induction in male fish is a biomarker for exposure to oestrogens and oestrogen mimics and that:

- induction in early life stage fish could have serious energetic consequences for the organisms;
- high levels of vitellogenin induction in fish are known to cause kidney failure and are associated with some haematological disturbances;
- a weak, but nevertheless significant correlation, has been shown between VTG induction in wild roach and the severity of the intersex condition in fish (that is male gonads show evidence of feminisation).

Routledge *et al* (1998) exposed adult male rainbow trout (*Oncorhynchus mykiss*) for 21 days to nominal 17β-oestradiol concentrations of 1, 10 and 100 ng l^{-1}. Analysis was undertaken to confirm the 100 ng l^{-1} test concentration, which was only 45 ng l^{-1} based on actual

concentration. Only the highest test concentration produced a significant increase in vitellogenin compared to controls. However, when the pre- and post-exposure samples of each fish were compared within each group, the 10 ng l^{-1} post-exposure group exhibited significantly higher levels of vitellogenin. It was, therefore, concluded that the threshold for vitellogenin induction in rainbow trout was between 1 and 10 ng l^{-1}. The same 21-day experiment was conducted in male and female roach (*Rutilus rutilis*) exposed to nominal concentrations of 1, 10 and 100 ng l^{-1} where a significant increase in plasma vitellogenin was only seen at the highest concentration of 100 ng l^{-1} in males.

Thorpe *et al* (2000) exposed female juvenile rainbow trout to 17β-oestradiol at nominal concentrations at 0, 1, 3.2, 10, 32, 100 and 320 ng l^{-1} for 7, 14 or 21 days. Analysis revealed measured concentrations to be less than nominal concentrations in all cases, with concentrations corresponding to <5, <5, <5, 9, 23, 72 and 247 ng l^{-1}, respectively, based on a time-weighted average for day 21. There was a dose-dependent increase in plasma vitellogenin after 7, 14 or 21 days of exposure for concentrations of <5-247 ng l^{-1} (time weighted means for day 21). In addition, there was a temporal effect (i.e. an increase in potency with duration of exposure) on plasma vitellogenin levels with 7 day LOEC and EC_{50} values of 24 and 28 ng l^{-1} decreasing to 8.9 and 15 ng l^{-1}, respectively, on day 14. There were no changes in the LOEC or EC_{50} values between days 14 and 21. The 14 day NOEC, LOEC and EC_{50} values for vitellogenin induction were thus reported as <5, 9 and 15 ng l^{-1}, respectively. Concentration-related effects on body weights or condition were not seen in any treatments over the 21-day period. The highest concentration tested (247 ng l^{-1}) resulted in an increase in the hepatosomatic index after 7, 14 and 21 days. However, none of the test concentrations had any effect on GSI values, although it was considered that the small size of the ovary in immature females relative to body weight would make any associated changes difficult to detect.

More recently, Thorpe *et al* (2001) exposed juvenile rainbow trout to measured 17β-oestradiol concentrations (0, 1, 3.2, 10. 32, 100 or 320 ng l^{-1}). Based on vitellogenin induction, LOEC and EC_{50} values of 12 and 25 ng l^{-1} were derived, which are similar to those reported previously by the author.

Panter *et al* (1998) exposed male fathead minnows (*Pimephales promelas*) to nominal concentrations of 17β-oestradiol at 10, 32, 100, 320 and 1000 ng l^{-1} for 21 days and determined the effect on plasma vitellogenin levels and gonad weight. A significant increase in plasma vitellogenin levels was seen at concentrations ≥100 ng l^{-1}. GSI values were significantly reduced at 320 and 1000 ng l^{-1} and, in comparison with the day 0 sample, testicular growth appeared to have been completely inhibited during the 21-day exposure period.

A later study investigated the effect of intermittent exposure to 17β-oestradiol in adult male fathead minnow (Panter *et al* 2000). In all cases, concentrations were nominal and the oestrogenic effects of the dosing regimes were evaluated by determination of plasma vitellogenin and changes in GSI value. Exposed male fathead minnow were dosed continuously or intermittently to 17β-oestradiol as follows:

(a) 30, 60 or 120 ng l^{-1} dosed continuously for 21 days or to 120 ng l^{-1} on alternate days or for 3 consecutive days in every six (both produced a time weighted average concentration equivalent to continuous dosing of 60 ng l^{-1}) over a total of 21 days. i.e. equal periods of exposure and 'clean water'.

(b) 30, 60 or 120 ng l^{-1} dosed continuously for 21 days or to 120 ng l^{-1} for 1 day in every 4 or for 3 consecutive days in every six (time weighted average concentration equivalent to continuous dosing of 30 ng l^{-1} and 60 ng l^{-1}, respectively) over a total of 21 days. i.e. longer intervals between exposure episodes.

(c) 120 ng l^{-1} continuously for 21 days or to 120 ng l^{-1} dosed for 3 consecutive days in every six over 21 days. After 21 days, seven fish from the control and two exposure groups were then exposed to a continuous flow of dilution water only (depuration). The remaining fish continued to be dosed under the original regimes for a further 21 days.

In experiment (a) all treatments induced a significant elevation in plasma vitellogenin when compared to the control and day 0 fish, which was dose-dependent. In addition, plasma vitellogenin in fish dosed intermittently were significantly different from the vitellogenin concentration in fish exposed continuously to the equivalent time weighted dose of 60 ng l^{-1}. Thus, the oestrogenic response was not a simple integration of concentration and duration. The corresponding GSI values showed no significant difference between exposed fish and controls, regardless of the dosing regime.

In experiment (b) exposure resulted in increased stimulation of vitellogenin synthesis in all cases, which was significantly greater than in control and 'day 0' fish. Plasma vitellogenin in fish exposed to 120 ng l^{-1} for 3 consecutive days in every six (50:50 treatment) was not significantly different from those in fish dosed continuously with 60 or 120 ng l^{-1}. In contrast, plasma vitellogenin in those exposed for 1 day out of every four (25:75 treatment) was significantly higher than continuous exposure to its corresponding equivalent time weighted average of 30 ng l^{-1}. In the 25:75 treatment, there was no evidence of inhibition of gonad growth, unlike both the continuous and 50:50 treatments in which growth inhibition was total.

Experiment (c) was designed to consider the effects of a longer period of continuous or intermittent exposure and incorporate a prolonged period of depuration for recovery. All treatment groups showed significant vitellogenin induction compared to fish at day 0 or those in the controls. Indeed, fish exposed intermittently responded as if they had been in continuous contact with 17β-oestradiol, suggesting that metabolism of vitellogenin in fathead minnow is slow. The results for GSI were considered inconclusive, due to small changes in the controls.

The absence of any significant reduction in the vitellogenin response in most of the intermittent treatments, compared with continuous exposure, suggests that this effect is not readily reversible within the time periods employed. In the 25:75 treatment (experiment b), there was no evidence of gonad inhibition, unlike both the continuous and 50:50 treatments in which inhibition was total. It was, therefore considered that this effect might be more readily reversible than plasma vitellogenin induction.

Fathead minnows were exposed to nominal 17β-oestradiol concentrations of 25, 50 and 100 ng l^{-1} during early life stages (Tyler *et al* 1999). Exposure to 17β-oestradiol was initiated in embryos (24 hr post-fertilisation) and continued throughout the 4 days until hatching and subsequently for another 30 days post-hatch. Two further groups were exposed to 100 ng l^{-1} at 24 hour post-fertilisation up until 10 and 20 days post-hatch and then maintained in clean water until the end of the experiment. At 30 days post-hatch, the survival of the larvae at all treatments ranged from 79 to 88%, with no indication of developmental abnormalities or growth inhibition due to exposure. Vitellogenin induction in fish exposed to 17β-oestradiol was dose-dependent and was significantly higher than controls at 50 and 100 ng l^{-1}.

Concentrations of vitellogenin were also significantly higher in fathead minnows exposed to 100 ng l^{-1} until 10 and 20 days post-hatch.

Folmar *et al* (2000) exposed male sheepshead minnow (*Cyprinodon variegatus*) to 17β-oestradiol for a total of 16 days, with sampling intervals of 2, 4, 7, 10, 13 days. Nominal concentrations were 20, 200, 500, 1000 and 2000 ng l^{-1} and were confirmed by radioimmunoassay and were found to be reasonably close to nominal values in all cases. 17β-oestradiol caused an upregulation of liver mRNA for vitellogenin by day 2 at 200 ng l^{-1} and also resulted in a significant induction in plasma vitellogenin over the 16 days. A NOEC of 20 ng l^{-1} was reported, although the large concentration difference between the NOEC and LOEC values needs to be recognised.

Histopathology

Miles-Richardson *et al* (1999) exposed sexually mature fathead minnows (*Pimephales promelas*) to 17β-oestradiol at nominal waterborne concentrations of 17, 27, 34, 68, 136, 272, 545, 2724, 27240 and 272400 ng l^{-1} for 19 days. These concentrations were confirmed by immunoassay and averaged 79±16% of the nominal concentration. The effects on the secondary sex characteristics (breeding tubercles and fatpads in males) and gonads were investigated. Male fathead minnows exposed to concentrations at or above 272 ng l^{-1} exhibited a reduction in size of breeding tubercles and were difficult to differentiate from females without histological examination of the gonads. Fatpads were also reduced in size in males exposed to 27240 ng l^{-1} but no significant differences were seen among fish exposed to 2724 ng l^{-1} or less. No gross changes were observed in exposed females exposed to any of the test concentrations. It should be noted that females do not have the prominent secondary sexual characteristics which males exhibit and therefore, the lack of effects on female morphology was not unexpected. Exposure of males caused alteration in the morphology of seminiferous tubules, which included proliferation of the Sertoli cells and degenerative changes, and which were significant at concentrations ≥136 ng l^{-1}. Statistically significant differences were seen in follicular development in female fish exposed to ≥27 ng l^{-1}. It was concluded that the LOEC values based on adverse histological effects were 27 and 136 ng l^{-1} for females and males, respectively; the corresponding NOEC values being 17 and 68 ng l^{-1}, respectively. It is unclear how such effects would impact on reproduction but, nonetheless, the study demonstrates that effects on female gonads cannot be ignored and also occur at low ng l^{-1} levels.

Toft and Baatrup (2001) exposed adult male guppies (*Poecilia reticulata*) to nominal 17β-oestradiol concentrations of 30, 100 and 1000 ng l^{-1} for 30 days. Behaviour, morphological changes, sperm count, body coloration and gonopodial length were measured in all fish. There was no difference in behaviour or obvious morphological changes except that the body colouration started to fade in the fish exposed to the highest concentrations of 17β-oestradiol. Unexpectedly, an increase in the number of ejaculated sperm cells was seen in all groups, the result being significant in the highest test concentration of 1000 ng l^{-1}. The colouration index was also affected in all groups but was only significantly lower at the 1000 ng l^{-1} test concentration. A further 30 day exposure was investigated in the 30 and 100 ng l^{-1} groups. Again the ejaculates of all treated fish still contained larger number of sperm cells than controls, although when compared with the counts after the first 30 day exposure, all treated fish now contained 5 to 20% fewer sperm cells in their ejaculate. Both treated groups had significantly smaller colouration indices than the controls. Although the GSI values were decreased compared to controls, this was not dose-related and was only significant ($p=0.04$) at the lowest test concentration of 30 ng l^{-1}. The relative length of the gonopodium was

unaltered by any of the treatments. This study is at variance with all other studies of 17β-oestradiol on gonad growth (albeit in other species) where a dose-dependent decrease has been observed but only at higher doses (>100 ng l^{-1}).

After exposure had ceased, 10 males were selected randomly from the 100 ng l^{-1} test group and transferred to mate with a virgin female. Each of the 10 females that were mated with the control males gave birth to an average of 6.2 young, whereas each female mated with a treated male only gave birth to an average of 1.8 young. This corresponds to 29% of the offspring produced in the control group. Animals exposed to 1000 ng l^{-1} were returned to clean water in order to study the reversibility of chemical-induced changes. Colouration, sperm count and gonodopodial length were measured after 1 and 3 months recovery and compared to a control group. Nearly twice the number of sperm cells in their ejaculates was seen in treated fish compared to controls. In addition, the colouration index remained significantly smaller than in the control group after 3 months, although the natural orange colour of the spots was fully regained during this period.The most likely explanation postulated for the increase in sperm count was that spermatozoa accumulated in the testis and sperm duct, and were driven out only because of the stripping. The effect on male orange colouration is likely to affect sexual behaviour given that females are known to be attracted to those males which are most coloured.

Changes in reproductive success and development

Duplicate groups of six fathead minnow (*Pimephales promelas*) fish (3 male and 3 female) were exposed to waterborne 17β-oestradiol at nominal concentrations of 27.24, 272.4 and 2724 ng l^{-1} for 19 days (Kramer *et al* 1998). Dissolved test concentrations, measured throughout the exposure period using immunoassay, showed high variability though measured concentrations averaged 79 ± 25% of the nominal concentration. Consequently results were analysed using linear and non-linear regression techniques. Egg production was reduced in a concentration manner and the EC_{50} for inhibition of egg production was 120 ± 16 ng l^{-1}. Although the mechanism was unclear, it is likely that the egg inhibition related to effects both in males and females (since both sexes were studied as a single experimental unit). Plasma vitellogenin was significantly induced in both males and females; the 19 day EC_{50} for induction of vitellogenin in males was 251 ng l^{-1}, but no vitellogenin induction plateau was observed in females and so no EC_{50} could be calculated. In addition, plasma vitellogenin concentration in males was inversely but weakly related to egg production (r^2 = 0.46), although this could not be described by a simple linear or curvilinear relationship. The relationship was much stronger in females (weighted linear regression r^2 = 0.81). Haematocrit, a non-specific indicator of fish health, was reduced in a concentration-response manner, the relationship being stronger in males than females (respective EC_{50} values of 812 and 741 ng l^{-1} were reported).

In a sex-reversal experiment, 50 male and 50 female sexually mature fathead minnows were exposed to a nominal concentration of 2724 ng l^{-1} of 17β-oestradiol for 10 days (Miles-Richardson *et al* 1999). Samples were taken from males on the final day of exposure and over a period of 16 weeks after exposure. As in previous experiments, exposure to this concentration induced gross changes in male secondary sex characteristics consisting of atrophy of the fatpad and breeding tubercules. For about 3 months post-exposure fish had secondary sex characteristics which remained at reduced size. Males exhibited morphological changes of Sertoli cells, which became less progressively pronounced from week 0 to week 16 post-exposure. Furthermore, no lesions were observed beyond 16 weeks post-exposure indicating that such changes were reversible.

In a study by Nimrod and Benson (1998) Japanese medaka (*Oryzias latipes*) larvae (5-8 days old) were exposed to measured 17β-oestradiol concentrations of 10, 120 and 1660 ng l^{-1} for 28 days following hatch. Following a further 55-day period in dilution water only, all fish in all three treatment groups were identified as female (using both external characteristics such as anal fin size and histological observation of the gonads). Subsequently, no egg batches were observed in any of the treated fish. No treatment-related differences were seen in either weight or length of fish. Fish were examined for their capability to spawn by placing them, one at a time, with a single unexposed male (taken from the breeding culture). Egg production was examined each morning and if spawning had occurred, batch size (number of eggs) was noted. If no eggs appeared, the male was exchanged for another unexposed one. In this reproductive phase, all females produced eggs except one female exposed to the highest dose. When the number of eggs was analysed, the group receiving the highest dose yielded significantly fewer eggs compared to the solvent control. It was, therefore, concluded that a concentration of 10 ng l^{-1} for 28 days was sufficient to result completely in female phenotypes in genetic males and that a decreased fecundity (based on egg production) was observed at 1660 ng l^{-1}.

Shioda and Wakabayashi (2000b) exposed adult female Japanese medaka to nominal 17β-oestradiol concentrations of 2.7, 27, 272, 2740 and 27 400 ng l^{-1} for 2 weeks. Following breeding with unexposed males, no eggs were spawned in female fish exposed to ≥2740 ng l^{-1}. The number of hatched eggs decreased significantly at 27 ng l^{-1} and the number of eggs both spawned and hatched decreased at levels of ≥ 272 ng l^{-1}. This study again illustrates that effects of steroid oestrogens such as 17β-oestradiol are not only important for male fish but that the impact on female reproduction is equally important.

Metcalfe *et al* (2001) exposed Japanese medaka early life stages (1 day after hatch) to nominal concentrations of 0, 1, 10, 100 and 1000 ng l^{-1} until medaka reached approximately 1.5 cm in length (which occurred at 85-110 days post hatch). The exposure period between renewals of test solutions was 48 hours and endpoints monitored included growth, general condition, alterations to sex ratios and the development of testis-ova. At 100 ng l^{-1}, the sex ratio was also significantly different from controls with a greater proportion of females. All 15 medaka identified as males developed the intersex condition, testis-ova. Several pathological lesions were observed in the kidney and liver, and all surviving fish were female and significantly larger relative to other treatments. Eosinophilic fluid was observed in organs and body cavity in fish exposed to 100 ng l^{-1}. At the 10 ng l^{-1} test concentration, testis-ova were observed in 3 of 30 males but there was no difference in the sex ratio. However, given that the natural occurrence of intersex has not been clearly established in this species, it is difficult to determine whether the small observed incidence of intersex medaka is a consequence of exposure. No testis-ova or difference in sex ratio was evident at the lowest concentration of 1 ng l^{-1}. It was concluded that the NOEC and LOEC values were 1 and 10 ng l^{-1} based on testis-ova induction. It is of note that a separate study was conducted to establish the degradation of 17β-oestradiol under the conditions used in the bioassays. Following analysis by GCMS/MS, concentrations of 10 and 1000 ng l^{-1} were found, on average, to be 42.9% of the nominals over a period of 0, 24 and 48 hours which was accompanied by increasing concentrations of oestrone. Although the study gives some indication of the relative stability of 17β-oestradiol, the authors attempted to report all of the bioassay results as 'actuals' on the basis of these findings which is considered inappropriate.

Tabata *et al* (2001) conducted experiments on Japanese medaka, which included the induction of female-specific proteins in adult males and an assessment of the influence on sex determination following exposure during early life stages. Adult males were continuously

exposed to nominal concentrations of 17β-oestradiol (5, 50 or 1000 ng l^{-1}) for up to 5 weeks under flow-through conditions. Following 1 week of exposure, female-specific proteins (largely vitellogenin) were detected in the blood of all males exposed to 50 ng l^{-1}. In the case of males treated with 5 ng l^{-1}, this was also observed but not until 5 weeks of exposure. In the second study, hatched medaka embryos were exposed to 100 - 100 000 ng l^{-1} in a static-renewal system for 200-230 days until the pre-mature stage. Numbers of males and females were counted based on observation of anal fins (secondary sex characteristic) and histological examination of the gonads for testes-ova. The lowest test concentration of 100 ng l^{-1} did not alter the sex ratio significantly (7 males: 11 females), with 5 males showing abnormalities in the gonads and 1 exhibiting the intersex condition. However, at 1000 ng l^{-1}, an exclusively female population (0 males:19 females) was observed.

Nash and Kime (2000) have conducted a multi-generation study in which zebrafish were exposed to 5 ng l^{-1} of 17β-oestradiol, although the full findings are yet to published. The study was conducted under flow-through conditions and offered a high reproducibility as there were 12 replicated breeding populations in the controls and the exposure group (each breeding colony consisted of 12 adults). Male and female F_0 adults were exposed to 17β-oestradiol for 4 weeks during spawning and the subsequent F_1 generation exposed throughout their lifetime. The embryo viability of F_1 progency (F_2 generation) was also investigated. In addition, various sub-exposures were conducted throughout the experiment. These included a cessation of 17β-oestradiol exposure at 52 days in the F_1 generation followed by a 5 month period in clean water before egg collection, and exposure of the F_0 generation only followed by an investigation of effects on the F_1 generation. Steroid concentrations were confirmed by ELISA analysis and end-points measured included egg production, embryo viability, survival to adulthood, sex ratio and adult gonadal histology.

For the F_0 generation, mean egg number and 14 hour egg mortality were measured for 10 days prior to exposure and no significant differences were seen between the controls and treatment groups (background mortality was <10%). Following a 28 day exposure period, no significant effects were seen on either egg production or mortality. No adverse effects were seen on embryo mortality or viability (deformities or development) in the F_1 generation. In addition, there were no differences in survival or weight of the F_1 juveniles (at 52 days), indicating the development of healthy adults. The reproductive success (egg production and egg mortality) of the F_1 generation was determined at 7 months. No adverse effects were seen on egg production, although there was a slight, but statistically significant, increase (20% vs. 10% in controls) in 14 hour egg mortality. No effects on F_1 reproductive success (egg production or mortality) were seen when exposure ceased at 52 days and was followed by a period of 5 months in clean water. In addition, no adverse effects on reproductive success of F_1 generation were observed following exposure of F_0 only. The results for gonadal histology and vitellogenin induction results have yet to be published.

Brion *et al* (2001, 2002) exposed zebrafish *Danio rerio* for 21 days to nominal concentrations of 5, 25 or 100 ng l^{-1} of 17β-oestradiol encompassing either their embryo-larvae (from fertilisation to 21 days post-fertilisation (dpf)), juvenile (from 21 dpf to 42 dpf) or adult stages (>200 dpf). Actual concentrations were confirmed by GC/MS (>80% of the nominals) and the experiment was conducted as single tank replicates. Whole body vitellogenin concentrations and gonadal development were analysed for treatments groups at various sampling points as follows: embryo-larvae (21 dpf, 42 dpf, 160 dpf), juveniles (42 dpf, 160 dpf) and adults (after exposure). Effects on secondary characteristics (manifestation of uro-genital papillae in males), gonadal growth and sex ratio were measured in adult fish for all treatment groups. In addition, for all the different life-stage exposures, reproductive performance of the F_0

generation was assessed (egg production) along with survival and development of the F_1 embryo-larvae.

Vitellogenin induction was observed in all exposed life-stage, although these effects were reversible after depuration. The effective concentration for vitellogenin induction during early-life stage exposure was 100 ng l^{-1}. In contrast, following adult exposure, the effective concentration for vitellogenin induction was ≥ 25 ng l^{-1} (males) and 100 ng l^{-1} (females). In the juvenile fish exposures, gonad histology revealed an increasing feminisation of the reproductive duct that was dose-dependent. At the test concentrations of 25 and 100 ng l^{-1}, 70% and 100% of the fish showed indications of female-like ducts whereas at 5 ng l^{-1}, this was only seen in 50% which was similar to the controls (Tyler, pers. comm 2001*)*. In addition, there was a delay in the age of the fish at their first laying when exposed to 100 ng l^{-1}. Following exposure during the embryo-larvae life stage, the sex ratio of subsequent adults (sampled at 160 dpf) was skewed towards females at 100 ng l^{-1} and an increased egg production was also observed at this dose. No effects on sex ratio or secondary sex characteristics were seen in adults following exposure during their juvenile life-stage, although a decrease in egg production was observed at test concentrations of 25 and 100 ng l^{-1}. Exposure of adult fish resulted in a modification of the secondary sexual characteristics in males at 25 and 100 ng l^{-1} as well as a dose-dependent inhibition of egg production. The design of the experiment (as single tank replicates) does not allow the results on egg production to be subjected to statistical analysis.

Hahlbeck *et al* (2001) conducted a semi-static study in which juvenile sticklebacks (*Gasterosteus aculeatus*) were exposed to 10 and 1000 ng l^{-1} 17β-oestradiol for 40 days. However, the full findings are yet to be finalised. At an exposure concentration of 10 ng l^{-1} there was no evidence of intersex or sex reversal, and at 1000 ng l^{-1} intersex condition or sex reversal in males was observed with a LOEC of 50 ng l^{-1}.

Changes in behaviour

The effects of oestradiol on fish sexual behaviour have been considered, along with biochemical and histopathological studies in a study of guppies (*Poecilia reticulata*) by Toft and Baatrup (2001).

In vivo studies in fish (Intra-peritoneal or intra-muscular injection)

A number of studies have investigated the effects of steroid hormones in fish when dosed via intraperitoneal or intramuscular injection (e.g. Van Bohemen *et al* 1982, Björnsson and Haux 1985, Flett and Leatherland 1989, Purdom *et al* 1994, Nimrod and Benson 1996, Madsen *et al* 1997, Hansen *et al* 1998, Christiansen *et al* 1998a,b, Papoulias *et al* 1999, Schwaiger *et al* 2000, Flammarion *et al* 2000, Westerlund *et al* 2000 and Solé *et al* 2000b) or by incorporation into the diet (e.g. Komen *et al* 1989, Herman and Kincaid 1991, 1988, Chang and Lin 1998, Blázquez *et al* 1998, Carlson and Williams 1999, Cooke and Hinton 1999 and Bjerselius *et al* 2001). These studies have demonstrated the oestrogenic activity of the steroid hormones but, whilst injection and feeding are convenient routes of administration, they are not the most relevant routes of exposure. Indeed, due to the likely kinetic difference (e.g. uptake, bioavailability), such studies are difficult to interpret in relation to exposure via water.

In vivo studies in invertebrates

Only five studies were located which have investigated the effect of waterborne 17β-oestradiol on potential endocrine mediated responses in marine invertebrates (Billinghurst *et al* 1998,

Andersen *et al* 1999, Hutchinson *et al* 1999, Anderson *et al* 2001 and Breitholtz and Bengtsson 2001).

Billinghurst *et al* (1998) exposed cypris larvae of the barnacle (*Balanus amphridite*) to nominal concentrations of 0.1, 1 and 10 μg l^{-1} of 17β-oestradiol for 24 hours. Settlement of the larvae was significantly reduced at all three test concentrations. In a second experiment, after 48 hours of exposure to a nominal concentration of 0.1 or 1 μg l^{-1}, replacement by clean water was undertaken in order to allow for a recovery period. Following the initial exposure, the percentage settlement was determined after 48, 72 and 96 hours and was only significantly lower than controls when exposed to a concentration of 1 μg l^{-1}.

In a life-cycle study, Anderson *et al* (1999) exposed *Acartia tonsa* (Crustacea, Copepoda) to a nominal concentration of 17β-oestradiol of 23 μg l^{-1} and found a significant stimulation of the relative daily egg production rate on day 10 of exposure. Although such an effect was also seen on days 9 or 11, this was not significant. An initial range finding experiment (covering 0.2-100 μg l^{-1}) had previously indicated that a concentration of approximately 20 μg l^{-1} produced a maximal induction of egg production.

Hutchinson *et al* (1999) conducted life-cycle studies investigating the effects of 17β-oestradiol on *Tibse battagliai* (Crustacea, Copepoda). Newly released (<24 h old) animals were exposed to nominal concentrations 0.1, 1, 10 and 100 μg l^{-1} over 21 days and effects monitored in terms of development, sex ratio and fecundity. None of the concentrations had an adverse effect on these life-cycle parameters and, thus, a 21-day of NOEC of ≥100 μg l^{-1} was reported.

Anderson *et al* (2001) tested the inhibitory effect of 17β-oestradiol to *Acartia tonsa* larval development. In the 5 day larval development test, EC_{10} and EC_{50} values were 370 and 720 μg l^{-1}, respectively.

Breitholtz and Bengtsson (2001) investigated the effects of 17β-oestradiol on *Nitocra spinipes* (Crustacea, Copepoda). Newly released (<24 h old) animals were exposed to nominal 17β-oestradiol concentrations of 1.6, 16 or 160 μg l^{-1} for up to 18 days and effects monitored in terms of larval development rate, sex ratio and fecundity. None of the test concentrations had a significant adverse effect on these life-cycle parameters.

Information is not available on the potential endocrine mediated responses of 17β-oestradiol in a wide range of invertebrate taxa.

B. Studies on terrestrial organisms

No information has been located on the potential endocrine disrupting effects of 17β-oestradiol on terrestrial species. Given that sorption to organic carbon is an important process resulting in the partitioning of 17β-oestradiol onto soils the absence of data on potential endocrine mediated responses in terrestrial organisms is a key area of uncertainty.

