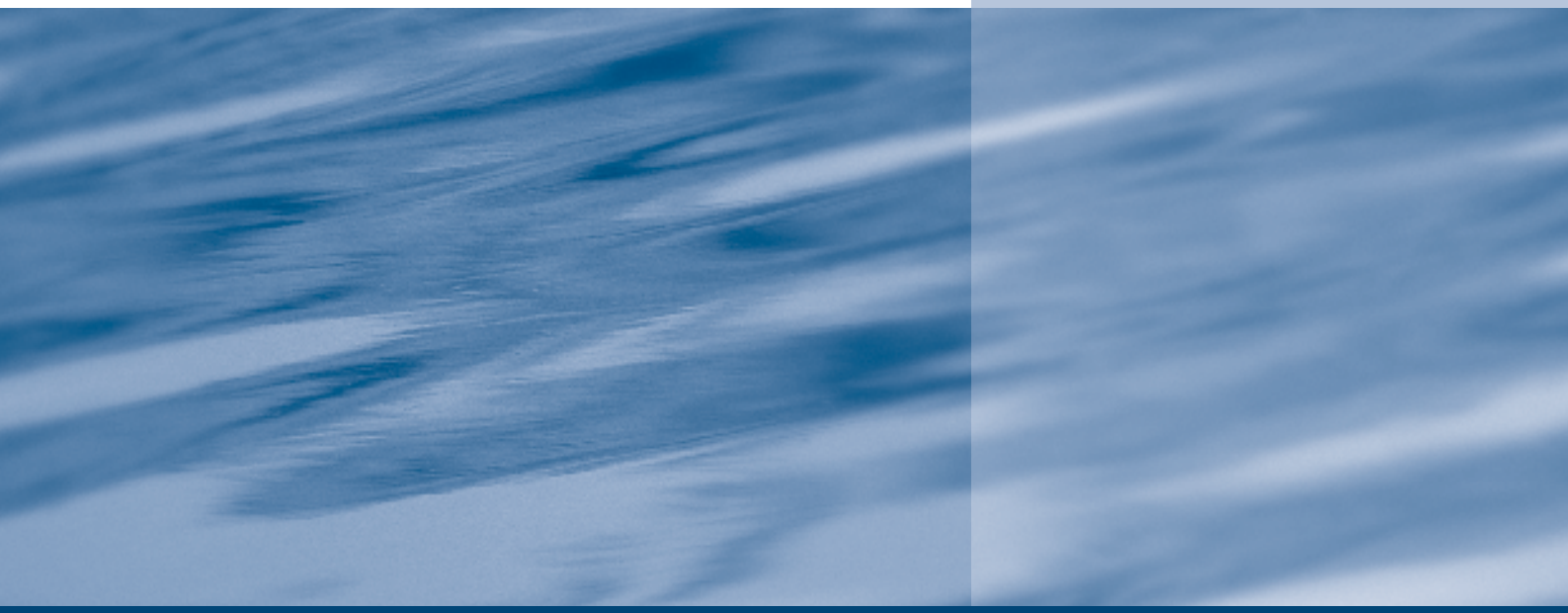

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Environmental Risk Evaluation Report: 4-tert-Octylphenol

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Professor Mike Depledge Head of Science

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In this work the Environment Agency has relied heavily on tonnage and use information submitted voluntarily by member companies of the Conseil Européen des Phénols Alkylés et Derivés (CEPAD[1]), working with the European Phenolic Resins Association (EPRA). Their helpful assistance is formally acknowledged. Additional useful information has also kindly been supplied by the Danish Environmental Protection Agency, the German and Austrian Federal Environment Agencies and the Norwegian Pollution Control Authority.

The Environment Agency would like to thank all contributors to this report. A list of organisations consulted is provided in Appendix 7.

[1] A trade association representing the major (although not necessarily all) producers and some users of octylphenol – it is part of the European Chemical Industry Council, CEFIC (as is the EPRA).

Executive summary

The Environment Agency commissioned this report to identify the potential risks that 4-*tert*-octylphenol – an important industrial chemical – might pose for fresh and marine waters and sediments, waste water treatment plants (WWTP), soil, air and predatory wildlife. The analysis applies to production and the major uses of this chemical.