C. Studies on aerial organisms

No information has been located on the potential endocrine disrupting effects of 17β-oestradiol on aerial species. Given that 17β-oestradiol is not considered to be volatile the

absence of data on potential endocrine mediated responses in aerial organisms is not a key area of uncertainty.

D. Summary of potential endocrine disrupting effects in wildlife

The data on potential endocrine mediated responses of 17β-oestradiol in wildlife is limited and restricted to aquatic organisms (amphibians, fish and invertebrates) An assessment of aquatic toxicity data indicates that, of the taxa for which data are available, fish are the most sensitive to the adverse effects of 17β-oestradiol. However, relatively few aquatic vertebrate taxa (where steroid oestrogens play a central role in reproduction), notably amphibians, have been studied in this regard and so this remains an area of uncertainty.

Laboratory studies in fish have measured a variety of end-points, although the ecological significance of some has not yet been fully established (e.g. plasma vitellogenin induction and effect on gonadosomatic index). Nevertheless, recent research in wild roach populations indicates that marked histological effects on the gonads, such as a severe intersex condition, can result in a reduced reproductive capacity. Alteration of the timing of maturation is also important in terms of reproductive success, as it may result in gametes being released outside the optimal breeding season and subsequently reduced recruitment. However, there can be no doubt that irreversible effects on reproductive parameters such as the production of markedly skewed sex ratios or single sex generations, marked reductions in egg production, significant increases in egg mortality and reduced fertilisation success are clearly of ecological significance, and have been shown to be sensitive end-points for vertebrate steroids.

The multi-generation study by Nash and Kime (2000)in zebrafish (*Danio rerio*) appeared to be a robust study, although the full findings are yet to be published. The main limitation of the study is that fish were only exposed to one test concentration of 5 ng l^{-1}. However, this exposure concentration resulted in a slight, but statistically significant, increase in egg mortality (20% compared to 10% in the controls).

The study by Brion *et al*. (2002) which involved early life stages of zebrafish (*Danio rerio*) being exposed to 17β-oestradiol for 3 weeks demonstrated a number of adverse effects at concentrations ≥ 25 ng l^{-1} (test concentration confirmed by analysis). These included gonad disruption (juvenile exposure), a decrease in egg production (juvenile and adult exposures), modification of secondary sexual characteristics (adult exposure) and significant vitellogenin induction in males (adult exposure). Whilst the reduction in egg production was seen in a concentration-dependent manner, the design of the experiment (use of pseudo-replicates in a single tank) did not allow formal statistical analysis to be performed.

Nimrod and Benson (1998) exposed early life stages (5-8 day larvae) for 28 days and reported a LOEC value of 10 ng l^{-1} based on 100% feminisation of Japanese medaka (*Oryzias latipes*). Clearly this would be expected to have adverse implications for recruitment. However, there are some concerns regarding the interpretation of this study, which is largely due to the fact the interval separating the three test concentrations was large (over two orders of magnitude). Consequently, a dose-response curve for alteration of sex ratio was not reported as even the lowest test concentration resulted in 100% feminisation.

It is of note that the alteration of sex ratio in the Nimrod and Benson study occurred at a much lower concentration compared to other studies involving early life stage Japanese medaka, and which were also of a longer duration but based on nominal concentrations. For example, Metcalfe *et al*. (2001) did not report any differences in sex ratio in Japanese medaka exposed

to a nominal concentration of 10 ng l^{-1} of 17β-oestradiol following 185 days exposure. Similarly, Tabata *et al*. (2001) did not report a significance difference in sex ratio following exposure of hatched medaka embryos to a nominal concentration of 17β-oestradiol of 100 ng l^{-1} for 200-230 days. Only at the much higher concentration of 1000 ng l^{-1} was an exclusively female population observed.

There are only a few other studies available which have studied egg production, fertilisation or hatchability of eggs (Kramer *et al* 1998 and Shioda and Wakabayshi 2000a,b). Kramer *et al* (1998) reported an EC_{50} of 120 ng l^{-1} based on reduction in egg production in fathead minnow following 19 days exposure of males and females. This result was based on measured concentrations, although high variability of test concentrations was evident. In the studies by Shioda and Wakabayshi (2000a,b), adult male Japanese medaka were exposed to varying nominal concentrations of 17β-oestradiol for 2 weeks after which they were kept together with unexposed females for spawning. Results indicated that exposure to ≥817 ng l^{-1} caused a significant decrease in the number of eggs and hatchings. However, when adult female Japanese medaka were exposed for 2 weeks and then mated with unexposed males, the number of hatched eggs decreased significantly at a much lower concentration of 27 ng l^{-1}. The number of eggs both spawned and hatched decreased at concentrations of ≥272 ng l^{-1}. This study illustrates that the effects of 17β-oestradiol are not only important for male fish but that the impact on female reproduction is equally important.

The lowest LOEC for vitellogenin induction based on measured concentrations, was for juvenile female rainbow trout following a 14 day exposure (Thorpe *et al* 2000). The LOEC was reported at 9 ng l^{-1}. Other studies, albeit based on nominal concentrations, support this finding, for example a LOEC of 10 ng l^{-1} in male adult rainbow trout (Routledge *et al* 1998) and a LOEC of 5 ng l^{-1} in adult male Japanese medaka (Shioda and Wakabayashi 2000b).

Overall the combined dataset for potential endocrine mediated responses on fish indicates that the threshold level above which potential effects are observed is in the range 5 –25 ng l^{-1} based on responses of a number of different endpoints.

Studies on the effects of 17β-oestradiol on the development and reproduction of the copepods *Tisbe battgaliai* (Hutchinson *et al* 1999, Breitholtz and Bengtsson 20001) showed no adverse effects up to concentrations of 160 μg l^{-1}. The lowest recorded NOEC for effects in invertebrates was 0.1 μg l^{-1} in a study of the reduction in the settlement of cypris larvae of the barnacle *Balanus amphridite* (Billinghurst *et al* 1998). However, the extent to which this endpoint is endocrine mediated is uncertain, but even if it was relevant the threshold for effects would still be higher than those for fish. Information is not available for a wide range of invertebrate taxa.

For amphibians exposure concentrations at 27.4 μg l^{-1} were required for effects, namely a change in sex ratios with incresed numbers of females (Kloas *et al* 1999).

Table 14.2 Summary of data on potential endocrine disrupting effects in aquatic organisms following exposure through the water column

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Fish						
Fathead minnow (*Pimephales promelas)*	Adult, male and female	Flow-through; 0, 17, 27, 34, 68, 136, 272, 545, 2724, 27240 and 272400 ng l^{-1} (Measured concentrations)	Significant changes in morphology of males based on atrophy of breeding tubules (relative to the controls) at 272 ng l^{-1} after 19 days exposure, with a NOEC of 136 ng l^{-1}. Significant changes in histopathology of male testis based on an alteration in the morphology of seminiferous tubules induced proliferation of the Setoli cells and degenerative changes (relative to the controls) at 136 ng l^{-1} after 19 days exposure, with a NOEC of 68 ng l^{-1}. Significant changes in histopathology of female ovaries based on a significant difference in follicular development (relative to the controls) at 27 ng l^{-1} after 19 days exposure, with a NOEC of 17 ng l^{-1}.	Miles-Richardson *et al* (1999)	Medium	Valid
	24 hr post-fertilisation	Flow-through; 0, 25, 50 or 100 ng l^{-1} (Nominal concentrations)	No significant change in development or growth of larvae (relative to the controls) at >100 ng l^{-1} after 35 days exposure, with a NOEC of >100 ng l^{-1}. Significant plasma vitellogenin induction (relative to the controls) at 50 ng l^{-1} after 35 days exposure, with a NOEC of 25 ng l^{-1}.	Tyler *et al* (1999)	Medium	Use with care

Table 14.2 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Fathead minnow (*Pimephales promelas)*	Adult, male	Flow-through; 0, 10, 32, 100, 320 and 1000 ng l^{-1}. (Nominal concentrations)	Significant increase in plasma vitellogenin (relative to the controls) at 100 ng l^{-1} after 21 days exposure, with a NOEC of 32 ng l^{-1}. Significant change in Gonadosomatic Index (relative to the controls) at 320 ng l^{-1} after 21 days exposure, with a NOEC of 100 ng l^{-1}.	Panter *et al* (1998)	Medium	Use with care
	Adult, male and female	Flow-through; 0, 27.24, 272.4, 2724 ng l^{-1}. (Measured concentrations)	Significant change in egg production (relative to the controls) at 120 ng l^{-1} (EC_{50}) after 19 days exposure. Significant plasma vitellogenin induction (males) (relative to the controls) at 251 ng l^{-1} (EC_{50}) after 19 days exposure.	Kramer *et al* (1998)	Medium	Valid
Guppy (*Poecilia reticulata*)	Adult, male	Flow-through; 0, 30, 100 and 1000 ng l^{-1} (Nominal concentrations)	Significant change in colouration index (relative to the controls) at 1000 ng l^{-1} after 30 days exposure, with a NOEC of 100 ng l^{-1}. Significant change in colouration index (relative to the controls) at 30 ng l^{-1} after 60 days exposure, with a NOEC of <30 ng l^{-1}. Significant reduction in birth rate (29% of that of the controls) after treated males were randomly selected to mate with a naïve females (relative to the controls) at 100 ng l^{-1} after 60 days exposure.	Toft and Baatrup (2001)	Medium	Use with care
Japanese medaka (*Oryzias lapites*)	Adult, male	Flow-through; 0, 5, 50 or 1000 ng l^{-1} (Nominal concentrations)	Significant plasma vitellogenin induction (relative to the controls) at 50 ng l^{-1} after 7 days exposure, with a NOEC of 5 ng l^{-1}. Significant change in plasma vitellogenin (relative to the controls) at 5 ng l^{-1} after 35 days exposure, with a NOEC of <5 ng l^{-1}.	Tabata *et al* (2001)	Medium	Use with care

Table 14.2 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Japanese medaka (*Oryzias lapites*)	Larvae (5-8 day post-hatch)	Flow-through; 0, 10, 120 and 1660 ng l^{-1} (Measured concentrations)	No significant change in growth (relative to the controls) at 1660 ng l^{-1} after 28 days exposure, (and 55 days in clean water) with a NOEC >1660 ng l^{-1}. Significant change in egg production (relative to the controls) where all females, except one, produced eggs when paired with naïve males at 1660 ng l^{-1}after 28 days exposure, with a NOEC of 120 ng l^{-1}. Significant change in sex (100% identified as female) (relative to the controls) at 10 ng l^{-1} after 28 days exposure.	Nimrod and Benson (1998)	High	Valid
	1 day post-hatch	Semi-static; 0, 1, 10, 100, 1000 ng l^{-1} (Nominal concentrations)	Significant change in the condition of surviving fish (showing general odema and pathological effects in the kidney and liver relative to the controls) at 100 ng l^{-1} after 85 to 110 days exposure, with a NOEC of 10 ng l^{-1}. Significant change in growth with surviving females being larger (relative to the controls) at 1000 ng l^{-1} after 85 to 100 days exposure, with a NOEC of 100 ng l^{-1}. Significant change in sex ratio, with a higher percent female (relative to the controls) at 100 ng l^{-1} after 85 to 100 days exposure, with a NOEC of 10 ng l^{-1}. No significant change in sex ratio, although 3 of 30 males developed testis-ova (relative to the controls) at 10 ng l^{-1} after 85 to 100 days exposure, with a NOEC of 1 ng l^{-1}.	Metcalfe *et al* (2001)	Medium	Use with care

Table 14.2 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study validity
Japanese medaka (*Oryzias lapites*)	Adult, female	Semi-static; 0, 2.7, 27, 272, 2740 and 27400 ng l^{-1} (Nominal concentrations)	Significant decrease in egg production (relative to the controls) at 272 ng l^{-1} after 14 days exposure, with a NOEC of 27 ng l^{-1}. Significant reduction in hatching success of eggs (relative to the controls) at 27 ng l^{-1} after 14 days exposure, with a NOEC value of 2.7 ng l^{-1}.	Shioda and Wakabayashi (2000b)	Medium	Use with care
	Early Life-stage	Semi-static; 0, 100 or 1000 ng l^{-1} (Nominal concentrations)	Significant change in sex ratio (100% female) (relative to the controls) at 1000 ng l^{-1} with a NOEC value of 100 ng l^{-1}.	Tabata *et al* (2001)	High	Use with care
Rainbow trout (*Oncorhynchus mykiss*)	Adult, male	Flow-through; 0, 1, 10 and 100 ng l^{-1} (Analysis of 100 ng l^{-1} ~ 45 ng l^{-1})	Significant plasma vitellogenin induction (relative to the controls) at 100 ng l^{-1} after 21 days exposure, with a NOEC of 10 ng l^{-1}. Significant plasma vitellogenin induction (relative to the controls) at 10 ng l^{-1} after 21 days exposure, with a NOEC of 1 ng l^{-1}.	Routledge *et al* (1998)	Medium	Use with care
	Juvenile, female	Flow- through; 0, 1, 3.2, 10, 32, 100 or 320 ng l^{-1} (Measured concentrations)	Significant plasma vitellogenin induction (relative to the controls) at 12 ng l^{-1} after 14 days exposure.	Thorpe *et al* (2001)	Medium	Valid

Table 14.2 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Rainbow trout (*Oncorhynchus mykiss*)	Juvenile, female	Flow-through; 0, <5, 9, 23, 72 and 247 ng l^{-1} (Measured concentrations)	Significant plasma vitellogenin induction (relative to the controls) at 24 ng l^{-1} after 7 days exposure, with a NOEC of 9 ng l^{-1}. Significant plasma vitellogenin induction (relative to the controls) at 9 ng l^{-1} after both14 and 21 days exposure, with a NOEC of <5 ng l^{-1}. Significant change in hepatosomatic index (relative to the controls) at 247 ng l^{-1} after 7, 14 and 21 days exposure. No significant change in gonadosomatic index (relative to the controls) at 247 ng l^{-1} after 7, 14 and 21 days exposure.	Thorpe *et al* (2000)	Medium	Valid
Roach (*Rutilus rutilus*)	Adult, male	Flow-through; 100 ng l^{-1} (Nominal concentrations)	Significant increase (relative to the controls) in plasma vitellogenin at 100 ng l^{-1} after 21 days exposure, with a NOEC of 10 ng l^{-1}.	Routledge *et al* (1998)	Medium	Use with care
	Adult, female	Flow-through; 100 ng l^{-1} (Nominal concentrations)	No significant effect (relative to the controls) on plasma vitellogenin at 100 ng l^{-1} after 21 days exposure, with a NOEC of >100 ng l^{-1}.	Routledge *et al* (1998)	Medium	Use with care
Sheepshead minnow (*Cyprinodon variegatus*)	Adult, male	20, 200, 500, 1000 and 2000 ng l^{-1} (Measured concentrations)	Significant upregulation of liver mRNA for vitellogenin, (relative to the controls) at 200 ng l^{-1} after 2 days exposure, with a NOEC of 20 ng l^{-1}. Significant induction in plasma vitellogenin (relative to the controls) at 200 ng l^{-1} after 16 days exposure, with a NOEC of 20 ng l^{-1}.	Folmar *et al* (2000)	Medium	Valid

Table 14.2 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Stickleback (*Gasterosteus aculeatus*)	Juvenile	Semi-static; 0, 10 and 1000 ng l^{-1} (Nominal concentrations)	Significant intersex condition or sex reversal in males (relative to the controls) at 50 ng l^{-1} after 40 days exposure, with a NOEC of 10 ng l^{-1}.	Hahlbeck *et al* (2001)	Medium	Use with care
Zebrafish (*Danio rerio*)	Embryo-larvae, juveniles and adults.	0, 5, 25 and 100 ng l^{-1} (Nominal concentrations)	Significant vitellogenin induction during early-life stage exposure and juveniles (relative to the controls) at 100 ng l^{-1} after 21 days exposure, with a NOEC 25 ng l^{-1}. Significant vitellogenin induction in adults (relative to the controls) at 25 ng l^{-1} and 100 ng l^{-1} for males and females respectively, after 21 days exposure, with corresponding NOECs of 5 ng l^{-1} and 25 ng l^{-1}. Significant gonadal disruption in juveniles (relative to the controls) at 25 ng l^{-1} after 21 days exposure, with a NOEC of 5 ng l^{-1}. Significant modification of secondary sex character-istics in adult males (relative to the controls) at ≥25 ng l^{-1} after 21 days exposure, with a NOEC of 5 ng l^{-1}. Significant decrease in egg production (relative to the controls) at ≥25 ng l^{-1} after 21 days exposure, with a NOEC of 5 ng l^{-1}.	Brion *et al* (2002)	Medium	Use with care
	Adult	Flow-through; 0, 5 ng l^{-1} (Nominal concentrations)	No significant effects on embryo survival, viability and development of juveniles of resulting F1 progeny (relative to the contols) at 5 ng l^{-1}after 28 days exposure, hence, NOEC 5 ng l^{-1}. No significant effects on egg production (relative to the contols) at 5 ng l^{-1} after 7 months, with a NOEC of <5 ng l^{-1}.	Nash and Kime (2000)	High	Use with care

Table 14.2 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Invertebrates						
Barnacle (*Balanus amphridite*)	Cypris larvae	0, 0.1, 1 and 10μg l^{-1} (Nominal concentrations)	Significant reduction in settlement of larvae (relative to the contols) at 0.1 μg l^{-1} after 24 hours exposure. Significant reduction in settlement of larvae (relative to the contols) at 1 μg l^{-1} after 48 hours exposure, with a NOEC of 0.1 μg l^{-1}.	Billinghurst *et al* (1998)	Low	Use with care
Copepod (*Acartia tonsa*)	Larvae	Up to 1000 μg l^{-1} (Nominal concentrations)	In a 5 day larval development test, EC_{10} and EC_{50} values of 370 μg l^{-1} and 720 μg l^{-1} , respectively, were reported.	Anderson *et al* (2001)	Medium	Use with care
Copepod (*Acartia tonsa*)	Larvae	0, 23 μg l^{-1} (Nominal concentrations)	Significant stimulation of the daily egg production rate (relative to the controls) at 23 μg l^{-1}after 10 days exposure.	Anderson *et al* (2001)	Medium	Use with care
Copepod (*Nitocra spinipes*)	Adult, juveniles (<24 hrs old)	0, 1.6, 16, or 160 μg l^{-1} (Nominal concentrations)	No significant effect on development, sex ratio and fecundity of juveniles (relative to the controls) at ≥160 μg l^{-1} after 18 days exposure, thus NOEC ≥160 μg l^{-1}.	Breitholtz and Bengtsson (2001)	High	Use with care
Copepod (*Tisbe battagliai)*	Newly released animals	Semi static; 0, 0.1, 1, 10 and 100 μg l^{-1} (Nominal concentrations)	No significant effect on development, sex ratio and fecundity (relative to the controls) at ≥100 μg l^{-1}.	Hutchinson *et al* (1999)	High	Use with care

14.5 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity

14.5.1 Studies relevant to the assessment of general toxicity in humans

Information on general toxic effects of oestradiol on human health has not been considered in this review.

14.5.2 Studies relevant to the assessment of general toxicity in wildlife

Table 14.3 summarises the acute and chronic data on the general toxicity of 17β-oestradiol to wildlife.

14.5.2.1 Studies on aquatic organisms

A. Fish

Acute toxicity

No information has been located on the general toxicity of 17β-oestradiol to fish.

Chronic toxicity

Thorpe *et al* (2000) exposed female juvenile rainbow trout to 17β-oestradiol at nominal concentrations at 0, 1, 3.2, 10, 32, 100 and 320 ng l^{-1} for 7, 14 or 21 days. Analysis revealed measured concentrations to be less than nominal concentrations in all cases, with concentrations corresponding to <5, <5, <5, 9, 23, 72 and 247 ng l^{-1}, respectively, based on a time-weighted average for day 21. Concentration-related mortalities were not seen in any treatments over the 21-day period.

Kramer *et al* (1998) exposed duplicate groups of six fathead minnows (3 male and 3 female) were exposed to waterborne 17β-oestradiol at nominal concentrations of 0, 27.24, 272.4 and 2724 ng l^{-1} for 19 days. Dissolved test concentrations, measured throughout the exposure period using immunoassay, showed high variability, but, measured concentrations averaged 79 ± 25% of the nominal concentration. Consequently results were analysed using linear and non-linear regression techniques. A total of 9 fish (of 60) died during the exposure period, of which four males died in a dose-dependent manner. There was no concentration-dependent mortality of female fish. A 19-day LC_{50} of 1150 ng l^{-1} was reported for males.

Fathead minnows (*Pimephales promelas*) were exposed to nominal 17β-oestradiol concentrations of 0, 25, 50 and 100 ng l^{-1} during early life stages (Tyler *et al* 1999). Exposure to 17β-oestradiol was initiated in embryos (24 hr post-fertilisation) and continued throughout the 4 days until hatching and subsequently for another 30 days post-hatch. Two further groups were exposed to 100 ng l^{-1} at 24 hour post-fertilisation up until 10 and 20 days post-hatch and then maintained in clean water until the end of the experiment. At 30 days post-hatch, the survival of the larvae at all treatment ranged from 79 to 88%.

Nimrod and Benson (1998) exposed Japanese medaka larvae (5-8 days old) to measured 17β-oestradiol concentrations of 10, 120 and 1660 ng l^{-1} for 28 days following. The survival rate was dose-dependently reduced and significantly altered for fish exposed to 1660 ng l^{-1}

compared to control groups. Following a further 55-day period in dilution water only, there was little mortality.

Shioda and Wakabayashi (2000b) exposed adult female Japanese medaka to nominal 17β-oestradiol concentrations of 0, 2.7, 27, 272, 2740 and 27 400 ng l^{-1} for 2 weeks. Following breeding with unexposed males, several female fish exposed to ≥2740 ng l^{-1} died in these groups.

Toft and Baatrup (2001) exposed adult male guppies (*Poecilia reticulata*) to nominal 17β-oestradiol concentrations of 30, 100 and 1000 ng l^{-1} for 30 days. There was no difference in survival.

Invertebrates

Acute toxicity

Breitholtz and Bengtsson (2001) investigated the effects of 17β-oestradiol on *Nitocra spinipes* (Crustacea, Copepoda) and a 96 hour LC_{50} value of 1600 μg l^{-1} was reported for the adult life-stage.

Anderson *et al* (2001) tested 17β-oestradiol for acute toxicity to *Acartia tonsa* and no lethal effects at concentrations up to 1000 μg l^{-1} were observed, resulting in a NOEC (mortality) of ≥1000 ng l^{-1}.

Chronic toxicity

Hutchinson *et al* (1999) conducted life-cycle studies investigating the effects of 17β-oestradiol on *Tibse battagliai* (Crustacea, Copepoda). Newly released (<24 hrs old) animals were exposed to nominal concentrations 0.1, 1, 10 and 100 μg l^{-1} over 21 days. None of the concentrations had a significant effect on mortality, thus, a 21-day of NOEC (mortality) of ≥100 μg l^{-1} was reported.

Breitholtz and Bengtsson (2001) investigated the effects of 17β-oestradiol on *Nitocra spinipes* (Crustacea, Copepoda). Newly released (<24 hrs old) animals were exposed to nominal 17β-oestradiol concentrations of 1.6, 16 or 160 μg l^{-1} for up to 18 days and none of the concentrations had a significant effect on mortality, resulting in a NOEC (mortality) of ≥160 μg l^{-1}.

14.5.2.2 Studies on terrestrial organisms

No general toxicity data for terrestrial organisms following exposure to 17β-oestradiol had been located.

14.5.2.3 Studies of aerial organisms

No general toxicity data for aerial organisms following exposure to 17β-oestradiol had been located.

Table 14.3 Summary of general toxicity data for aquatic organisms

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study validity
Chronic Fish Toxicity	Fathead minnow (*Pimephales promelas)*	35 days	0, 25, 50 or 100 ng l^{-1} (Nominal concentrations)	NOEC (embryo mortality LOEC (embryo mortality)	>100 ng l^{-1} >100 ng l^{-1}	Tyler *et al* (1999)	Use with care
	Fathead minnow (*Pimephales promelas)* males	19 days	0, 27.24, 272.4 and 2724 ng l^{-1} (Measured concentraions)	EC_{50}	1150 ng l^{-1}	Kramer *et al* (1998)	Valid
	Guppy (*Poecilia reticulata*)	30 days	0, 30, 100 and 1000 ng l^{-1} (Nominal concentrations)	NOEC (adult mortality)	1000 ng l^{-1}	Toft and Baatrup (2001)	Use with care
	Japanese medaka (*Oryzias lapites*)	28 days	0, 10, 120 and 1660 ng l^{-1} (Measured concentrations)	NOEC (larval mortality) LOEC (larval mortality)	120 ng l^{-1} 1660 ng l^{-1}	Nimrod and Benson (1998)	Valid
		200-230 days	0, 100 and 1000 ng l^{-1} (Nominal concentrations)	NOEC (early life-stage mortality)	1000 ng l^{-1}	Tabata *et al* (2001)	Use with care
	Adult, female	14 days	0, 2.7, 27, 272, 2740 and 27400 ng l^{-1} (Nominal concentrations)	NOEC LOEC	272 ng l^{-1} 2724 ng l^{-1}	Shioda and Wakabayashi (2000b)	Use with care
	Rainbow trout (*Oncorhynchus mykiss*) juvenile females	21 days	Flow-through; 0, <5, 9, 23, 72 and 247 ng l^{-1} (Measured concentrations)	NOEC (mortality)	247 ng l^{-1}	Thorpe *et al* (2000)	Valid

Table 14.3 Continued

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study validity
Acute Invertebrate Toxicity	Copepod (*Nitocra spinipes*)	96 hours	No data	LC_{50} (adult mortality)	1600 μg l^{-1}	Breitholtz and Bengtsson (2001)	Use with care
	Copepod (*Acartia tonsa*)	5 day	Up to 1000 μg l^{-1} (Nominal concentration)	NOEC (mortality)	1000 μg l^{-1}	Anderson *et al* (2001)	Use with care
Chronic Invertebrate Toxicity	Copepod (*Tisbe battagliai)*	21 days	0, 0.1, 1, 10 and 100 μg l^{-1} (Nominal concentration)	NOEC	≥100 μg l^{-1}	Hutchinson *et al* (1999)	Use with care
	Copepod (*Nitocra spinipes*)	18 day	0, 1.6, 16 and 160 μg l^{-1} (Nominal concentration)	NOEC (juveniles)	≥160 μg l^{-1}	Breitholtz and Bengtsson (2001)	Use with care

14.5.2.4 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity in wildlife

The chronic toxicity data for fish indicates that the levels of 17β-oestradiol required to cause general effects occur at levels > 100 ng l^{-1}. As a result it is evident that potential endocrine mediated responses in fish may be the mechanism responsible for the most toxic effects observed observed in fish. The relative insensitivity of the reproductive, developmental and lethality endpoints in the invertebrate group (crustaceans) exposed to to 17β-oestradiol means this taxonomic group is evidently not affected by exposure to vertebrate steroids.

14.6 Current classification of 17β-Oestradiol against European Commission or national regulations

As a natural steroid, oestradiol is not listed or classified under any of the major Council Directives.

In the United Kingdom consideration has been given to the derivation of Predicted No Effect Concentrations (PNEC) for the natural vertebrate steroids oestradiol and oestrone and the synthetic steroid ethinyloestradiol as part of the national strategy for "Endocrine disrupting substances in the environment" (EA 2000). The PNEC value derived for oestradiol was 1.0 ng $l^{-1,}$ expressed as an annual average.

14.7 Exposure data

14.7.1 Worker exposure data

The exposure of workers to 17β-oestradiol has not been considered in this review since no large scale production facilities are found in Europe.

14.7.2 Consumer exposure data

Natural steroid oestrogens are used in human medicine (Martingdale 1993), particularly;

- in hormone replacement therapy (HRT);
- for the treatment of other gynaecological disorders;
- in the treatment of prostate cancer in men;
- in the treatment of breast cancer in men;
- in the treatment of breast cancer in post-menopausal women

However, the levels administered for these specific purposes are designated by medical staff and designed to minimise adverse side-effects.

14.7.3 Environmental exposure data

14.7.3.1 Aquatic environment

The route of entry of natural steroid oestrogens into the aquatic environment will be primarily through human and animal excretion (see section 14.2.1) and subsequent transport through sewage treatment works.

The amount of oestradiol excreted in the urine or faeces from any individual will depend on a number of factors such as sex, race, hormonal status (e.g. pre- *vs*. post-menopausal), smoking, stage of menstruation, use of oral contraceptives and pregnancy. A summary of urinary and faecal excretion rates is given in Table 14.4. Pregnant women excrete the largest amount of natural oestrogens, followed by pre-menopausal women, oral contraceptive users and men, with post-menopausal women excreting the least.

Although the natural hormones are used therapeutically, for example, in hormone replacement, it has been estimated that the extra load to the environment due to the therapeutic use of 17β-oestradiol would contribute less than 5% when compared to natural excretion (Christensen 1998a).

Table 14.4 Summary of oestradiol levels released in urine and faeces by different human groups

Group	Urine Oestradiol concentrations (µg)	Faecal Oestradiol concentration (µg)
Pre-menopausal women	1.09[a] (Aldercruetz *et al* 1994) 2.88[d] (Aldercruetz *et al* 1994) 3.52 [c] (Key *et al* 1996) 0-14[b] (Eastham 1978)	0.955 (Aldercreutz and Järvenpää 1982) 0.563 (Aldercruetz *et al* 1994) 0.239 (Aldercruetz *et al* 1994)
Post-menopausal women	0.68[c] (Key *et al* 1996)	0.09 (Aldercreutz and Järvenpää 1982)
Pregnancy	127-900[i] (Altman 1961) 210-615[h] (Fostis 1987) 170[f] (Aldercreutz and Luukkainen 1970) 660[g] (Brown 1957)	203[e] (Aldercreutz and Martin 1976)
Men	-	0.065 (Aldercreutz and Järvenpää 1982)
Children <age 8 age 8-12	-	-

Key: a - Oriental women, b - Depending on stage of menstruation, c - Geometric mean, adjusted for age, stage of menstruation for non-smokers, d - Caucasian women, e - Weeks 33-37 of pregnancy, f - Weeks 36-40 of pregnancy, g - Last week of pregnancy, h - Depending on stage of pregnancy (lower value week 27; higher value week 37), i - Depending on stage of pregnancy (lower value for month 2, highest month 8).

Excretion by farm animals can also contribute to the natural loads of steroid oestrogen (including 17β-oestradiol) in the environment (Shore *et al* 1993, Archand-Hoy *et al* 1998, Blok and Wösten 2000). Certainly the amount of oestrogens excreted can be significant (Knight 1980, Turan 1995). Shore *et al* (1993) compared natural steroid oestrogen concentrations in treated sewage effluent from municipal STWs and small farm units based on data obtained using immunoassay techniques. Depending on time of year, effluent concentrations were 40-

120 and 150-350 ng l^{-1}, respectively, indicating that farm units can be a source of natural steroid oestrogens to the environment.

Recently, Dutch researchers from the Association of River Waterworks reviewed the magnitude of natural steroid oestrogen excretion (including 17β-oestradiol) by domestic animals in the Netherlands (Blok and Wösten 2000). The authors focussed specifically on cattle, pigs, chickens and horses, since contributions by other groups of animals, such as sheep and goats, were considered to be relatively low in comparison.

The studies included measured the concentration of natural steroid oestrogens in manure and urine of domestic animals. Where measurements were lacking for an excretion pathway, assumptions of suspected concentrations were made. The concentrations reported relate to total natural steroid oestrogens rather than specific compounds. The authors concluded that the highest average oestrogen contributions per animal were from pregnant cows and breeding female pigs, which excreted 37 and 7 mg day^{-1}, respectively. Contributions from other animals such as non-pregnant cows, female store pigs and chickens were expected to be much lower, with excretion concentrations of 1, 0.032 and 0.028 mg $animal^{-1}$ day^{-1}, respectively. Expected contributions from pregnant horses at the end of the gestation period were shown to be 100 mg $animal^{-1}$ day^{-1}. However, this was the only value available in the literature and no other reports could be cited to confirm this high concentration.

The average daily values per animal were used to calculate the total steroid oestrogen excretion by all domestic animals and humans in the Netherlands. These results indicate that pregnant cows and breeding female pigs contribute the greatest volume of natural steroid oestrogens in the Netherlands (22 and 10.6 kg day^{-1}, respectively). It is also worth noting that the total contribution of natural steroid oestrogens by Dutch livestock is estimated to be 10 times greater than contributed by the human population.

As a result, an important environmental source of 17β-oestradiol may be from diffuse sources which directly enter the aquatic environment.

Treatment works effluents and sewage sludges

Table 14.5 summarises the data on the concentrations of oestradiol in effluent discharges from treatment works and the levels recorded in sewage sludges at the treatment plants. Monitoring studies indicate that oestradiol concentrations in sewage treatment works effluents range from <0.15 to 88 ng l^{-1}. Further information on the studies in different countries are given in the following sections.

Table 14.5 Summary of the measured oestradiol concentrations in European treatment plant discharges

Location	Location	Oestradiol concentration (ng l^{-1})	Reference
Germany	16 STWs (preliminary and final clarification/aerator)	Nd[a], 2[b], 3[c]	Ternes *et al* (1999a)
	20 STWs	21[c]	Stumpf *et al* (1996)
	1 STW	<0.5 - 1.5	Hansen *et al* (1998)
		Nd - 11	Wegener *et al* (2001)
	3 STWs	<0.15 - 5.2 0.4[a] 0.9[d]	Kuch and Ballschmiter (2001)
Italy	6 STWs	0.35 - 3.5	Baronti *et al* (2000)
	5 STWs	<0.5 - 7	Johnson *et al* (2000)
Netherlands	5 STWs	Nd – 12, 0.9[a]	Belfroid *et al* (1999)
	3 STWs	<0.6 - 12	Johnson *et al* (2000)
Sweden	1 STW	1	Larsson *et al* (1999)
United Kingdom	7 STWs	2.7-48, 11[d]	Desbrow *et al* (1998)
	Chelmsford STW sampled during November-March Chelmsford STW sampled during July-December	7 - 88 4 - 8.8	Rodgers-Gray *et al* (2000)
	3 STWs on rivers Lea and Nene.	Nd - 4.26	Kanda *et al* (2001)
	2 STWs	Nd - 0.9	Niven *et al* (2001)

Key: Nd = not detected, STWs – sewage treatment works, a =median, b = 90 percentile, c = maximum, d = mean

Germany

Using GCMS/MS analysis, concentrations of 17β-oestradiol were measured in treated effluents from 16 German STWs (Ternes *et al* 1999a) and concentrations of 17β-oestradiol were up to 3 ng l^{-1}.

More recently, Kuch and Ballschmiter (2001) conducted monitoring of three German activated sludge STWs, sampled over 5 months. 17β-oestradiol was analysed by GC/MS (with LOD values of 0.15 ng l^{-1} being reported) and concentrations ranged from <0.15-5.2 ng l^{-1}.

Italy

Johnson *et al* (2000) measured 17β-oestradiol in effluents of five Italian activated sludge STWs. Based on LCMS/MS analysis, 17β-oestradiol was present in most of the samples analysed and ranged from <0.5-7.

Similar results were reported by Baronti *et al* (2000) who measured 17β-oestradiol in 6 Italian activated sludge STWs receiving domestic inputs. Analysis was by LCMS/MS and concentrations ranged from 0.35-3.5 (median value = 1), 17β-oestradiol being detected in all of the samples analysed (30).

Netherlands

Belfroid *et al* (1999) measured 17β-oestradiol in 5 STWs (3 receiving domestic waste, 2 receiving industrial waste) in the Netherlands using GCMS/MS analysis. In domestic effluents,

reported concentrations were up to 12 ng l^{-1}, which was higher than those found in the industrial effluents.

Johnson *et al* (2000) measured 17β-oestradiol concentrations in effluents from three Dutch STWs using GCMS/MS analysis and found these ranged from <0.6-12 ng l^{-1}.

United Kingdom

In the United Kingdom the Environment Agency funded work to identify oestrogenic substances in effluents from STWs (Desbrow *et al* 1998). The method development focused on three STWs receiving mainly domestic sewage and used fractionation techniques followed by GCMS analysis. The initial results from final effluents demonstrated that oestrogenic activity (as measured by the Yeast Estrogen Screen (YES) bioassay) was largely concentrated in only one fraction and indicated a polar organic structure for the causal agents. Subsequently, 21 effluent samples from seven STWs receiving mainly domestic waste were assessed. All were found to contain detectable concentrations of unconjugated 17β-oestradiol in the fraction with high oestrogenic activity. Concentrations ranged between 2.7-48 ng l^{-1} for 17β-oestradiol. Effluents for six STWs contained 17β-oestradiol at concentrations $\leq$10 ng l^{-1}.

Rodgers-Gray *et al* (2000) monitored Chelmsford STW effluent during two trials (November 1997-March 1998 and July 1998-December 1998) using GCMS analysis. Five-day composite samples of effluent were taken each month, and 17β-oestradiol was consistently detected throughout both the test periods. During the first trial, 17β-oestradiol concentrations varied from 7 to 88 ng l^{-1}. By comparison, the concentrations of 17β-oestradiol in the second trial ranged from 4 to 8.8 ng l^{-1}. However, it should be noted that the results from the first trial (which utilised GCMS analysis) are higher compared to monitoring data reported by other researchers.

Niven *et al* (2001) reported on monitoring results of effluent samples from two UK sewage treatment plants (Harpenden and Deephams). Trace concentrations of 17β-oestradiol were detected at concentrations <1 ng l^{-1}.

The most recent UK study involved monitoring 17β-oestradiol in effluent from one STW on the River Nene daily over 4 weeks (29 samples) and two STWs on the River Lea daily over 2 weeks (14 samples per works) (Kanda *et al* 2001, Williams *et al* 2001). Analysis was conducted using GCMS/MS and was performance tested, the LOD being 1 ng l^{-1}. For the STW on the River Nene, results indicated that 17β-oestradiol was generally below the LOD <1 ng l^{-1} with only 10 of 29 samples giving positive identifications up to ~ 2 ng l^{-1} in the dissolved fraction. For the two STWs on the River Lea, the maximum concentration of 17β-oestradiol measured was 4.26 ng l^{-1}.

Surface waters and sediments

Table 14.6 summaries the oestradiol concentrations that have been detected in surface waters, based on studies from the United Kingdom, Germany, Italy and the Netherlands. These studies indicate that when detected oestradiol occurs at trace ng l^{-1} concentrations in surface waters (generally less than 5 ng l^{-1} and often below 1 ng l^{-1}). Further information on the studies in different countries is given in the following sections.

Table 14.6 Summary of the measured oestradiol concentrations in European surface waters

Location	Location	Oestradiol concentration (ng l^{-1})	Reference
Germany	15 river and streams	Nd (LOD = 0.5 ng l^{-1})	Ternes *et al* (1999a)
	10 sites	Nd	Stumpf *et al* (1996), cited in Jurgens *et al* (1999).
	45 samples	Nd	Wegener *et al* (2001)
	The Danube (n=13), Nau (n=4) and Blau (n=4). The Illner River (n=4) and 3 creeks (n=2)	Nd-3.6 0.3[a] 0.6[b]	Kuch and Ballschmiter (2001)
Italy	Grab samples at 2 sites in the river Tiber; not detected downstream of activated sludge STW.	Nd, 0.11	Baronti *et al* (2000)
Netherlands	Positively detected in 4/11 locations sampled.	Nd[a], 5.5[c] (LOD = 0.5 ng l^{-1})	Belfroid *et al* (1999)
United Kingdom	5 sites in Severn Trent region,	Nd, 0.3, 25 (LOD = 0.3 ng l^{-1})	Fawell *et al* (2001)
	Rivers Nene and Lea.	Nd – 8.76 (LOD = 0.4 ng l^{-1})	Kanda *et al* (2001)

Key: Nd = not detected, STWs – sewage treatment works, [a] =median, [b] = mean, [c] = maximum

Germany

Ternes *et al* (1999a) reported on monitoring data for 17β-oestradiol in 15 German rivers but none was detected in any of the rivers (the reported LOD being 0.5 ng l^{-1}). Other German studies have supported these findings (Stumpf *et al* 1996, Wegener *et al* 2001).

More recently, Kuch and Ballschmiter (2001) analysed river and creek samples of the Danube (n=13 samples), Nau (n=4 samples) and Blau (n=4 samples) approximately 1 km downstream of STWs effluent sites. The Illner River (n=4) and three local creeks (n=2 each) were also analysed. All samples were analysed by GCMS and 17β-oestradiol concentrations which were positively idenitfied in 14 of 31 samples were <0.15-3.6 ng l^{-1}.

Italy

Baronti *et al* (2000) sampled two sites of the Tiber river; one was situated downstream of an activated sludge STW and the second was located 1 km before the mouth of the Tiber. After leaving Rome, the Tiber covers about 20 km and receives effluents of mechanical STWs located in small towns and also raw sewage. At the first site, no significant amounts of the four oestrogens including oestradiol were detected. However, analysis of the second site revealed concentrations of 0.11 ng l^{-1} of 17β-oestradiol.

Netherlands

Only trace concentrations (generally below the LODs of about 5 ng l^{-1}) of 17β-oestradiol were detected in eleven surface water sites in the Netherlands (Belfroid *et al* 1999).

United Kingdom

The most comprehensive monitoring survey on UK surface waters involved GCMS/MS analysis of two rivers for 17β-oestradiol (Kanda *et al* 2001, Williams *et al* 2001). Samples were taken at numerous sites downstream of sewage treatment works (5 sites from the River Nene daily over a period of 4 weeks; 5 sites from the River Lea daily over 2 weeks). The analytical method was performance tested, the LOD being 0.4 ng l^{-}. 17β-Oestradiol concentrations ranged from <0.4 to ~8.76 ng l^{-1}, with most values being below 1 ng l^{-1}.

Another recent study (Fawell *et al* 2001) conducted GCMS/MS analysis of 17β-oestradiol in five surface waters in the Severn Trent region of England with an LOD of 0.3 ng l^{-1} and measured concentrations at the two sites of 0.3 and 25 ng l^{-1}.

Williams *et al* (1999) applied the Exposure Assessment Modelling System (EXAMS) to predict the likely distribution of 17β-Oestradiol in the Rivers Thames, Calder and Aire. 17β-Oestradiol concentrations under average river conditions were predicted to be in the dissolved phase and were similar, ranging between 0.21-0.37 ng l^{-1}.

Only two studies were located which have measured contamination of sediments by 17β-oestradiol (Tanaka *et al* 2000, Kanda *et al* 2001).

Kanda *et al* (2001) conducted a survey of sediment contamination by, 17β-oestradiol at 10 sites downstream of three STWs in two UK rivers (Rivers Nene and Lea). Analysis was conducted using GCMS/MS techniques and 17β-oestradiol was below the LOD (<0.1 μg kg^{-1}) for all the samples analysed.

COMPREHEND Programme

In the EU funded COMPREHEND programme chemical analysis of industrial and domestic effluents was undertaken by two of the partners in the Netherlands and Switzerland using samples taken from across Europe. Analysis included the natural steroids oestrone, oestradiol and oestriol and ethinyloestradiol. Oestrone measurements were the most consistent in terms of recovery and a good correlation was obtained in a comparison of the techniques of the two laboratories for measurements of the same set of wastewater samples. There was poor agreement with oestradiol measurement and both laboratories experienced very poor recoveries with oestriol and low sensitivity with ethinylestradiol. Oestrone measurements in STW effluents showed a good degree of correlation with estrogenic activity (as measured with *in vitro* assays) and oestrone and oestradiol were generally in the 0 to 10 ng l^{-1} range. Ethinyloestradiol however, was often at or below the limit of detection (approximately 1 ng l^{-1}). Oestrogenic steroids were generally below the limits of detection for most industrial waste waters (unless there was a significant component of the effluent originating from domestic/human sources within the industrial plant).

Toxicity Identification and Evaluations

In the COMPREHEND programme Toxicity Identification and Evaluations (TIE) identified oestradiol, oestrone and ethinyloestradiol as the principal estrogenic components of domestic raw sewage, with ethinyloestradiol and oestrone dominating the oestrogenic activity of the final effluent. Taking into consideration the potencies of the various oestrogenic compounds measured in municipal STW effluents, it was concluded that natural and synthetic steroids, of human origin, are by far the most important estrogenic components and are responsible for

most of the estrogenic effects seen *in vivo* and *in vitro*. Ethinyloestradiol may be particularly important in this respect but the limitations of the analytical techniques used in the programme were a major constraint to confirming the importance of this component of the contraceptive pill. TIE also provided evidence of 'cooperative' effects between the different steroids, making the measured oestrogenic activity (in the yeast based YES assay) approximately three times greater than the sum of the activity of the individual components.

These results are generally consistent with those of Desbrow *et al* (1998) which used a fractionation system combined with a yeast based *in vitro* assay to isolate and identify the major oestrogenic chemicals present in seven sewage treatment works effluents receiving primarily domestic effluent. In all the effluents tested the most active fraction (>80% total activity in domestic effluent) was found to contain low levels of natural and synthetic steroidal oestrogens. The results obtained indicated that the concentrations of ethinyloestradiol, detected in the samples were generally too low to fully account for magnitude of the vitellogenin response observed when male fish were exposed to the effluent. (Sheahan *et al* 1994).

14.7.3.2 Terrestrial environment

Blok and Wösten (2000) also investigated the environmental fate of natural steroid oestrogens applied to land in the form of animal slurry. Two scenarios were tested:

a) A small-scale area with a surrounding ditch that had been intensively fertilised with breeding female pig or milk cow manure for three months.

b) A large-scale total catchment area scenario for the Rhine and Meuse rivers using the total numbers of animals within each catchment to estimate the volume of natural oestrogens released;

Results from the first scenario estimated that the concentration of natural oestrogens in water ditches surrounding the fields could rise to 150 ng l^{-1} if the highest volume of slurry permitted in a year was applied to the land over a period of three months. In the second scenario it was estimated that concentrations of natural oestrogens in the rivers could rise to 75 and 140 ng l^{-1} in the Rhine and Meuse, respectively, over a fertilising period of three months. Regular spreading of manure over a six-month period in summer was estimated to lead to water concentrations of 40 and 90 ng l^{-1}, respectively. These calculations were based on the assumption that 3% of the excreted oestrogens in animal slurry would eventually enter surface waters. Finlay-Moore *et al* (2000) have also investigated 17β-oestradiol and concentrations in soil and run-off water from soil to which broiler litter had been applied. Run-off concentrations ranged between 20 and 2530 ng l^{-1} and it was concluded that there was a significant risk of edge-of field losses.

14.7.3.3 Aerial environment

No data has been located on the concentrations of oestradiol in the aerial environment.

14.7.3.4 Comparison of environmental monitoring data and exposure concentrations causing potential endocrine mediated responses

The data on the concentrations of 17β-oestradiol in European surface waters (see Section 14.7.3.1) indicates that typical levels are generally less than 5 ng l^{-1}, though most values are usually below 1 ng l^{-1}. The key data on endocrine mediated responses of 17β-oestradiol in

aquatic organisms is for fish. The combined dataset for potential endocrine mediated responses on fish indicates that the threshold level above which effects are for observation is in the range 5 –25 ng l^{-1} based on responses of a number of different endpoints.

If a margin of safety (MOS)[1] approach is used to compare the threshold effects level above which endocrine mediated responses are observed (5-25 ng l^{-1}) with environmental concentrations (1-5 ng l^{-1}) then MOS values of 1 - 25 would result in the aquatic compartment. On the basis that an MOS of 100 should be required for the risk to be acceptable then 17β-oestradiol apparently presents a threat to fish (and other aquatic vertebrates) in terms of endocrine disrupting effects.

14.8 Overall Conclusions on 17β-Oestradiol

14.8.1 Data from studies assessing potential endocrine disrupting effects in wildlife

- Overall the combined dataset for endocrine mediated responses on reproductive and developmental endpoints in fish indicates that the threshold level above which effects are observed is in the range 5 –25 ng l^{-1} based on responses of a number of different endpoints.

- Studies on the effects of 17β-oestradiol on the development and reproduction of the copepods *Tisbe battgaliai* (Hutchinson *et al* 1999, Breitholtz and Bengtsson 2001) showed no adverse effects up to concentrations of 160 μg l^{-1}. The lowest recorded NOEC for effects in invertebrates was 0.1 μg l^{-1} in a study of the reduction in the settlement of cypris larvae of the barnacle *Balanus amphridite* (Billinghurst et al 1998). However, the extent to which this endpoint is endocrine mediated is uncertain, but even if it was relevant the threshold for effects would still be higher than those for fish. Information is not available on the potential endocrine mediated responses of 17β-oestradiol in a wide range of invertebrate taxa.

- For amphibians exposure concentrations at 27.4 μg l^{-1} were required for effects, namely a change in sex ratios with increased numbers of females (Kloas *et al* 1999).

14.8.2 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity in wildlife

- The chronic toxicity data for fish indicates that the levels of 17β-oestradiol required to cause general effects occur at levels > 100 ng l^{-1}. As a result it is evident that endocrine mediated responses in fish may be the mechanism responsible for the most toxic effects observed in fish.

- The relative insensitivity of the reproductive, developmental and lethality endpoints in the invertebrate group (crustaceans) exposed to to 17β-oestradiol means this taxonomic

[1] Margin of safety (MOS) = (Lowest NOEC for endocrine mediated responses)/Environmental concentrations

group is evidently not affected by exposure to vertebrate steroids. However, data are not available for a wide range of invertebrate taxa.

14.8.3 Exposure data

- The data on the concentrations of 17β-oestradiol in European surface waters (see Section 14.7.3.1) indicates that typical levels are generally less than 5 ng l^{-1}, though most values are usually below 1 ng l^{-1}. The key data on endocrine mediated responses of 17β-oestradiol in aquatic organisms is for fish. The combined dataset for endocrine mediated responses on fish indicates that the threshold level above which effects are observed is in the range 5 –25 ng l^{-1} based on responses of a number of different endpoints.

- If a margin of safety (MOS) approach is used to compare the threshold level above which endocrine mediated responses are observed (5-25 ng l^{-1}) with environmental concentrations (1-5 ng l^{-1}) then MOS values of 1 - 25 would result in the aquatic compartment. On the basis that an MOS of 100 should be required for the risk to be acceptable then 17β-oestradiol apparently presents a risk to fish (and other aquatic vertebrates) in terms of endocrine disrupting effects. This conclusion is consistent with the results of field surveys carried out in a number of European countries (for example the COMPREHEND programme) which have identified evidence of adverse effects on the development and reproductive capability of wild fish which are exposed to natural (and synthetic) steroids discharged from sewage treatment works (for review EA 2002).

- No information was located on terrestrial or aerial concentrations of oestrone.

14.9 Summary of the weight of evidence for endocrine disrupting effects in wildlife and associated uncertainties

The summary of the weight of evidence for endocrine disrupting effects of oestrone in wildlife along with associated uncertainties are given in Table 14.7.

Table 14.7 Summary of the weight of evidence conclusion and uncertainties associated with the assessment of the endocrine disrupting effects of 17β-oestradiol

	Target group	
	Humans	Wildlife
Weight of evidence	Not considered in the review	In fish it appears that the effects of 17β-oestradiol on reproduction and development which are considered to be endocrine mediated occur at markedly lower (and environmentally relevant) concentrations (> 5-25 ng l^{-1}) than those causing general toxicity. The processes of reproduction and development in certain invertebrate taxa (crustaceans) are evidently not generally affected by exposure to vertebrate steroids at typical environmental levels. However this may not be the case for other invertebrate taxa. The available aquatic exposure data (showing typical concentrations of 1 – 5 ng l^{-1}) indicates that 17β-oestradiol presents a risk to fish (and other aquatic vertebrates) in terms of endocrine disrupting effects. This is consistent with data from field surveys of fish populations exposed to natural and synthetic steroids discharged from sewage treatment works.
Uncertainties	Not considered in the review	The data on 17β-oestradiol induced and endocrine mediated effects on reproduction and development in wildlife is limited and restricted to aquatic organisms (invertebrates and fish). No data are available on potential endocrine mediated effects in terrestrial and aerial organisms. Given that sorption to organic carbon is an important process resulting in the partitioning of 17β-oestradiol onto soils the absence of data on potential endocrine mediated responses in terrestrial organisms is a key area of uncertainty.

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15. REVIEW OF DATA FOR 17α-ETHINYLOESTRADIOL

Table of Contents

Notes:

This section contains information on the effects of 17α-ethinyloestradiol on wildlife collected and collated from a range of sources including published papers, reports of studies conducted by industrial companies or research organisations, data compilations and a review of natural and synthetic steroids carried out by the United Kingdom Environment Agency (EA 2002).

This review has been carried out in accordance with the evaluation framework described in Section 2. In the review the International Programme for Chemical Safety (IPCS) definition of an endocrine disrupter has been adopted, namely that it is "*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*".

In the context of the review it is recognised that there are various laboratory-based *in vivo* and *in vitro* methods utilising a range of (eco)toxicological endpoints that are claimed by different sources to be relevant to the assessment of endocrine disruption in humans and wildlife. However, since this field is still in an early stage of development there is uncertainty regarding the significance of many of the current findings.

From the numerous recent reviews of potential test methods (such as the Detailed Review Paper prepared by OECD in 1997) there is a clear consensus in terms of the hierarchy of the relevance of test methods. In this hierarchy longer-term *in vivo* studies considering effects on reproduction and/or development (and including mechanistic information) are of greater relevance than short-term *in vivo* screening tests which are of greater relevance than *in vitro* assays. The greater relevance of chronic *in vivo* tests or those assessing effects during critical windows of sensitivity is also evidenced by the fact that these are the key (eco)toxicological methods being developed in the OECD Endocrine Disruption Testing and Assessment (EDTA) Programme. This hierarchy approach to data relevance has been adopted in the review along with a weight of evidence consideration of the available data.

The review has been carried out to address three key questions:

1. Does the available data indicate there is evidence that a chemical causes endocrine disrupting effects in target groups of wildlife?

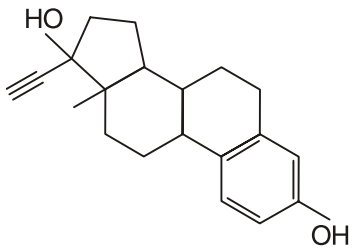
2. Do endocrine disrupting effects of the chemical in target groups of wildlife occur at lower concentrations than those causing effects on general systemic toxicological endpoints?

3. Are particular target groups of organisms in the environment likely to be exposed to concentrations of chemicals which exceed effects thresholds due to current emission patterns.

It should be recognised that this review is not designed to be a full Risk Assessment of a substance under the Existing Substances Regulation 793/93.

15.1 Physico-chemical data for 17α-ethinyloestradiol

15.1.1 Summary details on the substance

CAS Number	50-63-6
EINECS Number	-
IUPAC Name	(17α)-19-norpregna-1,3,5(10)-trien-20-yne-3,17-diol
Other names	17α-ethinyloestradiol, 17α-ethinylestradiol, 17α-ethynyloestradiol, 17α-ethynylestradiol, EE2
Molecular weight	296.4
Chemical formula	C_{20} H_{24} O_2
Chemical structure	

15.1.2 Physico-chemical properties and environmental fate information (from EA 2002)

The data on the physico-chemical properties of ethinyloestradiol and its environmental fate (see Table 15.1) indicate that the substance is relatively persistent in the aquatic environment. Sorption is the major removal process with photolysis being of lower importance and volatilisation being negligible.

No data are available on the persistence of 17α-ethinyloestradiol in soil though it is likely that adsorption to soil is a major removal process given the calculated organic carbon water partition coefficient (log Koc) of 3.8.

Table 15.1 Physico-chemical properties and environmental fate data (from EA 2002)

Physico-chemical property	Value (and comments)
Physical state at ambient temperature	Solid
Water solubility	4.7 - 19 mg l^{-1}
Octanol-water partition coefficient (log Kow)	3.67 - 4.2
Organic carbon water partition coefficient (log Koc)	3.8 (Calculated value)
Henry's Law Constant	No data
Type of degradation	
Aquatic - abiotic	Sorption is the major removal process with photolysis being of lower importance and volatilisation being negligible
Aquatic - biotic	A number of laboratory studies have indicated that 17α-ethinyloestradiol is relatively persistent.
Terrestrial	No data are available on the persistence of 17α-ethinyloestradiol in soil though it is likely that adsorption to soil is a major removal process
Atmospheric	No data

15.2 Production and Uses

15.2.1 Production patterns

The synthetic oestrogen hormone, 17α-ethinyloestradiol can be used in human medicine to treat various gynaecological disorders and post-menopausal breast cancer. However, its largest use is in oral contraceptives, when it is usually administered in combination with a synthetic progestin. Its concentration in the contraceptive pill ranges from 20 to 50 μg, with 35 μg most commonly prescribed (Archand-Hoy *et al* 1998).

15.2.2 Use patterns

An annual use of 0.029 tonnes of 17α-ethinyloestradiol has been estimated in the UK (Webb 2000). By comparison, it has been estimated that 0.088 tonnes of oral contraceptives (17α-ethinyloestradiol and mestranol) are used annually in the USA (Archand-Hoy *et al* 1998).

15.3 Toxicokinetics, metabolism and bioaccumulation

15.3.1 Toxicokinetics and metabolism

The major site of metabolism for 17α-ethinyloestradiol is the liver, with the two major metabolic pathways being 2-hydroxylation and 16β-hydroxylation. These pathways result in a number of metabolites, which are then conjugated with glucuronide and/or sulphate, and are considered to be biologically inactive (IARC 1979). However, a major portion of 17α-ethinyloestradiol is conjugated directly with glucuronic acid and excreted in the urine.

In contrast to the metabolites of natural oestrogens, a significant proportion of the metabolites of 17α-ethinyloestradiol are excreted by the faecal route. In radiolabelled studies, the ratio of faecal/urine radioactivity has been reported to be about 4:6, and the total recovery of radioactivity from both sources is about 90%. One study reported that about 30% is excreted in the faeces of which one-third is excreted as the unchanged form (which may be a result of deconjugation in the colon). The remainder is excreted in the urine mainly as the 17α-ethinyloestradiol glucuronide conjugate. Other glucuronide (and to a lesser extent sulphate) conjugates include:- 2-hydroxyoestradiol; 2-methoxy-ethynyloestradiol and 3-methoxy-2-hydroxy-ethynyloestradiol. It has been reported that only 1% of unchanged ethinyloestradiol is excreted in the urine, although a higher value of 16% has also been reported. De-ethynylated oestrogens (e.g. oestrone, 17β-oestradiol and oestriol) only account for 1-2% of the dose in women (Orme *et al* 1983).

15.3.2 Bioaccumulation

Only one study was located which has measured bioaccumulation factors (BCFs) following exposure to waterborne 17α-ethinyloestradiol (Länge *et al* 2001) whereas others have estimated BCFs on the basis of relationships with other parameters (Kramer *et al* 1998; Jürgens *et al* 1999).

Länge *et al* (2001) conducted a full life cycle study in fathead minnow and measured 17α-ethinyloestradiol in fish tissues after 153 days post hatch (at 64 ng l^{-1}) and 239 days post-hatch (at 16 ng l^{-1}). BCF values of 660 and 610 were calculated, respectively. The 0.2 and 1 ng l^{-1} test concentrations gave no detectable tissue concentrations (<0.38 ng g^{-1}) after 192 days post hatch and thus, a BCF value could not be calculated. It was noted that the 16 and 64 ng l^{-1} concentration induced toxic effects in F_0 fish, and was concluded that the BCF value in healthy fish is likely to be <500 and certainly below 2400.

A related study was conducted by Larsson *et al* (1999) in which caged juvenile rainbow trout of both sexes were placed upstream and downstream of a domestic Swedish STW serving approximately 3500 people. After 2 to 4 weeks of exposure, bile was collected from the fish. Effluent water was analysed for 17α-ethinyloestradiol (and 17β-oestradiol and oestrone) all of which were found at ng l^{-1} concentrations. The bile of fish caged downstream of the STW contained all three steroid oestrogens at concentrations that were 10^4-10^6 times higher than the water concentrations (up to 2.5 μg g^{-1} bile).

15.4 Studies relevant to the assessment of potential endocrine disrupting effects

15.4.1 Studies relevant to human health assessment of potential endocrine disrupting effects in humans

The effects of 17α-ethinyloestradiol on human health have not been considered in this review since the substance is used in prescribed medicines to act as a contraceptive and to treat various gynaecological disorders which involve planned modulation of hormonal activity. As such the doses prescribed are designed to operate in a specific manner where the potential adverse side-effects on consumers are minimal.

15.4.2 Studies relevant to the assessment of potential endocrine disrupting effects in wildlife

15.4.2.1 In vitro studies

No data on the responses of 17α-ethinyloestradiol in *in vitro* systems involving the cells and tissues from wildlife to species is presented.

15.4.2.2 In vivo studies

A. Studies on aquatic organisms

In vivo studies in amphibians

No data has been identified on the endocrine disrupting effects of 17α-ethinyloestradiol on amphibians.

In vivo studies in fish

Table 15.2 summarises the data on potential endocrine disrupting effects of 17α-ethinyloestradiol on aquatic organisms.

Biochemical changes

One of the key functions of endogenous oestrogens in fish is to stimulate the induction in the liver of a large phospholipoprotein vitellogenin (Chen 1983) which is released into the blood stream and sequestered by developing oocytes for production of egg yolk. In maturing female fish, vitellogenin is a major constituent of the blood proteins, while in male fish it is not normally present in appreciable amounts. However, if male fish are exposed to oestrogens or oestrogen mimics, vitellogenin can be produced at similar levels to that found in maturing females.

There is a degree of uncertainty as to the potential ecological relevance of the induction of vitellogenin in fish. Evidence from laboratory, semi-field and field studies carried out on fish exposed to natural and synthetic steroids in aquatic systems in Europe (CSTEE 1999, NRC 1999, Cheek *et al* 2001) has shown that VTG induction in male fish is a biomarker for exposure to oestrogens and oestrogen mimics and that:

- induction in early life stage fish could have serious energetic consequences for the organisms;
- high levels of vitellogenin induction in fish are known to cause kidney failure and are associated with some haematological disturbances;
- a weak, but nevertheless significant correlation, has been shown between VTG induction in wild roach and the severity of the intersex condition in fish (that is male gonads show evidence of feminisation).

In a 28 week study, Sheahan *et al* (1993) exposed rainbow trout to nominal concentrations of 0.1, 0.3 and 1 ng l^{-1} at mean temperatures of 11.4 and 17.4^{o}C. Plasma vitellogenin (sampled at 2, 12, 20 and 28 weeks) was determined, along with measurements of GSI, Hepatosomatic

Index (HIS) at 28 weeks. Histological examination of the liver and gonads was also undertaken at the end of the 28-week period. No treatment-related effects on condition were observed. In initial plasma samples, the general trend was one of increasing vitellogenin levels throughout the 28-week period, although they were not significantly different. However, at the end of the study, plasma vitellogenin levels in males were significantly higher in those treated at 1 ng l^{-1} at both temperatures, and also in those treated at 0.3 ng l^{-1} at 11.4°C. No significant difference in plasma vitellogenin concentration was seen between treated females and controls. In addition, neither GSI nor HSI values showed concentration-related trends. Histological changes in the livers of fish exposed to 1 ng l^{-1} indicated a depletion of storage material present in liver cells as would be expected for the production of vitellogenin.

Purdon *et al* (1994) found that 17α-ethinyloestradiol induced plasma vitellogenesis in male rainbow trout (*Oncorhynchus mykiss*) at nominal concentrations of 0.1, 0.5, 1 and 10 ng l^{-1} after 10 days exposure at 16.5°C. In the same study, significant vitellogenin induction occurred at concentrations ≥ 10 ng l^{-1} at 9.5°C in immature carp (*Cyprinus carpio*). Jobling *et al* (1996) also demonstrated that a nominal concentration of 2 ng l^{-1} (measured concentration 1.79 ng l^{-1}) resulted in significant vitellogenin induction in male rainbow trout over a 21 day period. This concentration also inhibited testicular growth (as demonstrated by a reduced Gonadosomatic Index (GSI) value) and affected the distribution of cell types in the testes.

More recently, Thorpe *et al* (2001) exposed groups of juvenile female rainbow trout for 14 days to measured 17α-ethinyloestradiol concentrations (0, 0.1, 0.32, 1, 3.2, 10 or 32 ng l^{-1}). Both LOEC and EC_{50} values of 1 ng l^{-1} were reported based on vitellogenin induction.

Allen *et al* (1999) exposed male and female flounder (*Platichthys flesus*) to nominal concentrations of 0, 1 and 10 ng l^{-1} of 17α-ethinyloestradiol for 3 weeks and GCMS analysis, showed the mean concentrations to be <1.5, <6 and 14.5 ng l^{-1}, respectively. A nominal concentration of 10 ng l^{-1} significantly induced vitellogenin induction in males and females (and increased liver weight in males) but failed to have an effect on the gonadosomatic index. In addition, histological examination of male and female gonads showed no serious abnormalities. As the nominal test concentration of 1 ng l^{-1} had no adverse effects on any of the parameters studied, it was considered to be the NOEC.

Folmar *et al* (2000) exposed male sheepshead minnow (*Cyprinodon variegatus*) to 17α-ethinyloestradiol for a total of 16 days, with sampling intervals of 2, 4, 7, 10, 13 days. Nominal concentrations were as follows: 20, 100, 200, 500 and 1000 ng l^{-1}. Test concentrations were confirmed by radioimmunoassay and were found to be reasonably close to nominal values in all cases. A 17α-ethinyloestradiol concentration of 100 ng l^{-1} caused an upregulation of liver mRNA for vitellogenin by day 2; a significant induction of plasma vitellogenin was also demonstrated at this concentration over the 16 day period. A NOEC of 20 ng l^{-1} was reported, although the large concentration difference between the NOEC and LOEC values should be noted.

Fenske *et al* (2001a) exposed adult male zebrafish (*Danio rerio)* to nominal 17α-ethinyloestradiol concentrations of 1.67, 3, 7.5, 10 and 20 ng l^{-1} over a period of 21 days under semi-static conditions. There was a clear concentration dependent induction of plasma vitellogenin, the lowest observed effect concentration being ≤1.67 ng l^{-1}. The highest exposure concentration induced more than a 30,000-fold increase in plasma VTG above the controls. In addition, there was a considerable variation in the responses of individual fish, especially at the lowest exposure doses, where there was more than a 13-fold difference in the concentration of vitellogenin between the male fish.

Histopathology

Schweinfurth *et al* (1996) reported on a preliminary study in which 3 life-stages of fathead minnow (embryo-larvae, juvenile and adult) were exposed over a period of 4 weeks to graduated 17α-ethinyloestradiol concentrations of 0, 10, 100, 1000 and 10000 ng l^{-1} (measured by radioimmunoassay) under flow-through conditions. Observations included growth, morphology, egg production of adult fish and histopathological examinations. Growth was reduced in the larvae at concentrations of $\geq$100 ng l^{-1} (this only occurred at 10000 ng l^{-1} in juveniles). Histopathological changes in the kidney and liver were noted in larvae and juvenile fish at concentrations as low as 10 ng l^{-1}. In adult fish there appeared to be a reduced deposition of eggs at all test concentrations, resulting in a LOEC of 10 ng l^{-1}being reported.

Changes in reproductive success and development

A full life-cycle study with fathead minnow revealed a variety of effects on survival, growth, gross development, gonad development, sex determination and reproductive maturity (Länge *et al* 2001). Newly fertilised embryos (<24 h old) were exposed to nominal concentrations of 0.2, 1, 4, 16 and 64 ng l^{-1} in flow-through conditions at 25 ±1°C for 305 days (4 days pre-hatch and 301 days post-hatch). Exposure concentrations were confirmed by radioimmunoassay analysis and ranged from 58-84% with mean measured values ≥ 70%. Hatching success of F_o embryos was not significantly different from controls at any exposure concentration (NOEC > 64 ng^{-1}). Larval growth was reduced at 16 ng l^{-1} and a NOEC of 4 ng l^{-1} identified at day 28. In addition, juvenile fish growth was reduced when sampled at days 28 and 56 and NOEC and LOEC values of 1 and 4 ng l^{-1} were reported, respectively.

Gross morphological changes were seen in F_o fish at test concentration of 16 and 64 ng l^{-1}, the most prominent finding being anal protrusion. No males (with appropriate secondary sexual characteristics and territorial behaviour) were seen after 172 days post hatch at a concentration of 4 ng l^{-1} or above. Histology of F_o exposed fish at 56 days post hatch revealed a female:male sex ratio of 84:5 (with ova-testes in 11% of fish) at a concentration of 4.0 ng l^{-1}. No significant effects were seen at lower test concentrations. After 172 days post hatch, no testicular tissue was observed in any fish exposed to 4 ng l^{-1}. Thus the NOEC and LOEC values based on gonad histology were 1 and 4 ng l^{-1}, respectively. Consequently, the initiation of breeding pairs and the spawning phase of the study could only be conducted with the control, 0.2 and 1 ng l^{-1} exposure groups. There were no statistically significant reductions in egg production and thus the NOEC and LOEC values for F_0 reproduction were >1 ng l^{-1}. It is also of note that only 50% of assumed females exposed to 4 ng l^{-1} (4 of 8 pairs), following a 29 depuration period, were able to breed when paired with males which had not been exposed to 17α-ethinyloestradiol. After 172 days post hatch, there was a concentration-related increase in plasma vitellogenin induction; the NOEC and LOEC being 4 and 16 ng l^{-1}, respectively. Extreme degeneration and tubular dilation was seen in the kidneys at the highest test concentration of 64 ng l^{-1} after 158 days; these effects were not evident at the lower treatments at 172 days.

The hatchability rate of F_1 embryos was not statistically significant from controls and, therefore, NOEC and LOEC values were considered to be > 1 ng l^{-1}. There was no significant reduction in the survival of F_1 fish and whilst statistically detectable changes in F_1 growth were evident at 0.2 ng l^{-1}, these were not considered to be biologically significant when compared to historical controls. Histological analysis of early life stage F_1 fish indicated that there was a slight increase in the number of females at 0.2 and 1 ng l^{-1}, but that the increase was not concentration-related and, therefore, not considered to be treatment related. No histopathological lesions were observed in the liver and kidney of any F_1 fish.

Van Aerle *et al* (2002) exposed newly fertilised fathead minnow (<24 hour old) to a nominal 17α-ethinyloestradiol concentration of 10 ng l^{-1} for five different exposure periods under flow-through conditions. The exposure regimes were: fertilised eggs through embryo development to 5 days post-hatch (dph), 5-10 dph, 10-15 dph, 15-20 dph and fertilised eggs through embryo development to 20 dph. Two control groups were employed (one dilution water and one ethanol solvent control). For each of the treatment and control groups, the fertilised eggs were divided equally into 4 replicate tanks. At 30 dph, 30 fish were randomly taken from each treatment for measurement of vitellogenin. At 100 dph, 30 fish were randomly taken from each treatment group for histological analysis of the gonad. Lengths and weights were taken, and where possible, the sex recorded (determined by their secondary sexual characteristics).

There were no effects on hatchability to either 30 dph or 100 dph, nor were there any indications of gross developmental abnormalities due to 17α-ethinyloestradiol exposure. All exposure regimes induced significant vitellogenin synthesis that was positively correlated with longevity of exposure (the highest induction occurring in the treatment 'fertilised eggs to 20 dph', where there was a 7-fold higher concentration of VTG than in the controls). In all of the treatments, disruption in duct development (a feminisation as demonstrated by an ovarian-like cavity) occurred in males, with a window of enhanced sensitivity between 10-15 dph (where 60% of the males had feminised ducts). However, no oocytes were seen in any of the males exposed to 17α-ethinyloestradiol. No apparent abnormalities in duct development were seen in females in any of the treatment groups. There was an altered pattern in sex cell development in males (inhibition of spermatogenesis) in all treatments and the solvent control when compared with the dilution water controls. Furthermore, fewer spermatozoa were observed in the testis of males exposed from 15-20 dph and egg fertilisation-20 dph, compared with both the solvent and dilution water controls. It was considered that the consequences of this, if any, on reproductive performance were uncertain. No apparent abnormalities in oocyte growth and development were seen in females of any of the treatment groups.

Scholz and Gutzeit (2000) exposed freshly hatched Japanese medaka (*Oryzias latipes*) of the d-rR strain for 2 months to nominal 17α-ethinyloestradiol concentrations of 0, 1, 10 or 100 ng l^{-1} under semi-static conditions. The exposure period was followed by a 6 week recovery period in order to detect long-lasting effects on sexual differentiation. Sex ratio, gonadal growth, spawning, fecundity, histology as well as ovarian gene expression of aromatase was monitored. Growth was unaffected in all treatment groups. At 100 ng l^{-1}, all XY medaka were sex reversed and had developed an ovary. At lower test concentrations, no alteration of testicular structure was detected (including testis-ova or ovarian-like structures) and male fertility appeared to be unchanged. In XX females, significantly reduced ovarian weight was observed at 10 and 100 ng l^{-1} as well as a significantly decreased egg production rate. There was a 80% reduction in egg production at 10 ng l^{-1} and complete inhibition occurred at the highest test concentration, likely to be caused by the absence of males. Aromatase, which is normally only expressed in ovaries, was also detectable in testis of XY males exposed to 10 ng l^{-1}.

Metcalfe *et al* (2001) exposed Japanese medaka early life stages (1 day after hatch) to nominal concentrations of 0, 0.1, 1, 10, 100 and 1000 ng l^{-1} until medaka reached approximately 1.5 cm in length (which occurred at 85-110 days post hatch). The exposure period between renewals of test solutions was 48 hours and end-points monitored included growth, general condition, alteration to sex ratios and the development of testis-ova. Fish exposed to 1000 ng l^{-1} showed eosinophilic fluid accumulation in all major organs (heart, kidney and liver) and the body cavity. All surviving fish in this treatment were female and were significantly smaller relative to other treatments. Eosinophilic fluid was also observed in

organs and body cavity in fish exposed to 100 ng l^{-1}. At this concentration, the sex ratio was also significantly different from controls with a greater proportion of females (91%). Testis-ova were observed in male medaka from all treatments except at 10 ng l^{-1} and in 1 male fish from the lowest test concentration (0.1 ng l^{-1}). Examination of several histological sections revealed a single oogonium within the testicular tissue. On this basis, a LOEC value of 0.1 ng l^{-1} was reported. However, given that the natural occurrence of intersex has not been clearly established in this species, it is difficult to determine whether the small observed incidence of intersex in medaka is a consequence of exposure. Furthermore, the interpretation of the results and the reported LOEC value is questionable given the lack of a clear concentration response. A LOEC of 0.1 ng l^{-1} was reported based on the induction of testis-ova in one of 33 males, although at a higher test concentration of 10 ng l^{-1}, testis-ova was not observed in any of the 23 males. Certainly, at 100 ng l^{-1} there was a significant difference in sex ratios with only 4 males being reported (all of which had testis-ova) as opposed to 43 females. Instead, it is considered that the 'true' LOEC is likely to be between 10 and 100 ng l^{-1}. It should be noted that a separate study was conducted to establish the degradation of 17α-ethinyloestradiol under the conditions used in the bioassays. Following analysis by GCMS/MS, concentrations of 10 and 1000 ng l^{-1} were found on average to be 28.6% of the nominals over a period of 0, 24 and 48 hours, accompanied by increasing concentrations of oestrone. Although the study gives some indication of the relative instability of 17α-ethinyloestradiol, the reporting of all of the bioassay results as 'actuals' on the basis of these findings is considered inappropriate.

Zillioux *et al* (2001) exposed sheepshead minnow (*Cyprinodon variegatus*) to nominal 17α-ethinyloestradiol concentrations of 0, 0.2, 2, 20, 200, 400, 800, 1600 and 3200 ng l^{-1} in a partial life-cycle test. Fish were exposed for either 43 or 59 days, from subadult stages to sexual maturity, under flow-through conditions. Two reproductive trials (evaluating egg production and hatching success) were initiated. The first trial commenced after the 43 day exposure period and consisted of replicate spawning groups/treatment, whereas the second commenced after the 59 day exposure period and consisted of only one spawning group/treatment. Survival of the progeny was also monitored. Test concentrations were analysed weekly for all treatments at or above 200 ng l^{-1} by HPLC analysis. In the case of the lower test concentrations only the stock solutions were analysed to confirm the respective dosing concentrations due to the insensitivity of HPLC analysis for such low doses.

Alterations to the architecture of the testis (fibrosis) were observed in male fish exposed to ≥2 ng l^{-1} for both exposure periods, the severity of which generally increased with test concentration. Testis-ova were observed in several males exposed to ≥200 ng l^{-1} and it was also noted in one male from the 20 ng l^{-1} group exposed for 59 days. Among the female fish, artresia of pre- and postvitellogenic occytes were noted in several female fish at 200 ng l^{-1}. This was also observed at the 2 and 20 ng l^{-1} treatments, albeit at a lower frequency. Egg production in the first spawning trial (43 day exposure) was reduced in fish exposed to 200 ng l^{-1} and, in the second (59 day exposure) where only one spawning group was available at the 20 ng l^{-1} treatment. However, it was not possible to conduct statistical analysis of these data because spawning groups were not replicated. In the first trial, hatching success was significantly reduced in the progeny of fish exposed to 200 ng l^{-1} (<10% compared to 60 and 84% in controls), although survival was unaffected among fry that successfully hatched. Survival in fry in the lower dose groups was also similar to controls. It was considered likely that the poor fertilisation at 200 ng l^{-1} was a consequence of the highly vacularized, fibrotic testis and reduced number of spermatogonia observed in all males at this concentration. The NOECs, LOECs and MATCs derived for different endpoints are summarised in the table below.

Data from study exposing sheepshead minnow to 17α-ethinyloestradiol (Zillioux *et al* 2001)

End-point	NOEC (ng l^{-1})	LOEC (ng l^{-1})	MATC (ng l^{-1})
Fish survival	200	400	283
Fibrosis of testis (males)	0.2	2	0.6
Testis-ova (males)	2	20	6.3
Reproductive success	20	200	63
Hatching success of progeny	20	200	63

Nash and Kime (2000) recently conducted a multi-generation study in which zebrafish were exposed to 0, 0.5, 5 or 50 ng l^{-1} of 17α-ethinyloestradiol, although the full findings are yet to be finalised. The study was conducted under flow-through conditions and offered a high reproducibility as there were 12 replicated breeding populations in the controls and the exposure group (each breeding colony consisted of 12 adults). Male and female F_0 adults were exposed to test concentrations for 4 weeks during spawning and the subsequent F_1 generation exposed throughout their lifetime. The embryo viability of F_1 progeny (F_2 generation) was also investigated. In addition, various sub-exposures were conducted throughout the experiment. These included a cessation of exposure at 52 days in the F_1 generation followed by a 5 month period in clean water before egg collection, and exposure of the F_0 generation only followed by an investigation of effects on the F_1 generation. Steroid concentrations were confirmed by ELISA analysis and end-points measured included egg production, embryo viability, sex ratio and adult gonadal histology.

For the F_0 generation, mean egg number and 14 hour egg mortality were measured for 10 days prior to exposure and no significant differences were seen between the controls and treatment groups (background mortality was <10%). Following the first 5 days exposure, no significant effects were seen on either egg production or mortality at any treatment. However, at days 6 to 15 of exposure, the high dose only caused rapid cessation of spawning and increased 14 hour egg mortality. In addition, by days 26-28 of exposure complete inhibition of egg production was seen following the 50 ng l^{-1} treatment. No adverse effects were seen on embryo mortality or viability (deformities or development) in the F_1 generation at the 0.5 or 5 ng l^{-1} treatments. In addition, there were no differences in survival or weight of the F_1 juveniles (at 52 days), indicating the development of healthy adults.

The reproductive success (egg production and egg mortality) of the F_1 generation was determined at 7 months. Highly significant effects were observed at 5 ng l^{-1} in that there was a 60% reduction in egg production and almost 100% mortality in these eggs (3 survived out of 12,000). Although there was no effect on egg production at 0.5 ng l^{-1}, there was a slight but statistically significant increase in egg mortality (~20 *vs* 10% in the controls). A significant increase (of similar magnitude) in egg mortality was observed even when F_1 exposure ceased at 52 days and was followed by a period of 5 months in clean water. Furthermore, there was a statistically significant increase (albeit slight) in the percentage of F_2 progeny exhibiting late development in both the treated groups. No adverse effects on reproductive success of F_1 generation were observed following exposure of F_0 only.

Gonadal histology and vitellogenin results are yet to be published, although it is understood that the data are imminent. In summary, the 5 ng l^{-1} resulted in a 100% female population phenotypically and gonad histology revealed that the testes had not formed. However, since spawning did occur, albeit at a reduced capacity, it would appear that a proportion of fish were behaviourally male in order to induce egg production. Nonetheless, no fertilisation occurred at this treatment.

In a paired breeding study, Van den Belt *et al* (2001) exposed adult male and female zebrafish under semi-static (renewed daily) conditions to 0, 5, 10, 25 and 50 ng l^{-1} of 17α-ethinyloestradiol for 3 weeks. Nominal concentrations were confirmed by LCMS analysis and were shown to be 76 ± 14% prior to each renewal. The experiments were performed twice with, respectively, n = 5 and n = 7 successful breeding pairs per treatment per group. Five days before the end of exposure, the males were separated from the females in all treatment groups to allow the females to mature new eggs and to synchronise spawning for the evaluation period. After 5 days the exposure was stopped and individual breeding pairs were formed and kept in tap water. To assess effects on the male reproductive systems apart from those on females, exposed males were paired with non-exposed females and exposed females with non-exposed males. These non-exposed males and females were kept in tap water and treated for 3 weeks in the same manner as the exposed fish, including the daily renewal of water and the 5 day separation period. To evaluate reproduction success after the 3 weeks of exposure, the breeding pairs were kept together for a period of 5 days and the % spawning females and males with a post-exposure fertilisation above 70% were counted. Other end-points investigated included gonadosomatic index and plasma vitellogenin levels.

A concentration related reduction in the number of females capable of spawning was observed at 10 ng l^{-1}, with complete inhibition of spawning at levels of 25 ng l^{-1}. At the 10 and 25 ng l^{-1} treatments, the ovaries of the non-spawning females were significantly regressed as well as being macroscopically different from controls. A reduced size and a lack of large mature oocytes were clearly visible when ovaries of 10 and 25 ng l^{-1} treated fish were compared with those from the solvent control group.

Adverse effects on male fertilisation capacity were seen at all treatment concentrations (i.e. fertilisation success all below 70%), with only 6% for the 25 ng l^{-1} exposure. In addition, the number of non-exposed females that spawned when bred with exposed males was below the expected breeding success with both non-exposed males and females. It was considered that this indicated a dual negative impact of 17α-ethinyloestradiol on male reproduction: reduced fertilisation success and disturbed sexual behaviour. A significant reduction in testes somatic index (TSI) was also seen in males following exposure to 10 and 25 ng l^{-1}. For both males and females, a concentration dependent vitellogenin induction was measured, the effect being significant for females at ≥10 ng l^{-1}. Statistical analysis could not be conducted on males as vitellogenin levels were below the detection limit for control fish. Using linear regression analysis, vitellogenin induction was found to be significantly correlated (-0.67, $p < 0.001$) with the decrease in OSI of females that did not spawn. In males, the observed vitellogenin induction was correlated with a decrease in TSI (-0.46 $P < 0.01$).

A full life cycle test in which zebrafish were exposed to 17α-ethinyloestradiol has recently been conducted (Wenzel *et al* 2001, also reported previously by Schäfers *et al* 2001). Fertilised eggs (F_1 generation) were exposed to nominal test concentrations of 0, 0.05, 0.28, 1.67 and 10 ng l^{-1} and exposure continued until the end of the early life stage of the next generation (F_2) (day 174) (Wenzel *et al* 2001). Each test concentration was conducted as replicates and there were two controls. Confirmatory chemical analysis was conducted by GCMS/MS and analysis confirmed that test concentrations during F_1 generation exposure were close to nominals (>80%-<120%), other than the 1.67 ng l^{-1} concentration which measured 1.1 ng l^{-1}. Actual test concentrations were subsequently reported as 0, 0.05, 0.3, 1.1 and 10 ng l^{-1}. In the case of the F_2 generation exposure, actual concentrations were consistently higher than nominals and considered to be 0, 0.1, 0.3 and 2 ng l^{-1}. End-points investigated included behavioural abnormalities, growth, time to first spawning, egg production and fertilisation capacity. Fertilisation capacity was based on the percentage of fertilised eggs per vessel and day. This was calculated by dividing the total egg number and

numbers of fertilised eggs by the number of females in the respective vessel (determined histologically after the study). The table below provides a brief overview of the study protocol along with the main end-points investigated.

Study design of a full life cycle test exposing zebrafish to 17α-ethinyloestradiol (Wenzel *et al* 2001)			
Period	Course	Days after test start	Key end-points
Fish, early life stage toxicity (FELS) (According to OECD 201 but with reduced surveillance) (first generation F_1)	Start with 100 fertilised eggs per vessel	0	
	Hatch	3	Hatching time and rate
	Feeding with breeding food	6	Survival rate
	Feeding with *Artemia salina*	9	
	First transfer	14	Survival rate
	End of FELS study in the first exposed generation; second transfer	35-42	Survival, length
Reproduction	Number of fish equated to 50 per vessel Juvenile growth Sexual maturation	35-42 75 75 onward	 Length development Time to first egg production
	Reproduction	91-120	Quantitative determination of daily egg production and fertilisation capacity
	End of the first exposed generation (F1)	135	Length, weight, survival rate
Fish, early life stage toxicity (FELS) (According to OECD 201 but with reduced surveillance) (Second generation F_2)	Start with 100 fertilised eggs per vessel, transferred from the vessels of period 2	135	
	Hatch	138	Hatching time and rate
	Feeding with breeding food	141	Survival rate (6 days)
	Feeding with Artemia salina	144	Survival rate (9 days)
		149	Survival rate (14 days)
	First transfer	155	Survival rate
	End of FELS study in the second exposed generation; end of whole study	174	Survival rate, length and weight

At the highest test concentration of 10 ng l^{-1}, no effect on performance of early life stages (up to day 42) of zebrafish exposed as fertilised eggs from unexposed parental fish was observed. Juvenile growth, time until first spawning, and fertilisation rate (based on cell cleavage) were found to be highly correlated. At concentrations ≥ 1.1 ng l^{-1}, juvenile growth decreased and time to first spawning increased compared to controls. Indeed, at the highest concentration of 10 ng l^{-1} no mating behaviour by males was observed and thus no spawning occurred. This inhibition of reproduction was virtually irreversible as after recovery in clean water (100 days), mating and spawning developed to normal levels but fertilisation was reduced to 4% of that of the controls. In addition, vitellogenin levels remained elevated in male zebrafish even after the recovery period and gross morphology did not fully regenerate. At 1.1 ng l^{-1}, there was a significant decrease in egg production rate. There was a concentration dependent reduction in fertilisation capacity, with normal rates seen at 0.3 ng l^{-1} whilst at 1.1 ng l^{-1} fertilisation capacity was only around 50%.

The early-life stage exposure in F_2 generation revealed no effects on survival rates at 6, 14, 21 or 28 days exposure. There was however, a small but significant decrease in length of those treated at 2 ng l^{-1} compared to untreated controls. In addition, growth of juveniles (days 35-75) was significantly reduced at concentrations of 0.3 and 2 ng l^{-1}. Time to reaching sexual maturity in the F_2 generation also was also delayed (by 6 and >8 weeks in the replicates) in the 2 ng l^{-1} exposure group. Fertilisation capacity in this group was also significantly decreased (at 2-6% of controls) whereas 0.3 ng l^{-1} had no effect on this parameter.

An EC_{50} based on fertilisation rate of 1.1 ng l^{-1} was calculated, whilst, at lower concentrations the fertilisation rate was normal. The corresponding LOEC and NOEC values were therefore, 1.1 and 0.3 ng l^{-1}. The LOEC for vitellogenin induction was also observed to be the same as that for fertilisation capacity.

The effects of 17α-ethinyloestradiol on different life stages of zebrafish have also been studied, in order to ascertain whether a critical period of sensitivity exists (Segner *et al* 2002). In this partial life cycle test, the same concentrations were used as in the full–life cycle study reported by Wenzel *et al* (2001). Zebrafish were exposed during different developmental stages: from fertilisation until a) the end of embryonal period (start of hatching at 3 dph), b) 21 dpf (phase of undifferentiated gonads) c) 42 dpf (phase of protogynic ovaries) d) 66-72 dpf (completion of male/female differentiation) and e) the reproductive age. In addition, fish were exposed only during the period or male/female differentiation (between days 42-75 dpf). At the end of exposures, fish were transferred to control conditions and reared until the adult stage after which reproductive parameters (time to spawning and mating behaviour, number of eggs per female, fertilisation capacity and embryo hatching), gonad histopathology and plasma vitellogenin were recorded for a 3 week period.

Estrogenic exposure of zebrafish during the embryonal (0-3 dpf), larval (0-21 dpf) or early life stage (dpf) period did not result in lasting effects on reproductive performance at the adult stage. However, a sensitive window for oestrogenic disturbances was identified during the period of sexual differentiation (0-75, 42-75 dpf). Exposure within this sensitive stage resulted in delayed and reduced spawning and lowered fertilisation rate at concentrations ≥3 ng l^{-1}. It is of note that whereas the decline in fertilisation capability was always clearly expressed, the response in time to first spawning and egg production could be obscured by high variation among individual or experimental groups. Significant induction of vitellogenin was also significant at ≥3 ng l^{-1}. Chronic (whole life span) and temporary (days 44 to day 71) exposure to 10 ng l^{-1} induced severe alterations of female and male gonads. This included a decrease in gamete cells (both oocytes and sperm cells), a decrease in gonadal size and an increase in connective tissue, phagocytic cells and residual bodies.

Changes in behaviour

No data has been identified on the endocrine disrupting effects of 17α-ethinyloestradiol on the sexual behaviour of fish.

In vivo **studies in invertebrates**

A number of studies have been conducted which have investigated the potential endocrine mediated responses of 17α-ethinyloestradiol on aquatic invertebrates.

The effects of 17α-ethinyloestradiol on the reproductive behaviour of the amphipod, *Gammarus pulex* has been investigated (Watts *et al* 2001a). Actual test concentrations were confirmed by GC/MS/MS analysis. Several aspects of reproductive behaviour including the

ability of males and females to detect each other, form precopulatory guarding pairs and to continue guarding behaviour were examined over a 24 hour period. No effect on direct or indirect effect on precopulatory guarding behaviour was seen at any test concentration (0.01-3700 μg l^{-1}). However, when forceably separated following placement in anaesthetic, it was possible to calculate the time taken to reform pairs. A significant increase in re-pairing time was observed, but only at the high doses of 540 and 3700 μg l^{-1}. The effect on population structure/recruitment was studied on mixed populations of the amphipod *Gammarus pulex* following 100 days exposure in a flow-through system (Watts *et al* 2002). Nominal test concentrations were 0, 0.1, 1 and 10 μg l^{-1}, which were confirmed by GC/MS/MS analysis at the start of the exposure. These revealed that actual concentrations were ≥70% of the nominals. Counts of total animal numbers revealed that, in all treatment groups, population size dramatically increased due to recruitment, with neonate gammarids the most abundant. At concentrations of 1 and 10 μg l^{-1}, the recorded mean population sizes of 385 and 411, respectively, were significantly greater than the control (257). The sex ratio of adults was biased by 2:1 in favour of females in all the treated groups compared to controls. The number of male adults, precopula guarding pairs, ovigerous females did not differ between treatments, nor were there any effects on the measurement of secondary characteristics (antenna and gnathopod length).

Full life cycle (4 - 6 weeks from gametogenesis or hatch, respectively, until adulthood) and multi-generation exposures (15 weeks) were performed to explore effects on developmental and reproductive parameters on the amphipod *Hyalella azteca*. Based on very limited details, it appears that the only adverse effects were reduced growth in male 2nd gnathopods (0.1-0.32 μg l^{-1}) and disturbed gonadal development (0.1-10 μg l^{-1}) in the second generation (Segner *et al* 2002).

Hutchinson *et al* (1999) conducted life-cycle studies investigating the effect of 17α-ethinyloestradiol on the copepod *Tibse battagliai* (Crustacea). Newly released (<24 hrs old) animals were exposed to nominal concentrations 0.1, 1, 10 and 100 μg l^{-1} over 21 days and effects monitored in terms of development, sex ratio and fecundity. None of the concentrations had an adverse effect on these life-cycle parameters and, thus, a 21-day of NOEC of ≥100 μg l^{-1} was reported.

Anderson *et al* (2001) tested 17α-ethinyloestradiol and its inhibitory effect on larval development of the copepod *Acartia tonsa*. In the 5 day larval development test, EC_{10} and EC_{50} values were 46 and 88 μg l^{-1}, respectively, for 17α-ethinyloestradiol.

Breitholtz and Bengtsson (2001) investigated the effects of 17α-ethinyloestradiol on the copepod *Nitocra spinipes* (Crustacea). Newly released (<24 hrs old) animals were exposed to nominal concentrations 0, 0.5, 5 and 50 μg l^{-1} (17α-ethinyloestradiol) for up to 18 days and effects monitored in terms of larval development rate, sex ratio and fecundity. None of the concentrations had a significant adverse effect on these life-cycle parameters.

A series of developmental and reproductive parameters were investigated in the freshwater snail (*Lymnea stagnalis)* following exposure to 17α-ethinyloestradiol, although experimental details are currently lacking (Segner *et al* 2002). Egg masses exposed for 3 weeks showed an altered protein pattern (0.05-0.5 μg l^{-1}), disturbed hatching (0.5-1 μg l^{-1}), delayed hatching (1 μg l^{-1}) and deformations in developing snails (0.1-1 μg l^{-1}). When exposed of 10 weeks, an altered protein pattern was similarly observed at 0.05-0.5 μg l^{-1}, and a reduced growth in hatchlings was observed at these same exposure concentrations. Exposure starting from the

juvenile stage until reaching sexual maturity, caused an increase in egg laying but hatching was disturbed at 0.5 μg l^{-1}.

A series of tests have been conducted which investigated the effects of 17α-ethinyloestradiol on the hydroid *Hydra vulgaris* (a primitive invertebrate), although experimental details are limited (Segner *et al* 2002). In male clones, regeneration of dissected polyps was inhibited at 320 μg l^{-1} following 72 hours of exposure, although no effect on degeneration of polyps was observed up to 1600 μg l^{-1}. There were no adverse effects at 0.01-58 μg l^{-1} concentrations. Following an exposure period of 6 weeks (both sexes), significant reductions in the number of oocytes and sperm activity were reported at 500 μg l^{-1}.

Watts *et al* (2001b) investigated the effect of 17α-ethinyloestradiol on development and reproduction in midge larvae *Chironomus riparius* over two generations in chronic sediment exposure assays. Test sediment was spiked at nominal concentrations of 0, 1, 10, 50 or 100 ng l^{-1}. GC/MS analysis was conducted on stock solutions, although sediment concentrations could not be established due to lack of analytical sensitivity. Following a settlement period, each replicate jar (four per treatment) received 20 individual instar *Chironomus riparius*. End-points investigated included median emergence times, the number and sex ratio of emerged adults, egg production and egg viability. Although the percentage of adults that emerged and their median emergence times were affected by exposure, there was no consistent dose-response relationship. For example, at 1 ng l^{-1}, both the first and second generation of adults emerged significantly earlier than control animals, although this was not seen at higher doses. At 50 and 100 ng l^{-1}, emergence of female adults was significantly delayed compared to controls, but male emergence in these treatments were unaffected. No effect on adult emergence time in either generation was noted at 10 ng l^{-1}. At a test concentration of 50 ng l^{-1} only, significantly more adults emerged in the second generation than in controls. Sex ratio appeared unaffected in all treatments in the F_1 generation, although the sex ratio of adults in the second generation (F_2) at 1, 10 and 50 ng l^{-1} was noticeably different with a prominence of males in each case. No effect on egg production by the first generation or egg viability was demonstrated.

Segner *et al* (2002) summarised toxicity studies on midge larvae *Chironomus riparius* exposed to 17α-ethinyloestradiol, including one that investigated effects on mouthpart deformities. However, experimental detail was lacking for all of the studies. Eggs were exposed for approximately 20 days and it was found that moulting was delayed/wet weight reduced at 1000 μg l^{-1}. The effect of 17α-ethinyloestradiol on mouthpart deformities was subsequently investigated in 4th instar life-stage. Although significant mouthpart deformities were noted at 0.01-10 μg l^{-1}, a dose-response relationship was not demonstrated as little or no effect was seen at higher (unspecified) concentrations (Segner *et al* 2002). Meregalli and Ollevier (2001) have also reported the lack of such effects. *Chironomus riparius* larvae (1dph first and second instar) were exposed to nominal 17α-ethinyloestradiol concentrations of 1, 10 or 100 μg l^{-1} for 9 days under semi-static conditions. No adverse effects on mouthpart deformity were observed.

In a 21-day reproductive study in the waterflea *Daphnia magna* conducted under semi-static conditions (according to FDA guideline 4.09) a slight shortening of maturation period in parent animals was observed, although there was no decrease in the number of offspring per surviving female (Schweinfurth *et al* 1996).

In a 3-generation study, the waterflea *Ceriodaphnia reticulata* and the cladoceran *Sida crystallina* were exposed to six different concentrations (10-500 μg l^{-1}) of 17α-ethinyloestradiol for 4 weeks. Every two days the medium was renewed and batch size, first appearance and

number of offspring produced were investigated. The juvenile phase of *Sida* was significantly shorter at >100 μg l^{-1} and no effects were seen on birth rate, number of juveniles per female and net reproduction rate for *Ceriodaphnia* (Jaser *et al* 2001).

B. Studies on terrestrial organisms

McMurry and Dickerson (2001) investigated the effects of 17α-ethinyloestradiol by oral gavage to bobwhite quail (*Colinus virginianus*) eggs. Six eggs were exposed to each test concentration (0.1, 0.3, 1, 3, 10 mg kg^{-1}) and quail were allowed to hatch before being sacrificed at 21 days of age. Blood, measurements and tissues were collected. Some trends for hatchling weight were reported in 17α-ethinyloestradiol dosed females, along with dose-response effects beyond those of survival of *in ovo* dosed quail. In addition, liver and kidney somatic index was significantly different from vehicle treatments (corn oil).

C. Studies on aerial organisms

No information has been located on the potential endocrine disrupting effects of 17α-ethinyloestradiol on aerial species. Given that oestrone is not considered to be volatile the absence of data on potential endocrine mediated responses in aerial organisms is not a key area of uncertainty. It should be recognised that there are currently no internationally agreed methods specifically developed to assess endocrine disrupting effects in aerial organisms.

D. Summary of potential endocrine disrupting effects in wildlife

Th data on potential endocrine mediated responses of 17α-ethinyloestradiol in wildlife is limited and restricted to aquatic organisms (fish and invertebrates) An assessment of aquatic toxicity data indicates that, of the taxa for which data are available, fish are the most sensitive to the adverse effects of l17α-ethinyloestradiol. However, relatively few aquatic vertebrate taxa (where steroid oestrogens play a central role in reproduction), notably amphibians, have been studied in this regard and so this remains an area of uncertainty.

Laboratory studies in fish have measured a variety of end-points, although the ecological significance of some has not yet been fully established (e.g. plasma vitellogenin induction and effect on gonadosomatic index). Nevertheless, recent research in wild roach populations indicates that marked histological effects on the gonads, such as a severe intersex condition, can result in a reduced reproductive capacity. Alteration of the timing of maturation is also important in terms of reproductive success, as it may result in gametes being released outside the optimal breeding season and subsequently reduced recruitment. However, there can be no doubt that irreversible effects on reproductive parameters such as the production of markedly skewed sex ratios or single sex generations, marked reductions in egg production, significant increases in egg mortality and reduced fertilisation success are clearly of ecological significance, and have been shown to be sensitive end-points for steroids.

Table 15.2 Summary of potential endocrine disrupting effects in aquatic organisms following exposure though the water column

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Fish						
Carp (*Cyprinus carpio)*	Juvenile carp	Static: 0, ≥ 10 ng l^{-1} (Nominal concentrations)	Significant vitellogenin induction (relative to the controls) at ≥ 10 ng l^{-1} after 10 days exposure.	Purdom *et al* (1994)	Medium	Use with care
Fathead minnow (*Pimephales promelas)*	Newly fertilised embryos	Flow-through; 0, 0.2, 1, 4, 16 and 64 ng l^{-1} (Measured concentrations)	Significant reduction in juvenile growth (relative to the controls) at 4 ng l^{-1} after 28 and 56 days exposure, with a NOEC of 1 ng l^{-1}. Significant changes in gonad histology (relative to the controls) with no testicular tissue observed in any fish at 4 ng l^{-1} after 172 days exposure, with a NOEC of 1 ng l^{-1}. No significant differences in egg production (relative to the controls) at 1 ng l^{-1} after 172 days exposure hence a NOEC of > 1 ng l^{-1}. Significant plasma vitellogenin induction (relative to the controls) at 16 ng l^{-1} after 172 days exposure, with a NOEC of 4 ng l^{-1}. No significant change in hatchability rate of F_1 embryos (relative to the controls) at 1 ng l^{-1} after 172 days exposure, hence a NOEC of >1 ng l^{-1}. Significant reduction in F_1 growth (relative to the controls) at 0.2 ng l^{-1}; after 172 days exposure, with a LOEC of <0.2 ng l^{-1}.	Länge *et al* (2001)	High	Valid

Table 15.2 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Fathead minnow (*Pimephales promelas)*	Newly fertilised eggs	Flow through; 0, 10 ng l^{-1} (Nominal concentrations)	No significant effects on hatchability or any indications of gross developmental abnormalities (relative to the controls) at 10 ng l^{-1}after 30 or 100 dph, hence a NOEC of 10 ng l^{-1}. Significant vitellogenin induction (relative to the controls) at 10 ng l^{-1} after 100 dph, thus NOEC <10 ng l^{-1}. Significant feminisation occurred in males (relative to the controls) at 10 ng l^{-1} after 100 dph, hence NOEC <10 ng l^{-1}. No significant abnormalities in duct development in females (relative to the controls) at 10 ng l^{-} After 100 dph, hence NOEC 10 ng l^{-1}. Significant inhibition of spermatogenesis (relative to the controls) at 10 ng l^{-1}after 100 dph, hence a NOEC of <10 ng l^{-1}.	Van Aerle *et al* (2002)	Medium	Valid
	Embryo-larval, juvenile and adult fish	Flow-through: 0, 10, 100, 1000 and 10000 ng l^{-1} (Measured concentrations)	Significant reduction in larvae growth (relative to the controls) at 100 ng l^{-1}after 28 days exposure, with a NOEC of 10 ng l^{-1}. Significant histopathological changes in the kidney and liver of larvae and juveniles (relative to the controls) at 10 ng l^{-1} after 28 days exposure, with a NOEC of <10 ng l^{-1}. Significant reduction in adult egg production at 10 ng l^{-1}after 28 days exposure, with a NOEC of <10 ng l^{-1}.	Schweinfurth *et al* (1996)	Medium	Valid

Table 15.2 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Flounder (*Platichthys flesus)*	Adult, male and female	Flow-through: 0, <1.5, <6 and 14.5 ng l^{-1} (Measured concentrations)	Significant vitellogenin induction (relative to the controls) at 10 ng l^{-1}after 21 days exposure, with a NOEC of 1 ng l^{-1}. No significant effect on gonado-somatic index (relative to the controls) at 10 ng l^{-1}after 21 days exposure, hence a NOEC of 1 ng l^{-1}.	Allen *et al* (1999)	Medium	Valid
Japanese medaka (*Oryzias latipes*)	1 day post-hatch	Semi-static; 0, 0.1, 1, 10, 100 and 1000 ng l^{-1} (Nominal concentrations)	Significant change in the condition of surviving fish (relative to the controls at 1000 ng l^{-1}, after 85 to 100 days, with a NOEC of 100 ng l^{-1}. Significant reduction in growth (relative to the controls) at 1000 ng l^{-1} after 85 to 100 days exposure, with a NOEC of 100 ng l^{-1}. Significant change in sex-ratio (higher % female) (relative to the controls) at 100 ng l^{-1} after 85 to 100 days exposure, with a NOEC of 1 ng l^{-1}. Significant testis-ova induction (relative to the controls) at 0.1 ng l^{-1} after 85 to 100 days exposure, with a NOEC of <0.1 ng l^{-1}.	Metcalfe *et al* (2001)	Medium	Valid

Table 15.2 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Japanese medaka (*Oryzias latipes*)	Newly hatched larvae	Semi-static; 0, 1, 10 and 100 ng l^{-1} (Nominal concentrations)	No significant change in growth (relative to the controls) at 100 ng l^{-1}after 60 days exposure, thus a NOEC of 100 ng l^{-1}. All male medaka were sex reversed and had developed an ovary at 100 ng l^{-1} after 60 days exposure, with a NOEC of 10 ng l^{-1}. Significant reduced ovarian weight in females (relative to the controls) at 10 ng l^{-1} after 60 days exposure, with a NOEC of 1 ng l^{-1}. Significant decreased egg production in females (relative to the controls) at 10 ng l^{-1} after 60 days exposure, with a NOEC of 1 ng l^{-1}.	Scholz and Gutzeit (2000)	High	Use with care
Rainbow trout (*Oncorhynchus mykiss)*	Adult, male	Static: 0, 0.1, 0.5, 1 and 10 ng l^{-1} (Nominal concentrations)	Significant plasma vitellogenin induction (relative to the controls) at 0.1 ng l^{-1} after 10 days exposure, with a NOEC of <0.1 ng l^{-1}.	Purdom *et al* (1994)	Medium	Use with care
	Adult, male	Flow-through: 0, 2 ng l^{-1} (Measured concentrations)	Significant vitellogenin (relative to the controls) at 1.79 ng l^{-1}after 21 days exposure, with a NOEC of <1.79 ng l^{-1}. Significant inhibition of testicular growth (relative to the controls) at 1.79 ng l^{-1}after 21 days exposure, with a NOEC of <1.79 ng l^{-1}.	Jobling *et al* (1996)	Medium	Use with care
	Juvenile, female	Flow-through; 0, 0.1, 0.32, 1, 3.2, 10 and 32 ng l^{-1} (Measured concentrations)	Significant plasma vitellogenin induction (relative to the controls) with an EC_{50} of 1 ng l^{-1} after 14 days exposure and a LOEC of 1 ng l^{-1}.	Thorpe *et al* (2001)	Medium	Valid

Table 15.2 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement Parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Rainbow trout (*Oncorhynchus mykiss)*	Adult, males and females	Flow-through: 0, 0.1, 0.3 and 1 ng l^{-1} (Nominal concentrations)	No significant effects on condition (relative to the controls) at 1 ng l^{-1} after 28 weeks exposure, thus a NOEC of > 1 ng l^{-1}. Significant plasma vitellogenin induction (relative to the controls) at 1 ng l^{-1} after 28 weeks exposure at a test temperature of 17.4 °C, with a NOEC 0.1 ng l^{-1}. Significant plasma vitellogenin induction (relative to the controls) at 1 ng l^{-1} after 28 weeks exposure at a test temperature of 11.4 °C, with a NOEC 0.3 ng l^{-1}. Significant histological changes in the liver (relative to the controls) at 1 ng l^{-1} after 28 weeks exposure, with a NOEC of 0.3 ng l^{-1}. No significant effects on either GSI or HIS values (relative to the controls) at 1 ng l^{-1} after 28 weeks exposure, with a NOEC of >1 ng l^{-1}.	Sheahan *et al*(1993)	Medium	Valid
Sheepshead minnow (*Cyprinodon variegatus*)	Adult, male	Flow-through: 0, 20, 100, 200, 500, and 1000 ng l^{-1} (Measured concentrations)	Significant upregulation of liver mRNA for vitellogenin, (relative to the controls) at 100 ng l^{-1} after 2 days exposure, with a NOEC of 20 ng l^{-1}. Significant induction in plasma vitellogenin (relative to the controls) at 100 ng l^{-1} after 16 days exposure, with a NOEC of 20 ng l^{-1}.	Folmar *et al* (2000)	Medium	Valid

Table 15.2 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Sheepshead minnow (*Cyprinodon variegatus*)	Sub-adult stages	Flow-through; 0, 0.2, 2, 20, 200, 400, 800, 1600 and 3200 ng l^{-1} (Measured concentrations)	End-point (after 59 days exposure) — NOEC (ng l^{-1}) — LOEC (ng l^{-1}) Fibrosis of testis (males) — 0.2 — 2 Testis-ova (males) — 2 — 20 Reproductive success — 20 — 200 Hatching success of progeny — 20 — 200	Zillioux *et al* (2001)	High	Valid
Zebrafish (*Danio rerio*)	Males and females	Flow-through; 0, 0.5, 5 and 50 ng l^{-1} (Measured concentrations)	No significant effects on egg production (F_1 generation) (relative to the controls) at 50 ng l^{-1} with a NOEC of 5 ng l^{-1}. No significant effects on embryo mortality or viability (F_1 generation) (relative to the controls) at 5 ng l^{-1} with a NOEC of 0.5 ng l^{-1}. No significant effects on juvenile mortality or weight (F_1 generation) (relative to the controls) at 5 ng l^{-1} after 52 days exposure, thus a NOEC of 5 ng l^{-1}. Significant reduction in egg production (relative to the controls) at 5 ng l^{-1} after 7 months exposure, with a NOEC of 0.5 ng l^{-1}. Significant increase in egg mortality (relative to the controls) at 0.5 ng l^{-1}, with a NOEC of <0.5 ng l^{-1}.	Nash and Kime (2000)	High	Valid
	Adult, male	Semi-static; 0, 1.67, 3, 7.5, 10 and 20 ng l^{-1} (Nominal concentrations)	Significant plasma vitellogenin induction (relative to the controls) at 1.67 ng l^{-1}after 21 days exposure, with a NOEC of ≤1.67 ng l^{-1}.	Fenske *et al* (2001a)	Medium	Use with care

Table 15.2 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	NOEC (ng l^{-1})	LOEC (ng l^{-1})	Reference	Test Relevance	Study Validity
Zebrafish (*Danio rerio*)	Fertilised eggs	F_1 generation exposed to; 0, 0.05, 0.3, 1.1 and 10 ng l^{-1}	Reduced juvenile growth (F1)	0.3	1.1	Wenzel *et al* (2001)	High	Valid
			Increase in time to first spawning (F1)	0.3	1.1			
			Reduced egg production (F1)	0.3	1.1			
			Reduced fertilisation capacity (F1)	0.3	1.1			
		F_2 generation exposed to; 0, 0.1, 0.3 and 2 ng l^{-1}	Survival (F2 larvae)	>2	>2			
			Reduced larval length (F2)	0.3	2			
			Reduced juvenile 'psuedo' growth (F2)	0.1	0.3			
			Increase in time to first spawning (F2)	0.3	2			
		(Measured concentrations)	Fertilisation capacity (F2)	0.3	2			
			An EC_{50} of 1.1 ng l^{-1} for fertilisation rate was calculated. At 10 ng l^{-1}, no mating behaviour by males in F1 generation and thus no spawning occured. The reduced juvenile growth in F2 was based on subtracting the mean length the respective vessel population at day 35 and the individual lengths at day 75 (expressing pseudo specific growth from day 35 to day 75).					

Table 15.2 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Zebrafish (*Danio rerio*)	Adult, male and female	Semi-static; 0, 5, 10, 25 and 50 ng l^{-1} (Measured concentrations)	Significant inhibition of females spawning (relative to the controls) at 10 ng l^{-1} after 21 days exposure, with a NOEC of 5 ng l^{-1}. Significant reduced GSI in females (ovaries significantly regressed) and males (reduction in testes somatic index) (relative to the controls) at 10 ng l^{-1} after 21 days exposure, with a NOEC of 5 ng l^{-1}. Significant effects on male fertilisation capacity (relative to the controls) at 5 ng l^{-1} after 21 days exposure with a NOEC of <5 ng l^{-1}. Significant vitellogenin induction in females (relative to the controls) at 10 ng l^{-1}after 21 days exposure, with a NOEC of 5 ng l^{-1}.	Van den Belt *et al* (2001)	Medium	Valid
	Different stages	Flow-through; 0, 0.05, 0.28, 1.67 and 10 ng l^{-1} (Nominal concentrations)	No significant effect on reproductive performance at the adult stage (relative to the controls) when embryonal (0-3 dpf), larval (0-21 dpf) or early life stage period were exposed to 10 ng l^{-1}. Significant induction of vitellogenin, delayed and reduced spawning and lowered fertilisation rates (relative to the controls) when exposure to 3 ng l^{-1} occurred during the period of sexual differentiation (0-75, 42-75 dpf), with a NOEC of 1.67 ng l^{-1}.	Segner *et al* (2002)	Medium	Valid

Table 15.2 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Invertebrates						
Amphipod (*Gammarus pulex)*	Adult	Static; 0, 0.01 – 3700 μg l^{-1} (Measured concentrations)	No significant effect on direct or indirect effect on precopulatory guarding behaviour (relative to the controls) at 3700 μg l^{-1}, hence NOEC of 3700 μg l^{-1}. Significant increase in re-pairing time (relative to the controls) at 540 μg l^{-1}, with a NOEC of 0.01 μg l^{-1}.	Watts *et al* (2001a)	High	Valid
	Mixed age populations	Flow-through; 0, 0.1, 1, and 10 μg l^{-1} (Measured concentrations)	Significant increase in mean population size (relative to the control) at 1 μg l^{-1} after 100 days exposure, with a NOEC of 0.1 μg l^{-1}. Significant change in sex ratio of adults (biased by 2:1 in favour of females) (relative to the controls) at 0.1 μg l^{-1}.	Watts *et al* (2002)	High	Valid
Amphipod (*Hyalella azteca)*	4-6 weeks from gametogenesis	No data (Nominal concentrations)	Reduced growth in male 2nd gnathopods (0.1 – 0.32 μg l^{-1}) and disturbed gonadal development (0.1 – 10 μg l^{-1}) in the second generation.	Segner *et al* (2002)	Valid	Use with care
Cladoceran (*Sida crystallina)*	Larvae	Semi-static: 0, 10 – 500 μg l^{-1} (Nominal concentrations)	No significant effects on birth rate, number of juveniles per female and net reproduction rate (relative to the controls) at 500 μg l^{-1}after 28 days exposure, hence a NOEC of 500 μg l^{-1}. Significantly shorter juvenile phase (relative to the controls) at 100 μg l^{-1} after 28 days exposure, with a NOEC at 10 μg l^{-1}.	Jaser *et al* (2001)	High	Valid
Copepod (*Acartia tonsa*)	Larvae	No data (Nominal concentrations)	In the 5 day larval development test, LC_{10} and LC_{50} values were 46 μg l^{-1} and 88 μg l^{-1}, respectively.	Anderson *et al* (2001)	Medium	Use with care

Table 15.2 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Copepod (*Tisbe battagliai*)	Newly released (<24 h old animals)	Semi static; 0, 0.1, 1, 10 and 100 μg l^{-1} (Nominal concentrations)	No significant effect on development, sex ratio and fecundity (relative to the controls) at 100 μg l^{-1}, hence a NOEC of ≥100 μg l^{-1}.	Hutchinson *et al* (1999)	High	Valid
Copepod (*Nitocra spinipes*)	Adult, juveniles (<24 h old)	0, 0.5, 5 and 50 μg l^{-1} (Nominal concentrations)	No significant effect on development, sex ratio and fecundity for juveniles (relative to the controls) at 50 μg l^{-1} after 18 days exposure, with a NOEC of ≥50 μg l^{-1}.	Breitholtz and Bengtsson (2001)	High	Use with care
Freshwater snail (*Lymnea stagnalis)*	Egg masses	0, 0.05, 0.1, 0.5, 1.0 μg l^{-1} (Nominal concentrations)	Significant altered protein pattern (relative to the controls) at 0.05 μg l^{-1} after 21 days and 10 weeks exposure, with a NOEC of <0.05 μg l^{-1}. Significant disturbed hatching (relative to the controls) at 0.5 μg l^{-1}after 21 days exposure, with a NOEC of 0.1 μg l^{-1}. Significant delayed hatching (relative to the controls) at 1 μg l^{-1}after 21 days exposure, with a NOEC of 0.05 μg l^{-1}. Significant deformations in developing snails (relative to the controls) at 0.1 μg l^{-1} after 21 days exposure, with a NOEC of 0.05 μg l^{-1}. Significant reduced growth of hatchlings (relative to the controls) at 0.05 μg l^{-1} after 10 weeks exposure, with a NOEC of <0.05 μg l^{-1}.	Segner *et al (*2002)	Medium	Use with care

Table 15.2 Continued

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Hydroid (*Hydra vulgaris)*	Adult, male and female	Up to 1600 µg l^{-1} (Nominal concentrations)	Significant inhibition of regeneration of dissected polyps in male clones (relative to the controls) at 320 µg l^{-1}, after 72 hours exposure. Significant reductions in the number of oocytes (females) and sperm activity (males) (relative to the controls) at 500 µg l^{-1}after 6 weeks exposure.	Segner *et al (*2002)	Medium	Use with care
Midge larvae (*Chironomus riparius)*	Larvae	Static: 0, 1, 10, 50 and 100 µg l^{-1} (Nominal concentrations in sediment)	Significant delay of emergence of female adults (relative to the controls) at 50 µg l^{-1} with a NOEC of 10 µg l^{-1}. No significant delay in emergence of male adults (relative to the controls) at 100 µg l^{-1}, hence NOEC of >100 µg l^{-1}. At 50 µg l^{-1} only, significantly more adults emerged in the second generation than in the controls. No significant effect on egg production or viability by the first generation (relative to the controls) at 100 µg l^{-1}, hence NOEC of >100 µg l^{-1}.	Watts *et al* (2001b)	High	Use with care
	No data	Static: (Nominal concentrations)	Significant delay in moulting and reduced wet weight (relative to the controls) at 1000 µg l^{-1}after 20 days exposure.	Segner *et al* (2002)	Medium	Use with care

Species	Life stage of the test organism at start of test	Exposure route and concentration series	Description of endocrine disruption measurement parameter(s) and effect concentrations	Reference	Test Relevance	Study Validity
Water flea (*Daphnia magna)*	Juvenile	Semi-static (Nominal concentrations)	No significant immobilisation or decrease in the number of offspring (relative to the controls) at 387 μg l^{-1} after 21 days exposure.	Schweinfurth *et al (*1996)	High	Use with care
Water flea (*Ceriodaphnia reticulata)*	Larvae	Semi-static: 0, 10 – 500 μg l^{-1} (Nominal concentrations)	No significant effects on birth rate, number of juveniles per female and net reproduction rate (relative to the controls) at 500 μg l^{-1}. A higher mortality of newly hatched juveniles (relative to the controls) at 200 μg l^{-1} after 28 days exposure.	Jaser *et al* (2001)	High	Valid

Although there were a number of highly relevant studies, three were considered key in terms of the potential endocrine disrupting effect of 17α-ethinyloestradiol (Länge *et al* 2001, Nash and Kime 2000, Wenzel *et al* 2001).

Analytical monitoring was conducted which confirmed nominal test concentrations (mean measured value >70%)

- A number of end-points of potential demographic relevance were measured including survival, growth, gross development, gonad development, sex determination and reproductive maturity, as well as measuring the induction of vitellogenin.

- Sensitive early life stages were included (embryos and juveniles).

- Exposure was chronic (i.e. full–life cycle studies including F_0 and F_1 generations, and in two cases the F_2 generation); and

- The intervals between test concentrations were small, thereby increasing confidence in the reported NOEC and LOEC values.

In the full life-cycle study in fathead minnow (Länge *et al* 2001), LOEC and NOEC values were reported for each of the toxic end-points studied, with the authors reporting an overall NOEC value of 1 ng l^{-1} (nominal value). In particular, after 172 days exposure of the F_o generation, 4 ng l^{-1} caused total feminisation of the population (no testicular tissue in any of the males). This is a severe effect given that total absence of phenotypic males would inevitably affect population sustainability. Consequently, the next lowest exposure concentration of 1 ng l^{-1} may be taken as the NOEC, where the sex ratio was 65% males to 35% females. It is also of note that only 50% of assumed females exposed to 4 ng l^{-1}, following a 29 day depuration period, were able to breed when paired with unexposed males.

In the full-life cycle in zebrafish by Wenzel *et al* (2001) the most sensitive end-point reported in this study was an EC_{50} value of 1.1 ng l^{-1} (measured concentration) for fertilisation success. This value was essentially a LOEC as the next lower test concentration of 0.3 ng l^{-1} did not reduce fertilisation success compared to controls. A concentration of 10 ng l^{-1} resulted in complete inhibition of fertilisation, which would support the view of a steep dose-response curve as in the Länge *et al* (2001) study. In addition, a number of other end-points were investigated, all of which either produced the same or a higher LOEC. Thus, fertilisation success, which is a highly relevant ecological end-point, was clearly sensitive to the effect of this steroid.

A multi-generation study by Nash and Kime (2000) has been performed although the full findings are yet to be published. The most sensitive end-points reported in the study were for egg production (60% reduction compared to controls) and egg mortality (100%) in the F_1 generation at 5 ng l^{-1}, both of which are of high ecological significance. There was also a slight but statistically significant, increase in egg mortality at 0.5 ng l^{-1} (20% compared to a background of 10% in the controls The ecological significance of such an increase is less certain, particularly given that OECD Test Guideline 210 (fish early-life stage toxicity test) permits up to 30% mortality of controls (up to the post hatch stage) in tests with zebrafish.

The other study worthy of discussion is by Metcalfe *et al* (2001) as it involved early life stages of Japanese medaka and was of chronic duration (85-110 days). However, the end-points measured were more limited than those studied by the above authors and only involved growth, survival, sex ratios and induction of testis-ova. In addition, the results are based on

nominal concentrations (a separate persistence study using similar conditions indicated that the actual concentrations were, on average, only 28.6% of the nominal concentrations of 10 and 1000 ng l^{-1}). A further deficiency was that the difference between test concentrations was large (an order of magnitude each time). A LOEC of 0.1 ng l^{-1} was reported but this was based on induction of testis-ova in a single male out of 33. However, given that the natural occurrence of intersex has not been clearly established in this species, it is difficult to determine whether the small observed incidence of intersex medaka is a consequence of exposure. Furthermore, at a higher test concentration of 10 ng l^{-1}, testis-ova was not observed in any of the 23 males. At this concentration, sex ratios were not significantly different and the numbers of males were even slightly higher than females. Thus, the interpretation of the results and the reported LOEC value is questionable, particularly given the lack of a clear concentration-response. Certainly, at 100 ng l^{-1} there was a significant difference in sex ratios with only 4 males being reported (all of which had testis-ova) as opposed to 43 females.

Overall the combined dataset for potential endocrine mediated responses on fish indicates that the threshold level above which effects are observed is in the range 0.3 – 1.0 ng l^{-1} based on responses on a number of different endpoints including feminisation of male reproductive organs and changes in fertilisation success.

Studies on the effects of 17α-ethinyloestradiol on the development and reproduction of the copepods *Tisbe battgaliai* (Hutchinson *et al* 1999, Breitholtz and Bengtsson 2001) showed no adverse effects up to concentrations of 100 μg l^{-1}. The lowest recorded NOECs for effects in invertebrates were a value of 0.05 μg l^{-1} in a study of reduced growth of freshwater snail (*Lymnea stagnalis*) hatchlings and a value of 0.1 μg l^{-1} in a study of the change in sex ratios of the amphipod *Gammarus pulex* (Watts et al 2002). However, the extent to which this endpoint is endocrine mediated is uncertain, but even if it was relevant the threshold for effects would still be higher than those for fish. Information is available for a range of invertebrate taxa including crustaceans, hydroids, molluscs and insects.

15.5 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity

15.5.1 Studies relevant to the assessment of general toxicity in humans

Information on general toxicity effects on human health has not been considered in this review (see section 15.4.1).

15.5.2 Studies relevant to the assessment of general toxicity in wildlife

15.5.2.1 Studies on aquatic organisms

Fish

Acute toxicity

The only acute toxicity study for fish was by Wenzel *et al* (2001) which reported a 96 hour LC_{50} value of 1700 000 ng l^{-1} (1700 μg 1^{-1}) when zebrafish (*Danio rerio*) were exposed to 17α-ethinyloestradiol.

Chronic toxicity

In a 28 week study, Sheahan *et al* (1994) exposed rainbow trout (*Oncorhynchus mykiss*) to nominal concentrations of 0.1, 0.3 and 1 ng l^{-1} at mean temperatures of 11.4 and 17.4°C. No treatment-related effects on survival or condition were observed.

Schweinfurth *et al* (1996) reported on a preliminary study in which 3 life-stages of fathead minnow (*Pimephales promelas*) (embryo-larvae, juvenile and adult) were exposed over a period of 4 weeks to graduated 17α-ethinyloestradiol concentrations of 0, 10, 100, 1000 and 10,000 ng l^{-1} (measured by radioimmunoassay) under flow-through conditions. An increase in mortality in all 3 life-stages was seen at concentrations of 1000 ng l^{-1} or above.

In a subsequent full life-cycle study Länge *et al* (2001) exposed newly fertilised fathead minnow embryos (<24 hrs old) to nominal concentrations of 0, 0.2, 1, 4, 16 and 64 ng l^{-1} in flow-through conditions at 25 ±1°C for 305 days (4 days pre-hatch and 301 days post-hatch). Exposure concentrations were confirmed by radioimmunoassay analysis and ranged from 58-84% with mean measured values ≥ 70%. Hatching success of F_o embryos was not significantly different from controls at any exposure concentration (NOEC > 64 ng^{-1}) and a NOEC of 16 ng l^{-1} was identified based on survival in larvae and juvenile F_o fish. No significant differences in survival of adult fish during pairing and laying (176-301 days post hatch) at 1 ng l^{-1} were seen, and thus the reported NOEC value was >1 ng l^{-1}.

Van Aerle *et al* (2002) exposed newly fertilised fathead minnow embryos (<24 hours old) to a nominal 17α-ethinyloestradiol concentration of 10 ng l^{-1} for five different exposure periods under flow-through conditions. The exposure regimes were: fertilised eggs through embryo development to 5 days post-hatch (dph), 5-10 dph, 10-15 dph, 15-20 dph and fertilised eggs through embryo development to 20 dph. Two control groups were employed (one dilution water and one ethanol solvent control). For each of the treatment and control groups, the fertilised eggs were divided equally into 4 replicate tanks. The percentage embryo mortality was monitored and subsequently any larval mortalities were recorded on a daily basis. There were no effects on survival to either 30 dph or 100 dph due to 17α-ethinyloestradiol exposure.

Scholz and Gutzeit (2000) exposed freshly hatched Japanese medaka (*Oryzias latipes*) larvae of the d-rR strain for 2 months to nominal 17α-ethinyloestradiol concentrations of 1, 10 or 100 ng l^{-1} under semi-static conditions. Survival (and growth) was unaffected in all treatment groups.

Metcalfe *et al* (2001) exposed Japanese medaka early life stages (1 day after hatch) to nominal concentrations of 0, 0.1, 1, 10, 100 and 1000 ng l^{-1} until medaka reached approximately 1.5 cm in length (which occurred at 85-110 days post hatch). The exposure period between renewals of test solutions was 48 hours. Only a few medaka survived at 1000 ng l^{-1} whereas mortality was low at other test concentrations.

Zillioux *et al* (2001) exposed sheepshead minnow (*Cyprinodon variegatus*) to nominal 17α-ethinyloestradiol concentrations of 0, 0.2, 2, 20, 200, 400, 800, 1600 and 3200 ng l^{-1} in a partial life-cycle test. Fish were exposed for either 43 or 59 days, from subadult stages to sexual maturity, under flow-through conditions. Survival of exposed fish was severely affected in a dose-dependent manner at concentrations ≥ 400 ng l^{-1}, which was considered to be due to generalised oedema.

In a paired breeding study, Van den Belt *et al* (2001) exposed adult male and female zebrafish under semi-static (renewed daily) conditions to 5, 10, 25 and 50 ng l^{-1} of 17α-ethinyloestradiol for 3 weeks. Nominal concentrations were confirmed by LCMS analysis

and were shown to be 76 ± 14% prior to each renewal. Slight mortality occurred in both male and female fish during the 3 week exposure. During the subsequent 5-day breeding period in control water, cumulative mortality increased up to 60% for the 50 ng l^{-1} exposure group.

Wenzel *et al* (2001) reported a 28 day LC_{50} value of 100 ng l^{-1} when juvenile and adult male and female zebrafish were exposed to 17α-ethinyloestradiol.

B. Invertebrates

Acute toxicity

In a study by Schweinfurth *et al* (1996) 17α-ethinyloestradiol was of low acute toxicity to the water flea *Daphnia magna,* although the experimental details available are limited. 48 hr EC_{50} and NOEC values of 6400 and 3000 μg l^{-1}, respectively, have been reported based on the immobilisation endpoint.

The effects of 17α-ethinyloestradiol on the survival (and reproductive behaviour) of the amphipod, *Gammarus pulex* have also been investigated under static conditions (Watts *et al* 2001a). Actual test concentrations were confirmed by GC/MS/MS analysis and 24 hour, 48 hour and 10 day LC_{50} values of 7920, 4190 and 840 μg l^{-1} were reported.

Anderson *et al* (2001) tested 17α-ethinyloestradiol for acute toxicity to the copepod *Acartia tonsa* and 48 hour LC_{10} and LC_{50} values of 680 and 1100 μg l^{-1} (based on nominal concentrations) respectively for adults, were reported for 17α-ethinyloestradiol following exposure.

Jaser *et al* (2001) reported a 24 h EC_{50} value of 1814 μg l^{-1} for the waterflea (*Ceriodaphnia reticulata*) and a 24 h EC_{50} value of >4100 μg l^{-1} for the cladoceran (*Sida crystallina*) based on nominal concentrations.

Breitholtz and Bengtsson (2001) investigated the effects of 17α-ethinyloestradiol on the copepod *Nitocra spinipes* (Crustacea) and a 96 hour LC_{50} values of 510 μg l^{-1} was reported for the adult life-stage.

A series of tests have been conducted which investigated the effects of 17α-ethinyloestradiol on the hydroid *Hydra vulgaris* (a primitive invertebrate), although experimental details are limited (Segner *et al* 2002). A 96 hour LC_{50} value of 3780 μg l^{-1} was reported, indicating a relatively low acute toxicity.

Segner *et al* (2002) summarised toxicity studies on midge larvae*Chironomus riparius* exposed to 17α-ethinyloestradiol, however, experimental details were lacking for all of the studies. A 10 day LC_{50} value of 8830 μg l^{-1} was reported for 2nd instar midge larvae *Chironomus riparius*. Meregalli and Ollevier (2001) also reported on *Chironomus riparius* larvae (1dph first and second instar) exposed to nominal 17α-ethinyloestradiol concentrations of 1, 10 or 100 μg l^{-1} for 9 days under semi-static conditions. No adverse effects on survival or mouthpart deformity were observed.

Chronic toxicity

In a 21-day reproductive study with the water flea *Daphnia magna* conducted under semi-static conditions (according to FDA guideline 4.09), no dose-dependent immobilisation or

mortality was observed at a concentration of 387 μg l^{-1} of 17α-ethinyloestradiol (Schweinfurth *et al* 1996). Kopf (1995) reported NOEC, EC_{10} and EC_{50} values of 10, 12.5 and 105 μg l^{-1}, respectively, for *Daphnia magna* but no further details were available.

In a 3-generation study, the water flea *Ceriodaphnia reticulata* and the cladoceran *Sida crystallina* were exposed to six different concentrations (10-500 μg l^{-1}) of 17α-ethinyloestradiol for 4 weeks. Every two days the medium was renewed and survival of adults and juveniles, batch size, first appearance and number of offspring produced were investigated. The juvenile phase of *Sida* was significantly shorter at >100 μg l^{-1} and at 200 μg l^{-1} a higher mortality of newly hatched juveniles was seen for *Ceriodaphnia* (Jaser *et al* 2001).

Hutchinson *et al* (1999) conducted life-cycle studies investigating the effect of 17α-ethinyloestradiol on the copepod *Tibse battagliai* (Crustacea). Newly released (<24 h old) animals were exposed to nominal concentrations 0.1, 1, 10 and 100 μg l^{-1} over 21 days. None of the concentrations had an effect on mortality and, thus, a 21-day of NOEC of ≥100 μg l^{-1} was reported.

Anderson *et al* (2001) tested 17α-ethinyloestradiol for its inhibitory effect on larval development of the copepod *Acartia tonsa* over a 5 day period. EC_{10} and EC_{50} values of 46 and 88 μg l^{-1}, respectively, for 17α-ethinyloestradiol were reported.

Breitholtz and Bengtsson (2001) investigated the effects of 17α-ethinyloestradiol on the copepod *Nitocra spinipes* (Crustacea). Newly released (<24 h old) animals were exposed to nominal concentrations 0.5, 5 and 50 μg l^{-1} (17α-ethinyloestradiol) for up to 18 days. None of the concentrations had a significant effect on mortality.

15.5.2.2 Studies on terrestrial organisms

No general toxicity data for terrestrial organisms following exposure to 17α-ethinyloestradiol have been located.

15.5.2.3 Studies on aerial organisms

No general toxicity data for aerial organisms following exposure to 17α-ethinyloestradiol have been located.

15.5.2.4 Comparion of potential endocrine mediated responses and general systemic toxicity in wildlife

The chronic toxicity data for fish indicates that the levels of 17α-ethinyloestradiol required to cause general effects occur at levels > 1 ng l^{-1}, based on adult mortality in a life cycle study of fathead minnows (Länge *et al* 2001). As a result it is evident that potential endocrine mediated responses in fish may be the mechanism responsible for the most toxic effects observed in fish .

Table 15.3 Summary of the general toxicity toxicity data for aquatic organisms

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study validity
Acute Fish Toxicity	Zebrafish (*Danio rerio)*	96 hours	No data (Nominal concentrations)	96 hour LC_{50}	1700 000 ng l^{-1}	Wenzel *et al* (2001)	Use with care
Chronic Fish Toxicity	Fathead minnow (*Pimephales promelas)*	32 days	0, 0.2, 1, 4, 16 and 64 ng l^{-1} (Measured concentrations)	NOEC (embryo-larval mortality) LOEC (embryo-larval mortality)	16 ng l^{-1} 64 ng l^{-1}	Länge *et al* (2001)	Valid
		60 days		NOEC (juvenile mortality) LOEC (juvenile mortality)	16 ng l^{-1} 64 ng l^{-1}		
		305 days		NOEC (adult mortality)	>1 ng l^{-1}		
		30 and 100 days post-hatch	0, 10 ng l^{-1} (Nominal concentrations)	NOEC LOEC	10 ng l^{-1} ≥10 ng l^{-1}	Van Aerle *et al* (2002)	Use with care
		28 days	0, 10, 100, 1000 and 10000 ng l^{-1} (Measured concentrations)	NOEC (Embryo-larval, juvenile and adult) LOEC (Embryo-larval, juvenile and adult)	100 ng l^{-1} 1000 ng l^{-1}	Schweinfurth *et al* (1996)	Valid
	Japanese medaka (*Oryzias latipes*)	85-110 days	0, 0.1, 1, 10, 100 and 1000 ng l^{-1} (Nominal concentrations)	-	Increased mortality at 1000 ng l^{-1}	Metcalfe *et al* (2001)	Use with care
		60 days	0, 1, 10 and 100 ng l^{-1} (Nominal concentrations)	NOEC LOEC	100 ng l^{-1} >100 ng l^{-1}	Scholz and Gutzeit (2000)	Use with care

Table 15.3 Continued

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study reliability
Chronic Fish Toxicity	Rainbow trout (*Oncorhynchus mykiss)*	28 weeks	0, 0.1, 0.3 and 1 ng l^{-1} (Nominal concentrations)	NOEC LOEC	>1 ng l^{-1} >1 ng l^{-1}	Sheahan *et al* (1993)	Use with care
	Sheepshead minnow (*Cyprinodon variegatus*)	59 days	0, 0.2, 2, 20, 200, 400, 800, 1600 and 3200 ng l^{-1} (Nominal concentrations)	NOEC LOEC	200 ng l^{-1} 400 ng l^{-1}	Zillioux *et al* (2001)	Valid
	Zebrafish (*Danio rerio*)	28 days	No data (Nominal concentrations)	LC_{50}	100 ng l^{-1}	Wenzel *et al* (2001)	Use with care
Acute Invertebrate Toxicity	Amphipod (*Gammarus pulex)*	24 hour 48 hour 10 day	0, 0.01 – 3700 µg l^{-1} (Measured concentrations)	24 h LC_{50} (24 hour) 48 h LC_{50} (48 hour) 10 day LC_{50}	7920 µg l^{-1} 4190 µg l^{-1} 840 µg l^{-1}	Watts *et al* (2001a)	Valid
	Cladoceran (*Sida crystallina)*	24 hour	No data (Nominal concentrations)	EC_{50}	>4100 µg l^{-1}	Jaser *et al* (2001)	Use with care
	Copepod (*Nitocra spinipes)*	96 hour	0, 0.5, 5 and 50 µg l^{-1} (Nominal concentrations)	LC_{50}	510 µg l^{-1}	Breitholtz and Bengtsson (2001)	Use with care
	Copepod (*Acartia tonsa*)	48 hour	No data (Nominal concentrations)	LC_{50}.	1100 µg l^{-}	Anderson *et al* (2001)	Use with care
	Hydroid (*Hydra vulgaris)*	96 hours	Up to 1600 µg l^{-1} (Nominal concentrations)	NOEC LC_{50}	58 µg l^{-1} 3780 µg l^{-1}	Segner *et al* (2002)	Use with care
	Midge larvae (*Chironomus riparius)*	10 days	No data (Nominal concentrations)	LC_{50}	8830 µg l^{-1}	Segner *et al* (2002)	Use with care

Table 15.3 Continued

Test type	Test species	Exposure period	Test concentrations series used	Endpoint	Effect concentration	Reference	Study reliability
Acute Invertebrate Toxicity	Water flea (*Daphnia magna)*	48 hours	No data (Nominal concentrations)	NOEC EC_{50}	3000 μg l^{-1} 6400 μg l^{-1}	Schweinfurth *et al (*1996)	Use with care
	Water flea (*Ceriodaphnia reticulata)*	24 hour	10 – 500 μg l^{-1} (Nominal concentrations)	EC_{50}	1814 μg l^{-1}	Jaser *et al* (2001)	Use with care
Chronic Invertebrate Toxicity	Cladoceran (*Sida crystallina)*	28 days	0, 10 – 500 μg l^{-1} (Nominal concentrations)	NOEC (adult mortality)	>500 μg l^{-1}	Jaser *et al* (2001)	Valid
	Copepod (*Nitocra spinipes)*	18 days	0, 0.5, 5 and 50 μg l^{-1} (Nominal concentrations)	NOEC (mortality)	50 μg l^{-1}	Breitholtz and Bengtsson (2001)	Valid
	Copepod (*Acartia tonsa*)	5 days	No data (Nominal concentrations)	LC_{50}	88 μg l^{-1}	Anderson *et al* (2001)	Use with care
	Copepod (*Tisbe battagliai)*	21 days	0, 0.1, 1, 10 and 100 μg l^{-1} (Nominal concentrations)	NOEC (mortality)	≥100 μg l^{-1}	Hutchinson *et al* (1999)	Valid
	Water flea *Ceriodaphnia reticulata*	28 days	0, 10 – 500 μg l^{-1} (Nominal concentrations)	NOEC (adult survival)	>500 μg l^{-1}	Jaser *et al* (2001)	Valid
	Water flea (*Daphnia magna)*	21 days	No data (Nominal concentrations)	NOEC (mortality)	387 μg l^{-1}	Schweinfurth *et al (*1996)	Use with care

The relative insensitivity of the reproductive, developmental and lethality endpoints in one invertebrate group (crustaceans) exposed to 17α-ethinyloestradiol means this taxonomic group are evidently not affected by exposure to the synthetic vertebrate steroid.

15.6 Current classification of 17α-ethinyloestradiol against European Commission and national regulations

The synthetic steroid 17α-ethinyloestradiol is not listed or classified under any of the major Council Directives.

In the United Kingdom consideration has been given to the derivation of Predicted No Effect Concentrations (PNEC) for the natural vertebrate steroids oestrone and oestradiol and the synthetic steroid ethinyloestradiol as part of the national strategy for "Endocrine disrupting substances in the environment" (EA 2000). A PNEC value of 0.1 ng l^{-1} was derived for 17α-ethinyloestradiol as an annual average.

15.7 Exposure data

15.7.1 Worker exposure data

The exposure of workers to 17α-ethinyloestradiol has not been considered in this review.

15.7.2 Consumer exposure data

The exposure of consumers to 17α-ethinyloestradiol has not been considered in this review.

15.7.3 Environmental exposure data

15.7.3.1 Aquatic environment

The synthetic steroid 17α-ethinyloestradiol is a component of the contraceptive pill, and therefore originates entirely from humans. The primary route of entry of 17α-ethinyloestradiol into the aquatic environment will be through domestic sewage, as a result of its excretion following use of the contraceptive pill by women.

Effluents from synthetic steroid manufacturers could also potentially result in local concentrations, although this will only be the case in European countries where manufacturers are located. Following consultation with the largest formulator in the UK, it is understood that GCMS analytical monitoring of waste effluents have shown 17α-ethinyloestradiol concentrations to be below the limit of detection (LOD) (specified to be 1 μg l^{-1} for the methodology used). It is of note that the LOD is higher than the concentrations considered to be of biological potency (which are in the trace ng l^{-1} range). In addition, formulating processes are increasingly being improved in order to prevent any potential contamination of waste effluents (for example by using dry rather than wet scrubbing) (Pharmacia, *pers. comm*).

Another potential route into the environment is via the disposal of unwanted or out of date 17α-ethinyloestradiol by users. With regard to disposal of unused drugs by the general population, the correct procedure in European countries is to return these to the pharmacy, which is then responsible for disposal (ultimately by licensed waste disposal contractors).

Depending on the nature of the waste, it may then go to STW (or undergo incineration or be taken to designated landfill sites). Although householders are encouraged to return unused or life-expired medicines to pharmacists for safe disposal they are under no obligation to do so. Furthermore, such action is dependent on whether clear advice is given, for example, in any accompanying patient information leaflet. In practice, the majority of people will either flush unused drugs down the toilet (ultimately passing to STWs) or dispose of them in domestic refuse which ultimately will enter domestic waste landfill sites or, to a lesser extent, be incinerated.

Treatment works effluents and sewage sludges

Table 15.4 summarises the data on the concentrations of 17α-ethinyloestradiol in effluent discharges from treatment works and the levels recorded in sewage sludges at the treatment plants. Monitoring studies indicate that 17α-ethinyloestradiol in sewage treatment works effluents range from <0.1 to 62 ng l^{-1}. Further information on the studies in different countries is given in the following sections.

Table 15.4 Summary of the measured 17α-ethinyloestradiol concentrations in European treatment plant discharges

Location	Location	17α-Ethinyloestradiol concentration (ng l^{-1})	Reference
Germany	20 STWs	17[a], 62[c]	Stumpf *et al* (1996)
	13 STWs	Nd	Wegener *et al* (1999)
	16 STWs	1[a], 4[b], 15[c]	Ternes *et al* (1999)
	1 STW	<0.2 - 3	Hansen *et al* (1998)
	3 STWs	<0.1 - 8.9, 0.7[a], 1.4[d]	Kuch and Ballschmiter (2001)
Italy	6 STWs	Nd-1.7, 0.45[a]	Baronti *et al* (2000)
	5 STWs	Nd - 2.2	Johnson *et al* (2000)
Netherlands	5 STWs (2 industrial, 3 domestic)	Nd[a] Nd - 7.5	Belfroid *et al* (1999)
	3 STW	<0.2 - <1.4	Johnson *et al* (2000)
Spain	24 hr composite samples from 4 STWs.	Nd	Solé *et al* (2000a)
Sweden	1 STW	4	Larsson *et al* (1999)
United Kingdom	Activated sludge STWs, 8 samples	Nd - 7	Aherne and Briggs (1989)
	7 STWs (Detected in 2 STWs on all 3 occasions; one STW on 1 occasion)	Nd - 7	Desbrow *et al* (1998)
	Chelmsford STW	1.7 - 3.4	Rodgers-Gray *et al* (2000)
	3 STWs on rivers Lea and Nene	Nd - 1.85	Niven *et al* (2001)
	2 STWs	Nd	Kanda *et al* (2001)

Key: Nd = not detected, STWs – sewage treatment works, a =median, b = 90 percentile, c = maximum

Only a limited number of studies are available that have either estimated concentrations of 17α-ethinyloestradiol in sewage influents (Wilson 1978, Rathner and Sonneborn 1979) or undertaken analytical monitoring (Tabak *et al* 1981, Johnson *et al* 2000, Baronti *et al* 2000).

These studies indicate that its concentration in sewage influent is likely to range between 5-10 ng l^{-1}. There are only limited data on the concentrations of 17α-ethinyloestradiol discharged from STWs in European countries (see Table 15.4). Reported concentrations can be highly variable (ranging from 0.13-62 ng l^{-1}), although they have typically been found to be below the LOD (≈ 0.1 ng l^{-1}) up to 10 ng l^{-1}.

Germany

Stumpf *et al* (1996) surveyed German sewage effluents and detected 17α-ethinyloestradiol above the LOD of 1 ng l^{-1} in all 20 STWs investigated; the median concentration being 17 ng l^{-1} with a maximum value of 62 ng l^{-1}. However, these reported concentrations are surprisingly high bearing in mind the concentrations in reported other countries and it is possible that there was another elutant that had the same retension time as 17α-ethinyloestradiol. Using GCMS/MS analysis, concentrations of 17α-ethinyloestradiol were measured in treated effluents from 16 German STWs (Ternes *et al* 1999). Maximum concentrations were up to 15 ng l^{-1} and the substance was positively identified in 9 of 16 samples. The corresponding median value was 9 ng l^{-1}.

More recently, Kuch and Ballschmiter (2001) conducted monitoring of three German activated sludge STWs, sampled over 5 months. 17α-ethinyloestradiol was analysed by GC/MS with an LOD value of 0.1 ng l^{-1}, and concentrations ranged from <0.1-8.9 ng l^{-1}.

Italy

Johnson *et al* (2000) estimated inputs of 17α-ethinyloestradiol into activated sludge STWs based on population equivalents, contraceptive use and water flow rates. These estimates were then tested against measured concentrations for five Italian activated sludge STWs. Predictions were particularly sensitive to assumptions on the number of women taking the oral contraceptive and were not very accurate. Measured sewage influent levels for the Italian STWs ranged from <0.5-10 ng l^{-1}.

These values compare well with measurements made by Baronti *et al* (2000) of 17α-ethinyloestradiol concentrations in sewage influents of six Italian activated sludge STWs over a period of 5 months. In the study an average concentration of 3 ± 2.6 ng l^{-1} was reported.

Netherlands

Using GCMS/MS analysis, Belfroid *et al* (1999) did not detect 17α-ethinyloestradiol above the LOD (0.3-1.8 ng l^{-1}) for 8 of 10 samples taken from five STWs (3 receiving domestic waste, 2 receiving industrial waste) in the Netherlands. When identified, resulting 17α-ethinyloestradiol concentrations were 7.5 ng l^{-1} in domestic effluents and 2.6 ng l^{-1} in industrial effluents.

Johnson *et al* (2000) estimated inputs of 17α-ethinyloestradiol into activated sludge STWs based on population equivalents, contraceptive use and water flow rates. These estimates were then tested against measured concentrations for three Dutch activated sludge STWs. Predictions were particularly sensitive to assumptions on the number of women taking the oral contraceptive and were not very accurate. Measured sewage influent levels for the Dutch STWs ranged from <0.2-8.8 ng l^{-1}.

United Kingdom

In one UK survey, up to 7 ng l^{-1} of 17α-ethinyloestradiol was detected in some STW effluents (Aherne and Briggs, 1989). However, the specificity of the immunoassay and whether both

the free steroid and its conjugates were determined was unclear which renders the results of limited value.

A UK study in which seven STWs effluents were assessed using GC/MS analysis on three separate occasions found 17α-ethinyloestradiol to be below the limit of detection (<0.2 ng l^{-1}) for 14 of the 21 samples. When identified, 17α-ethinyloestradiol concentrations ranged from 0.2±0.1 to 7±3.7 ng l^{-1}, with concentrations of <1 ng l^{-1} for 4 of the samples (Desbrow *et al* 1998). Rodgers-Gray *et al* (2000) similarly reported 17α-ethinyloestradiol concentrations of 1.7-3.4 ng l^{-1} in effluents from Chelmsford STW, and more recently values of <1 ng l^{-1} (Rodgers-Gray *et al* 2001).

In a recent UK study monitoring of 17α-ethinyloestradiol in effluent from one STW on the River Nene over 4 weeks (29 samples) and two on the River Lea over 2 weeks (14 samples per works) was conducted (Kanda *et al* 2001, Williams *et al* 2001). Analysis was by GCMS/MS and was performance tested. 17α-ethinyloestradiol was rarely detected above the LOD of 1 ng l^{-1}, the maximum value recorded being 1.85 ng l^{-1}.

In sewage treatment works the extent to which ethinyloestradiol is degraded depends on the processes operating within the works. Many organic compounds are biodegraded by organisms that utilise these compounds for growth and this pathway is probably responsible for the biodegradation of natural oestrogens. Cometabolism, in which an organic compound is modified but not utilised for growth, is another important degradation process which may be important in the fate and potential effects of ethinyloestradiol. Vader *et al* (2000) investigated the degradation of ethinyloestradiol by nitrifying activated sludge with micro-organisms grown in a reactor with feedback of sludge fed with only a mineral salts medium containing ammonium as the sole energy source. This activated sludge was capable of degrading ethinyloestradiol whereas sludge with an insignificant nitrifying capacity did not result in the degradation of the steroid. Oxidation of ethinyloestradiol by nitrifying sludge resulted in the formation of hydrophilic compounds. It was postulated that degradation by nitrifying sludge results in a loss of oestrogenic activity, as hydroxylated derivatives of ethinyloestradiol are know to have a substantially lower pharmacological activity than ethinyloestradiol (Bergink *et al* 1983).

Surface waters and sediments

Table 15.5 summaries the 17α-ethinyloestradiol concentrations that have been detected in surface waters, and includes studies from the Germany, Italy, the Netherlands and the United Kingdom. These studies indicate that when detected, 17α-ethinyloestradiol occurs at trace ng l^{-1} concentrations in surface waters (generally less than 5 ng l^{-1} and often below 1 ng l^{-1}).

Table 15.5 Summary of the measured 17α-ethinyloestradiol concentrations in European surface waters

Location	Location	17α-ethinyoestradiol concentration (ng l^{-1})	Reference
Germany/Czech Republic	-	0.6 - 18.8	Lebietzka (1996)
Germany	10 river sites	Nd - 4	Stumpf *et al* (1996)
	52 river samples	Nd (LOD = 1 ng l^{-1}) – 3.4	Wenzel *et al* (1998)
	15 river and streams	Nd (LOD = <0.5 ng l^{-1})	Ternes *et al* (1999)
	158 river samples from 79 sites	Nd (LOD = 0.05-0.1 ng l^{-1}) - 2	Adler *et al* (2001)
	River and creek samples of the Danube (n=13), Nau (n=4) Blau (n=4) and Illner (n=4) and three creeks (n=2 each)	Nd (LOD = <0.5 ng l^{-1}) - 5.1 0.4[a] 0.8[b]	Kuch and Ballschmiter (2001)
Italy	River Tiber (2 locations)	Nd - 0.04	Baronti *et al* (2000)
Netherlands	16 samples from 11 sites	Nd[a] (LOD = <0.1-0.2 ng l^{-1}) - 4.3[c]	Belfroid *et al* (1999)
United Kingdom	9 samples	Nd	Aherne *et al* (1985)
	13 river samples at medium flow level, 3 impounding reservoir samples	1 - 15	Aherne and Briggs (1989)
	7 samples from 5 sites in Severn Trent region	Nd (LOD = 0.3 ng l^{-1})	Fawell *et al* (2001)
	Rivers Nene and Lea	Nd (LOD = 0.3 ng l^{-1}) - 4.6	Kanda *et al* (2001), Williams *et al* (2001)

Key: nd = not detected, STWs – sewage treatment works, a =median, b = mean, c = maximum

In receiving waters, the available evidence supports the view that 17α-ethinyloestradiol is more persistent than the natural steroids in surface waters, possibly by an order of magnitude (that is with a half-life of 46 days *vs*. 3-4 days for natural steroids in surface waters), and that its potential to sorb to sediments is greater. 17α-ethinyloestradiol is not generally detected above the current limit of analytical detection (~ 0.1- 0.5 ng l^{-1}).

Germany

In a report by Wenzel *et al* (1998) on the analyses of 17α-ethinyloestradiol in German surface waters, 3 samples of the 52 tested had concentrations above the limit of detection (of 1 ng l^{-1}) and these showed values of 1.1, 3.4 and 3.4 ng l^{-1}.

Ternes *et al* (1999) reported on monitoring data for 17α-ethinyloestradiol in 15 German rivers and streams, but it was not detected in any of the rivers (the reported LOD being 0.5 ng l^{-1}). Other German studies also have supported these findings (Stumpf *et al* 1996, Wegener *et al* 1999).

Adler *et al* (2001) conducted a comprehensive survey of 17α-ethinyloestradiol in middle and southern German rivers. A series of 79 stations were sampled twice within 9 months and from these 158 samples 92% were below the limit of detection (0.05 – 0.1 ng l^{-1}). Only 5 showed

concentrations above the limit of detection with values ranging from 0.2 to 2 ng l^{-1}, while a further 8 were at the limit of detection (0.1 ng l^{-1}).

More recently, Kuch and Ballschmiter (2001) analysed river and creek samples of the Danube (n=13), Nau (n=4) and Blau (n = 4) approximately 1 km downstream of STWs effluent sites. Samples from the Illner River (n=4) and three creeks (n=2 each) were also analysed. All samples were analysed by GC/MS and 17α-ethinyloestradiol concentrations were in the range from below the limit of detection (<0.1 ng l^{-1}) to 5.1 ng l^{-1} with the substance being positively identified in 15 of 31 samples.

Italy

Baronti *et al* (2000) sampled two sites of the River Tiber; one of which was situated downstream of an activated sludge STW and the second was located 1 km before the mouth of the Tiber. After leaving Rome, the Tiber covers about 20 km and receives effluents of mechanical STWs located in small towns and discharges of raw sewage. At the first site, no significant amounts of 17α-ethinyloestradiol were detected. However, analysis of the second site revealed concentrations of 0.04 ng l^{-1} of 17α-ethinyloestradiol. It was suggested that this finding supports the hypothesis that oestrogens in environmental waters come primarily from untreated wastewater rather than from activated sludge STW effluents.

Netherlands

Only trace concentrations (generally below the LODs up to about 5 ng l^{-1}) of 17α-ethinyloestradiol was detected in eleven surface water sites in the Netherlands (Belfroid *et al* 1999). 17α-ethinyloestradiol was only detected in 3 of 16 samples with value of 0.4, 1.2 and 4.3 ng l^{-1} being reported.

United Kingdom

Aherne *et al* (1985) did not detect 17α-ethinyloestradiol in any of nine UK river samples which were analysed by immunoassay (LOD = 5 ng l^{-1}). A later study, utilising the same methodology, measured 17α-ethinyloestradiol at concentrations of between 2-15 ng l^{-1} in 13 samples of surface water (Aherne and Briggs 1989). However, no specificity or performance data were provided and the validity of these values is questionable.

Williams *et al* (1999) applied the Exposure Assessment Modelling System (EXAMS) to predict the likely distribution of oestrogen steroids in the Rivers Thames, Calder and Aire. 17α-ethinyloestradiol concentrations were estimated to be varying between 0.024 and 0.038 ng l^{-1}. However, under low-flow conditions, predicted concentrations increased by a factor of 4 to 10 times above the average concentrations at the point of discharge.

The most comprehensive monitoring survey on UK surface waters involved GCMS/MS analysis of two rivers for 17α-ethinyloestradiol (Kanda *et al* 2001, Williams *et al* 2001). Samples were taken at numerous sites downstream of sewage treatment works (5 sites from the River Nene daily over a period of 4 weeks and 5 sites from the River Lea daily over 2 weeks). The analytical method was performance tested with the LOD being 0.5 ng l^{-1} for 17α-ethinyloestradiol. 17α-ethinyloestradiol concentrations ranged from <0.5 to 4.6 ng l^{-1}, although most values were below 1 ng l^{-1}.

Another recent study conducted using GCMS/MS analysis of 17α-ethinyloestradiol in five surface waters in the Severn Trent region of England (Fawell *et al* 2001) found the steroid was not detected above the LOD in any of the 7 samples (<0.3 ng l^{-1}).

Kanda *et al* (2001) conducted a survey of sediment contamination by 17α-ethinyloestradiol at 10 sites downstream of three STWs in two UK rivers (Rivers Nene and Lea). Analysis was conducted using GCMS/MS techniques and 17α-ethinyloestradiol was below the LOD (<0.1 μg kg^{-1}) for all the samples analysed.

COMPREHEND Programme

In the EU funded COMPREHEND programme chemical analysis of industrial and domestic effluents was undertaken by two of the partners in the Netherlands and Switzerland using samples taken from across Europe. Analysis included the natural steroids oestrone, oestradiol and oestriol and ethinyloestradiol. Oestrone measurements were the most consistent in terms of recovery and a good correlation was obtained in a comparison of the techniques of the two laboratories for measurements of the same set of wastewater samples. There was poor agreement with oestradiol measurement and both laboratories experienced very poor recoveries with oestriol and low sensitivity with ethinylestradiol. Oestrone measurements in STW effluents showed a good degree of correlation with estrogenic activity (as measured with *in vitro* assays) and oestrone and oestradiol were generally in the 0 to 10 ng l^{-1} range. Ethinyloestradiol however, was often at or below the limit of detection (approximately 1 ng l^{-1}). Oestrogenic steroids were generally below the limits of detection for most industrial waste waters (unless there was a significant component of the effluent originating from domestic/human sources within the industrial plant).

Toxicity Identification and Evaluations

In the COMPREHEND programme Toxicity Identification and Evaluations (TIE) identified oestradiol, oestrone and ethinyloestradiol as the principal estrogenic components of domestic raw sewage, with ethinyloestradiol and oestrone dominating the oestrogenic activity of the final effluent. Taking into consideration the potencies of the various oestrogenic compounds measured in municipal STW effluents, it was concluded that natural and synthetic steroids, of human origin, are by far the most important estrogenic components and are responsible for most of the estrogenic effects seen *in vivo* and *in vitro*. Ethinyloestradiol may be particularly important in this respect but the limitations of the analytical techniques used in the programme were a major constraint to confirming the importance of this component of the contraceptive pill. TIE also provided evidence of 'cooperative' effects between the different steroids, making the measured oestrogenic activity (in the yeast based YES assay) approximately three times greater than the sum of the activity of the individual components.

These results are generally consistent with those of Desbrow *et al* (1998) which used a fractionation system combined with a yeast based *in vitro* assay to isolate and identify the major oestrogenic chemicals present in seven sewage treatment works effluents receiving primarily domestic effluent. In all the effluents tested the most active fraction (>80% total activity in domestic effluent) was found to contain low levels of natural and synthetic steroidal oestrogens. The results obtained indicated that the concentrations of ethinyloestradiol, detected in the samples were generally too low to fully account for magnitude of the vitellogenin response observed when male fish were exposed to the effluent (Sheahan *et al* 1994).

15.7.3.2 Terrestrial environment

No data has been located on the concentrations of 17α-ethinyloestradiol in the terrestrial enviroment, though it is believed that data may become available in the near future.

15.7.3.3 Aerial environment

No data has been located on the concentrations of 17α-ethinyloestradiol in the aerial environment.

15.7.3.4 Comparison of environmental monitoring data and exposure concentration causing potential endocrine mediated responses

The data on the concentrations of 17α-ethinyloestradiol in European surface waters (see Section 15.7.3.1) indicates that although levels in the region of 1 ng l^{-1} are reported, most values (90% of those published) are below the limit of detection which is typically between 0.1 and 0.3 ng l^{-1}. This coincides with the theoretical calculations of 17α-ethinyloestradiol surface water concentrations based on market volumes, which approach 0.5 ng l^{-1} in Germany, if human metabolism or degradation under waste water treatment are not considered. (Ericson *et al* 2002). Human metabolites include the much less active hydroxylation products which account for approximately 30-50% of the administered 17α-ethinyloestradiol (Bolt 1975). The elimination in sewage treatment plants can be assumed to be at least 50% based on adsorption potential. If these processes are accounted for then calculated surface water concentration would be lower by a factor of 4, that is approximately 0.13 ng l^{-1}.

The key data on endocrine mediated responses of 17α-ethinyloestradiol in aquatic organisms is for fish. The combined dataset for endocrine mediated responses on fish indicates that the threshold level above which effects are observed is in the range 0.3 - 1 ng l^{-1} based on responses of a number of different endpoints.

If a margin of safety (MOS)[1] approach is used to compare the threshold level above which endocrine mediated responses are observed (0.3 - 1 ng l^{-1}) with environmental concentrations (0.1- 1 ng l^{-1}) then MOS values of 1 - 10 would result for the aquatic compartment. On the basis that an MOS of 100 should be required for the risk to be acceptable then 17α-ethinyloestradiol apparently presents a risk to fish (and other aquatic vertebrates) in terms of endocrine disrupting effects. However, it needs to be recognised that there are issues with the environmental concentration data for 17α-ethinyloestradiol since the vast majority of samples contain levels which are below the limit of detection and, therefore, no robust assessment of the MOS can be made due to current analytical limitations in terms of sensitivity.

Clearly there may be locations where the release of 17α-ethinyloestradiol presents a risk to fish (and other aquatic vertebrates) in terms of endocrine disrupting effects. This will be seen as adverse effects on the development and reproductive capability of wild fish which are exposed to natural and/or synthetic steroids discharged from sewage treatment works (for review EA 2002). However, typical aquatic concentrations are slightly below threshold levels capable of resulting in endocrine disrupting effects. It is, therefore, more probable that such effects result from the discharge of natural steroids such as oestradiol and oestrone.

[1] Margin of safety (MOS) = (Lowest NOEC for endocrine mediated responses)/Environmental concentrations

15.8 Overall Conclusions on 17α-ethinyloestradiol

The following conclusions have been drawn from the review of the effects on 17α-ethinyloestradiol on wildlife:

15.8.1 Data from studies assessing potential endocrine disrupting effects in wildlife

- Overall the combined dataset for endocrine mediated responses on fish indicates that the threshold level above which effects are observed is in the range 0.3 – 1.0 ng l^{-1} based on effects on a number of different endpoints including feminisation of male reproductive organs and changes in fertilisation success.

- Studies on the effects of 17α-ethinyloestradiol on the development and reproduction of the copepods *Tisbe battgaliai* (Hutchinson *et al* 1999, Breitholtz and Bengtsson 20001) showed no adverse effects up to concentrations of 100 μg l^{-1}. The lowest recorded NOECs for effects in invertebrates were a value of 0.05 μg l^{-1} in a study of reduced growth of freshwater snail (*Lymnea stagnalis*) hatchlings and a value of 0.1 μg l^{-1} in a study of the change in sex ratios of the amphipod *Gammarus pulex* (Watts *et al* 2002). However, the extent to which these endpoints are endocrine mediated is uncertain, but even if it was relevant the threshold for effects would still be higher than those for fish. Information is available for a range of invertebrate taxa including crustaceans, hydroids, molluscs and insects.

15.8.2 Comparison of data from studies assessing potential endocrine disrupting effects and/or general toxicity in wildlife

- The chronic toxicity data for fish indicates that the levels of 17α-ethinyloestradiol required to cause general effects occur at levels > 1 ng l^{-1} , based on adult mortality in a life cycle study of fathead minnows (Lange *et al* 2001). As a result it is evident that endocrine mediated responses in fish may be the mechanism responsible for the most toxic effects observed in fish.

- The relative insensitivity of the reproductive, developmental and lethality endpoints in one invertebrate group (crustaceans) exposed to 17α-ethinyloestradiol mean this taxonomic group are evidently not affected by exposure to vertebrate steroids.

15.8.3 Exposure data

- Risk to consumers are based on the use of pharmaceutical products which are only administered under controlled conditions.

- The data on the concentrations of 17α-ethinyloestradiol in European surface waters (see Section 15.7.3.1) indicates that although levels in the region of 1 ng l^{-1} are reported, most values (90% of those published) are below the limit of detection which is typically between 0.1 and 0.3 ng l^{-1}. The key data on endocrine mediated responses of 17α-ethinyloestradiol in aquatic organisms is for fish. The combined dataset for endocrine mediated responses on fish indicates that the threshold level above which effects are observed is in the range 0.3 - 1 ng l^{-1} based on responses of a number of different endpoints.

- If a margin of safety (MOS)[2] approach is used to compare the threshold level above which endocrine mediated responses are observed (0.3 - 1 ng l^{-1}) with environmental concentrations (0.1-1 ng l^{-1}) then MOS values of 1 - 10 would result in the aquatic compartment. On the basis that an MOS of 100 should be required for the risk to be acceptable then 17α-ethinyloestradiol apparently presents a threat to fish (and other aquatic vertebrates) in terms of endocrine disrupting effects. However, it needs to be recognised that there are issues with the environmental concentration data for 17α-ethinyloestradiol since the vast majority of samples contain levels which are below the limit of detection and, therefore, no robust assessment of the MOS can be made due to current analytical limitations in terms of sensitivity.

- Clearly there may be certain locations (hotspots) where the release of 17α-ethinyloestradiol presents a risk to fish (and other aquatic vertebrates) in terms of endocrine disrupting effects. This will be seen as adverse effects on the development and reproductive capability of wild fish which are exposed to natural and/or synthetic steroids discharged from sewage treatment works (for review EA 2002). However, it is more probable that such effects result from the discharge of natural steroids such as oestradiol and oestrone.

- No information was located on terrestrial or aerial concentrations of 17α-ethinyloestradiol.

15.9 Summary of the weight of evidence for endocrine disrupting effects in wildlife and associated uncertainties

The summary of the weight of evidence for endocrine disrupting effects of 17α-ethinyloestradiol in wildlife along with associated uncertainties are given in Table 15.6.

[2] Margin of safety (MOS) = (Lowest NOEC for endocrine mediated responses)/Environmental concentrations

Table 15.6 Summary of the weight of evidence conclusion and uncertainties associated with the assessment of the endocrine disrupting effects of 17α-ethinyloestradiol

	Target group	
	Humans	Wildlife
Weight of evidence	Not considered in the review	In fish it appears that threshold effects of 17α-ethinyloestradiol on reproduction and development which are considered to be endocrine mediated occur at markedly lower (and environmentally relevant) concentrations (> 0.3-1 ng l^{-1}) than those causing general toxicity. The processes of reproduction and development in certain aquatic invertebrate taxa (crustaceans) are evidently not affected by exposure to vertebrate steroids at typical environmental concentrations. However this may not be the case for other invertebrate taxa. The available exposure data indicates that 17α-ethinyloestradiol can in certain circumstances present a risk to fish (and other aquatic vertebrates) in terms of endocrine disrupting effects. However, detectable aquatic concentrations in surface waters near populated areas (<0.3 ng l^{-1}) are in most cases below threshold levels capable of resulting in endocrine disrupting effects. The assessment of risk is confounded by the current analytical limitations in the sensitivity of detection of 17α-ethinyloestradiol.
Uncertainties	Not considered in the review	The data on 17α-ethinyloestradiol induced and endocrine mediated effects on reproduction and development in wildlife is limited and restricted to aquatic organisms (invertebrates and fish). No data are available on potential endocrine mediated effects in terrestrial and aerial organisms. Given that sorption to organic carbon is an important process and sewage sludge may be applied to land the absence of data on potential endocrine mediated responses in terrestrial organisms is an area of uncertainty.

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16. CONCLUSIONS

Based on the data which has been collated in this project this section draws conclusions on:

- The need for further data on the potential endocrine disrupting effects of the 12 individual substances to humans and/or wildlife within the context of information on general systemic toxicity and data on the potential for exposure of target groups of humans and/or wildlife;

- General issues related to the assessment of endocrine disruption;

- Implementation of a framework for reviewing the potential endocrine disrupting effects of other substances of concern.

However, the conclusions drawn need to be considered within the overall scope and aims of the project and, therefore, some background information has been presented below to set this section in context with the rest of the report.

In order to carry out the review process an evaluation framework has been developed to review the nature and extent of endocrine disrupting effects of identified chemicals (and potentially others in the future) based on robust datasets. However, it needs to be recognised that the framework does **not** involve carrying out a full Risk Assessment of a substance under the Existing Substances Regulation 793/93.

In the review the International Programme for Chemical Safety (IPCS) definition of an endocrine disrupter has been adopted, namely that it is "*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*". As a result the review considers other mechanisms of action than oestrogenicity and anti-oestrogenicity including androgenicity and anti-androgenicity, effects on thyroid function and effects on hormone secretion and synthesis and steroidogenesis, where relevant data are available.

The conclusions from the reviews are based on currently available reproduction and developmental toxicity data which has generally not been produced with a view to specifically assessing the potential endocrine disrupting effects of a substance. As a result there may be evidence of reproductive, embryotoxic or foetotoxic effects of a substance but not necessarily data on accompanying changes in endocrine function (for example changes in hormone levels). The conclusions have also taken into account whether reproductive and/or developmental changes in target groups of humans and/or wildlife occur at or below those doses/concentrations causing general systemic toxicity.

In considering the conclusions it also needs to be recognised that:

- The results of this study can serve as an input to policy discussions which might then, for example, mandate a full risk assessment within the context of existing legislation (under Directive EEC 793/93). This may require further testing or monitoring data for certain of the substances reviewed.

- In the future new techniques will be developed to assess endocrine disrupting properties of chemicals and these may provide data on the 12 substances which means the current conclusions have to be revisited. The approach taken at that

moment will be determined by the Commission in consultation with stakeholders and the Scientific Committees of the Commission.

16.1 Conclusions on the need for further data on the 12 substances

In Sections 4 to 15 the available data relating to potential endocrine disrupting effects in target groups of humans and wildlife of the identified substances have been reviewed and compared with data for general systemic toxicity in these groups, as well as information on the levels of the substances to which target groups are exposed.

The reviews of each substance have identified data gaps for the assessment of effects and exposure and these have been summarised in Tables ES1 and ES2 and at the end of each section. In Table 16.1 a series of defined conclusions on the status of each substance, particularly in relation to addressing additional data requirements are shown.

In the assessment of potential endocrine disrupting effects in humans it was evident that for a number of substances (4-chloro-3-methylphenol, 2,4-dichlorophenol, 4-nitrotoluene and resorcinol) there was a need for more reliable multi-generation developmental and reproductive studies to reduce uncertainty. This information was needed to substantiate the weight of evidence from currently available information which shows that no adverse reproduction and/or developmental effects were evident for these substances at exposure doses causing general systemic toxicity. For 2,4-dichlorophenol and resorcinol these tests have or will be initiated in 2002 and will provide additional robust data relevant to the assessment of endocrine disrupting effects. Further targeted exposure data for workers and/or consumers are also required for a number of substances, including 4-chloro-3-methylphenol 2,4-dichlorophenol, 4-nitrotoluene, o-phenylphenol and 4-*tert* octylphenol to allow the potential risks to these target groups to be quantified.

In the assessment of endocrine disrupting effects in wildlife it was evident that relevant data for the individual substances were generally limited to that for certain aquatic species (crustaceans and fish) and absent for other aquatic taxa and terrestrial and aerial invertebrates. Depending on the key environmental exposure pathways for a substance (based on its partitioning between compartments) this may represent an area of uncertainty. These data gaps also reflect the current absence of validated methods to assess endocrine disrupting effects in invertebrates, although it is also important to recognise that data on the general toxicological effects of 'hazardous' substances on terrestrial and aerial species is generally lacking for the majority of high production volume industrial chemicals. For most substances there is also clearly a need for targeted monitoring of the concentrations of substances in certain environmental compartments based on the key exposure pathways for wildlife species (that is to consider those taxonomic groups most at risk of exposure).

One of the key objectives of the review of the 12 substances was to identify specific cases of consumer or ecosystem exposure to these substances, with particular attention to potentially vulnerable consumer groups such as children. The following conclusions on potential risks to consumers from the 9 industrial substances can be drawn from the available data:

- A number of the industrial substances (2,4-dichlorophenol, 4-nitrotoluene and 4-*tert* octylphenol) are used in the manufacture of products from which it is probable that there is no or extremely limited consumer exposure. However, information on consumer exposure is limited or absent and it is difficult to draw robust conclusions on the potential risks to vulnerable groups. Further targeted monitoring may, therefore, be needed to quantify potential risks to consumers.

- For substances where there is the potential for consumer exposure (BADGE through epoxy lining of food and drink cans, 4-chloro-3-methylphenol in pharmaceutical products and resorcinol through hair dyeing and pharmaceutical products) the data indicates that there is evidently no risk to consumers including children from current exposure patterns[1].

In terms of risks to the ecosystem the greatest risks to aquatic ecosystems (and principally the vertebrates) relate to exposure to the natural steroids (17β-oestradiol and oestrone). The extent of the problem depends on the route and magnitude of the releases and the assimilative capacity of the receiving water (in particular the volume of the receiving water relative to the size of the discharge). There are also potential risks to aquatic organisms from exposure to the synthetic steroid 17-ethinyloestradiol and the industrial substances 2,4-dichlorophenol and 4-tert octylphenol, though these are more likely to be associated with discharge 'hotspots'.

Information on risks to the terrestrial environment is limited since there is little monitoring data and almost no information on the potential endocrine disrupting effects of the substances to terrestrial species. This is largely a consequence of the absence at present of standardised methods with which to evaluate such effects.

16.2 General issues related to the assessment of endocrine disruption

In addition to the conclusions on the individual substances reviewed in the report it is evident that there are a number of generic issues associated with the assessment of endocrine disrupting effects which apply to all potential substances of interest and not just those considered in the review.

The assessment of endocrine disrupting effects in humans and wildlife is an evolving area and a considerable body of activity is on-going at both national and international levels. It was evident from the recent Report of a European Workshop on Endocrine Disrupters held in Aronsborg, Sweden (EC 2001) that there are a number of key areas of uncertainty which need to be addressed to enhance the evaluation of the extent of endocrine disrupting effects of substances of concern and the risks they present to humans and/or wildlife. Key areas requiring further activity are:

- The development of validated methods which provide robust information on endocrine disrupting effects in particular target groups, specifically invertebrates where there is a currently a lack of knowledge on the endocrinology of many taxonomic groups;

[1] For 4-chloro-3-methylphenol in pharmaceutical products and resorcinol in hair dyeing and pharmaceutical products this is based on the assumption that these are used as described in the accompanying literature.

- The conduct and interpretation of mammalian and non-mammalian tests in relation to potential low-dose effects (see Section 2.3);

- Collation (and if required generation) of information on the normal background variability in reproduction and developmental responses of mammalian and wildlife species;

- Assessment of the risks presented by the potential endocrine disrupting effects of synthetic substances in relation to background exposure to natural compounds (for example vertebrate steroids and phyto-oestrogens).

- Consideration of the effects of mixtures since target groups of humans and/or wildlife may be exposed to combinations of both natural and/or synthetic substances with varying degrees of endocrine disrupting potency.

16.3 Implementation of a framework for reviewing the potential endocrine disrupting effects of other substances of concern

Following the conduct of the reviews in this project a framework for conducting reviews of other substances identified as potential endocrine disrupters in a prioritisation exercise has been proposed (see Figure 16.1). The framework (Boxes 2 and 3 in Figure 16.1) follows the approach described in Section 2 of the report and incorporates a step-wise evaluation of data on the substances against the three issues given in Section 2 of the report, namely whether:

- the weight of evidence for a substance indicates endocrine disrupting effects occur in target groups of humans and/or wildlife;

- endocrine disrupting effects occur at or below doses/concentrations of the substance than those causing general non-endocrine mediated (eco)toxicological effects in the target groups;

- the target groups of humans and/or wildlife are likely to be exposed to the substance in the environment at doses/concentrations capable of causing endocrine disrupting effects.

In the framework it is also important to consider:

1. Is there sufficient data of definitive significance available to draw robust and meaningful conclusions on the extent to which a substance causes or has the potential to cause endocrine disruption effects in target groups of humans and/or wildlife at levels at or below those causing general non-endocrine (eco)toxicological responses?;

2. What additional information is needed to allow robust and meaningful conclusions to be drawn if there is currently insufficient data for a substance?.

The framework is designed to build on a prioritisation exercise using the procedure being developed by BKH (Box 1 in Figure 16.1) and it is envisaged that this screening approach will be used to prioritise the substances for which a review is conducted.

At the prioritisation stage when sufficient data is available substances for which there is a) evidence of endocrine disruption in humans and/or wildlife and b) a potential for exposure of the target group(s) should be considered a priority for a more detailed review using the procedure described in Section 2. When there is evidence of endocrine disruption in a target group but an absence of data on the potential for exposure this situation should lead to the acquisition of relevant basic exposure data, so that a decision can be made on whether a more detailed review of the substance is required. If there is evidence of exposure potential but no information on potential endocrine disrupting effects then no detailed review should be conducted until some robust data indicating a potential for endocrine disrupting effects has been generated.

Where there is sufficient data and a) an absence of evidence or negative weight of evidence for potential endocrine disrupting effects in a target group and b) no potential for exposure then the further detailed consideration of the substance is not a priority action.

For the assessment of endocrine disrupting effects of a substance at the prioritisation exercise stage the emphasis should be placed on *in vivo* data where this is available. The review of the 9 industrial substances has shown that effects observed *in vitro* assays are difficult to extrapolate to effects in whole organisms, especially at doses/concentrations which may reflect the lowest observed toxicity in a target group.

References

EC (2001) Report of a European Workshop on Endocrine Disrupters on 18-20 June 2001 in Aronsborg, Sweden, European Commission, Brussels.

Table 16.1 Summary of the conclusions on the 12 substances with regard to additional data requirements

Substance	Humans	Wildlife
BADGE[1,2]	• A comprehensive mammalian toxicology dataset is available and the weight of evidence from the available information shows that no adverse reproductive and/or developmental effects occur at doses causing general systemic toxicity. However, further testing of potential endocrine disrupting effects may be required when agreed test methods become available. • Exposure data are available for workers and consumers	• Further testing of potential endocrine disrupting effects in aquatic and terrestrial organisms may be needed when agreed test methods are available. • Further environmental exposure data are required for the terrestrial compartment.
Carbon disulphide[1,2]	• Mammalian toxicology data and human epidemiological data are available which indicates that reproductive and/or developmental effects occur in workers when current European regulatory thresholds are exceeded. Further testing of potential endocrine disrupting effects may be required when agreed test methods become available. • Exposure data are available for workers and consumers	• Further testing of potential endocrine disrupting effects in wildlife species may be needed when agreed test methods are available. • Further environmental exposure data are required for the aerial compartment.
4-Chloro-3-methylphenol[1,2]	• There is a need for more reliable multi-generation developmental and reproductive studies to substantiate the weight of evidence from the available information which shows that no adverse reproductive and/or developmental effects occur at doses causing general systemic toxicity. • Further targeted exposure data are required.	• Further testing of potential endocrine disrupting effects in aquatic organisms (particularly fish) may be needed when agreed test methods are available. • Further environmental exposure data are required for the terrestrial compartment.
2,4-Dichlorophenol[1,2]	• There is a need for more reliable multi-generation developmental and reproductive studies to substantiate the weight of evidence from the available information which shows that no adverse reproductive and/or developmental effects occur at doses causing general systemic toxicity. A multi-generational study in rats has been initiated in 2002 • Further targeted exposure data for consumers are required.	• Further testing of potential endocrine disrupting effects in aquatic organisms (particularly fish) may be needed when agreed test methods are available. • Further environmental exposure data are required for the terrestrial compartment.

Table 16.1 Continued

Substance	Humans	Wildlife
4-Nitrotoluene[1,2]	• There is a need for more reliable multi-generation developmental and reproductive studies to substantiate the weight of evidence from the available information which shows that no adverse reproductive and/or developmental effects occur at doses causing general systemic toxicity. • Further targeted exposure data for consumers are required.	• Further testing of potential endocrine disrupting effects in aquatic organisms (particularly fish) and aerial organisms may be needed when agreed test methods are available. • Further environmental exposure data are required for the aerial compartment.
o-Phenylphenol[1,2]	• The weight of evidence from the available information shows that no adverse reproductive and/or developmental effects occur at doses causing general systemic toxicity. Further testing of endocrine disrupting effects may be required when agreed test methods become available. • Further targeted exposure data are required.	• Further testing of potential endocrine disrupting effects in terrestrial organisms may be needed when agreed test methods are available. • Further environmental exposure data are required for the aquatic and terrestrial compartments.
Resorcinol	• There is a need for more reliable multi-generation developmental and reproductive studies to substantiate the weight of evidence from the available information which shows that no adverse reproductive and/or developmental effects occur at doses causing general systemic toxicity. A multi-generational study in rats has been initiated in 2002	• Further testing of potential endocrine disrupting effects in aquatic organisms (particularly fish) may be needed when agreed test methods are available.
4-tert-Octylphenol[1,2]	• The weight of evidence from the available information shows that no adverse reproductive and/or developmental effects occur at doses causing general systemic toxicity. Further testing of endocrine disrupting effects may be required when agreed test methods become available. • Further exposure data for consumers are required.	• Further testing of potential endocrine disrupting effects in terrestrial organisms may be needed when agreed test methods are available. • Further environmental exposure data are required for the terrestrial compartment.

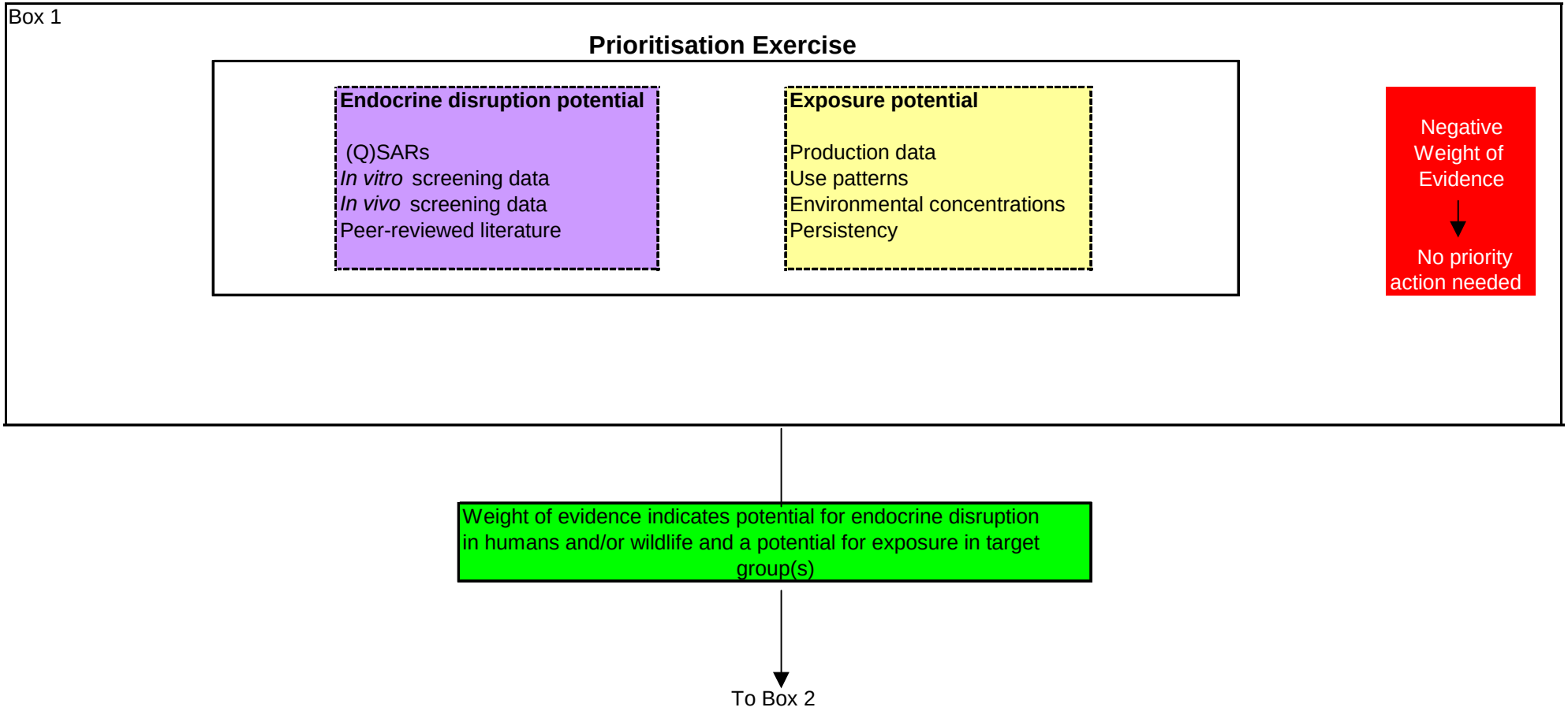
Table 16.1 Continued

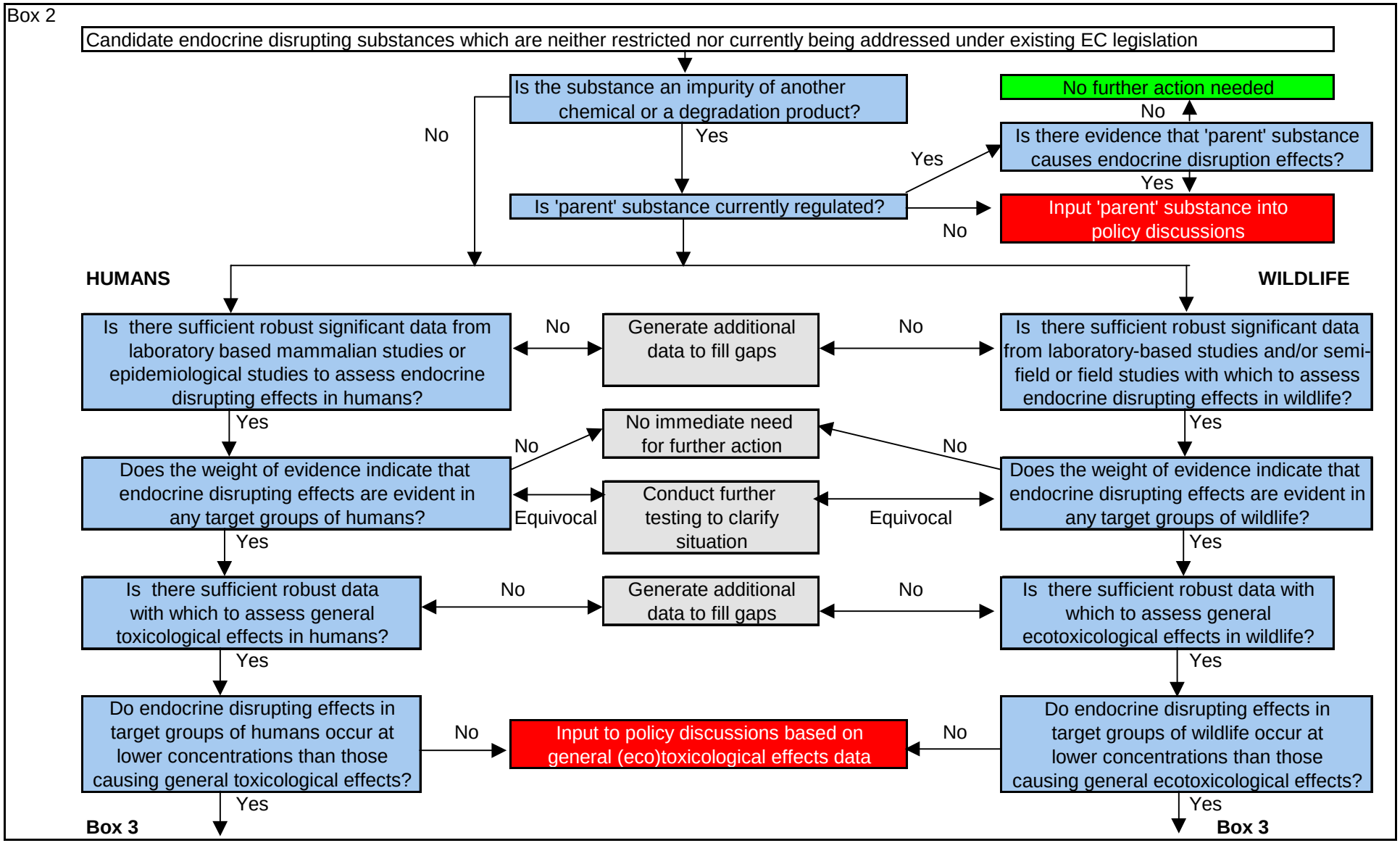
Substance	Humans	Wildlife
Tetra BDE	No review completed	No review completed
Oestrone	Not considered in the review	• Further testing of potential endocrine disrupting effects in terrestrial organisms may be needed when agreed test methods are available. • Further environmental exposure data for the terrestrial compartment are required.
17β Oestradiol	Not considered in the review	• Further testing of potential endocrine disrupting effects in terrestrial organisms may be needed when agreed test methods are available. • Further environmental exposure data for the terrestrial compartment are required.
17α Ethinyloestradiol	Not considered in the review	• Further testing of potential endocrine disrupting effects in terrestrial organisms may be needed when agreed test methods are available. • Further environmental exposure data for the terrestrial compartment are required. • Improvements in the limits of detection of chemical analysis of 17α Ethinyloestradiol in aquatic samples are needed.

Notes:

[1] - The results of this study can serve as an input to policy discussions which might then, for example, mandate a full risk assessment within the context of existing legislation (Directive EEC 793/93). This may require further testing or monitoring data for certain of the substances reviewed.

[2] - As new data becomes available on the 12 substances it may mean the current conclusions have to be revisited. The approach taken at that moment will be determined by the Commission in consultation with stakeholders and the Scientific Committees of the Commission.


Box 1
Prioritisation Exercise
Endocrine disruption potential
(Q)SARs
In vitro screening data
In vivo screening data
Peer-reviewed literature
Exposure potential
Production data
Use patterns
Environmental concentrations
Persistency
Negative Weight of Evidence
No priority action needed
Weight of evidence indicates potential for endocrine disruption in humans and/or wildlife and a potential for exposure in target group(s)
To Box 2


Box 2
Candidate endocrine disrupting substances which are neither restricted nor currently being addressed under existing EC legislation
Is the substance an impurity of another chemical or a degradation product?
No
Yes
No further action needed
No
Is there evidence that 'parent' substance causes endocrine disruption effects?
Yes
Yes
Is 'parent' substance currently regulated?
Yes
Input 'parent' substance into policy discussions
No
HUMANS
WILDLIFE
Is there sufficient robust significant data from laboratory based mammalian studies or epidemiological studies to assess endocrine disrupting effects in humans?
No
Generate additional data to fill gaps
No
Is there sufficient robust significant data from laboratory-based studies and/or semi-field or field studies with which to assess endocrine disrupting effects in wildlife?
Yes
Yes
No immediate need for further action
No
No
Does the weight of evidence indicate that endocrine disrupting effects are evident in any target groups of humans?
Conduct further testing to clarify situation
Does the weight of evidence indicate that endocrine disrupting effects are evident in any target groups of wildlife?
Equivocal
Equivocal
Yes
Yes
Is there sufficient robust data with which to assess general toxicological effects in humans?
No
Generate additional data to fill gaps
No
Is there sufficient robust data with which to assess general ecotoxicological effects in wildlife?
Yes
Yes
Do endocrine disrupting effects in target groups of humans occur at lower concentrations than those causing general toxicological effects?
No
Input to policy discussions based on general (eco)toxicological effects data
No
Do endocrine disrupting effects in target groups of wildlife occur at lower concentrations than those causing general ecotoxicological effects?
Yes
Yes
Box 3
Box 3

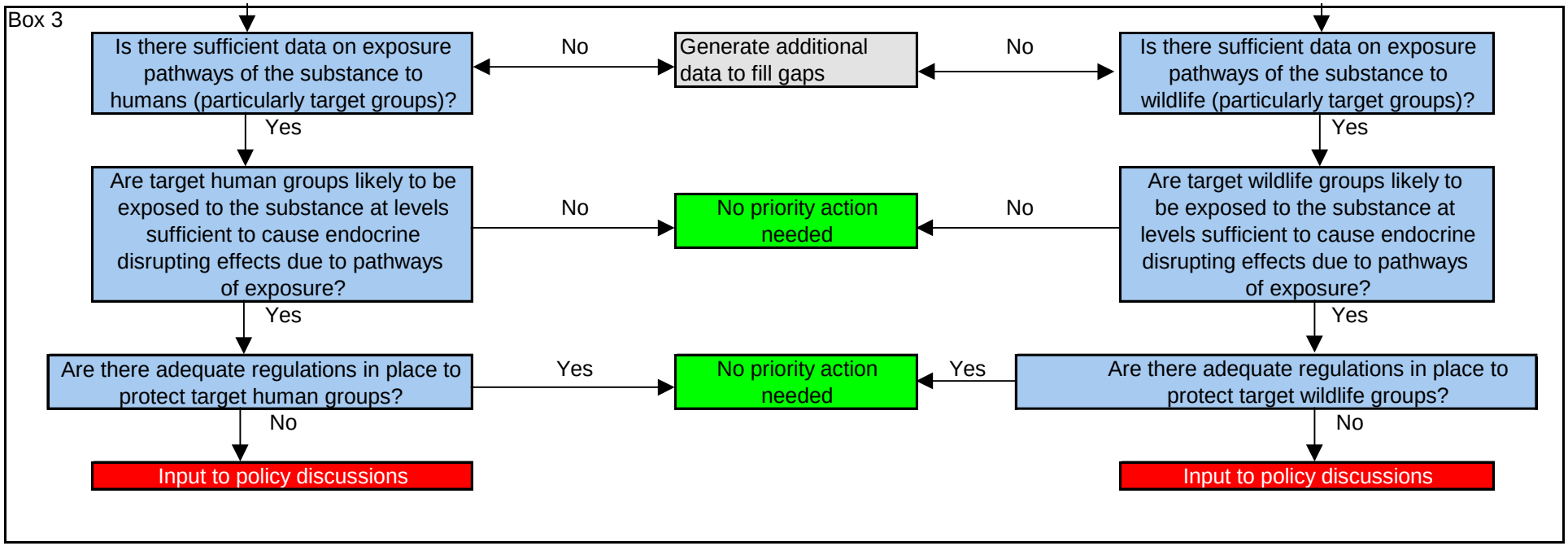


Figure 16.1 Framework for the proiritisation and review of potential endocrine disrupting substances