4-*tert*-Octylphenol (CAS no. 140-66-9) is a high production-volume substance. European Union (EU) consumption was approximately 23,000 tonnes in 2001, and demand was expected to grow. It is a chemical intermediate, and is mainly used to make phenolic resins (98%). The remainder is converted into ethoxylates to produce surfactants. It can also be present as an impurity in nonylphenol (with other octylphenol isomers, typically up to a level of around 5%).

The phenolic resins are used in rubber processing to make tyres (82%). Minor uses include as a component in printing inks and electrical insulation varnishes, and in the production of ethoxylated resin for offshore oil recovery. Octylphenol ethoxylates are mainly used in emulsion polymerisation, textile processing, water-based paints, pesticide and veterinary medicine formulations, and to produce octylphenol ether sulphates. Importantly, the parent substance can be reformed in the environment through degradation of the ethoxylate chain.

The substance is a solid (melting point 79-82ºC, boiling point 280-283ºC). It has a vapour pressure of 0.21 Pa at 20ºC, a water solubility of 19 mg/L at 22ºC and a log octanol–water partition coefficient (log K_{ow}) of 4.12. Hydrolysis and photolysis are believed to be negligible removal processes for 4-*tert*-octylphenol in the aquatic environment. It is not readily biodegradable, although it is considered to meet the criteria for inherent biodegradation. The log K_{ow} implies a moderate bioaccumulation potential in aquatic biota, which is supported by measured bioconcentration factor (BCF) values of a few hundred for fish (the estimated BCF is around 600). The substance mainly partitions to soil and sediment when it is released to the environment.

Only limited monitoring has been carried out in the UK. In general, the concentration of 4-*tert*-octylphenol in surface water was below 1 μg/L and in many cases it was below the limit of detection of the analytical method. Similar values are reported from elsewhere in Europe. Some higher concentrations were found in trade and sewage effluents, with the highest value (10.8 mg/L) reported from an untreated trade effluent from a 4-*tert*-octylphenol manufacturing plant. The limited information on measured environmental levels meant that environmental concentrations had to be estimated using data on emissions and modelling. Emissions were predicted for each stage of the life cycle using the assumptions of a European Technical Guidance Document (TGD) for industrial chemical risk assessment, supplemented where possible with industry-specific information (including emission scenario documents). These predicted values are likely to be overestimates in some cases.

The substance adsorbs to sewage sludge, and the spreading of sludge from WWTP that treat effluent that contains 4-*tert*-octylphenol is the major route of exposure to soil. Only limited data about the properties of the substance in soil are available at present. Rapid degradation in soil is not anticipated, although one study appears to show little or no accumulation in soil from sludge application. Atmospheric transport and deposition to soil is expected to be negligible.

A reasonable amount of validated information is available to assess the environmental hazard potential of 4-*tert*-octylphenol. It can be considered acutely toxic to aquatic organisms, and may cause long-term adverse effects in the aquatic environment. The predicted no-effect concentration (PNEC) for freshwater aquatic organisms selected for the environmental risk assessment is 0.122 µg/L. Further long-term toxicity testing on suitable invertebrate species could refine this. 4-*tert*-Octylphenol can also adversely affect the endocrine systems of certain organisms, in some cases (e.g. aquatic snails) at concentrations that might be below the PNEC. Further research is needed in this area. The PNEC in other compartments are largely derived from the surface water PNEC, with the exception of a PNEC of 10 mg/kg body weight/day for predators exposed through food. 4-*tert*-Octylphenol does not meet the TGD marine risk-assessment criteria for a persistent, bioaccumulative and toxic (PBT) chemical (since it does not meet the bioaccumulation criterion), although it does meet the UK Chemicals Stakeholder Forum PBT criteria for a substance of concern.

The assessment identifies a potential risk from production and most uses to the freshwater and marine aquatic (including sediment) compartments, WWTP and soil. This is based on exposure estimates that have many uncertainties. More detailed data on emissions, biodegradation and measured concentrations in the environment are required before more realistic estimates can be made. There is also scope to refine the toxicity assessment for all compartments through further testing, although initially further information on releases needs to be collected.

The substance does not pose a risk to the atmosphere at current levels of use, and there are no indications of risk to predators exposed via the food chain. In addition, there is no concern for WWTP micro-organisms in several uses.

An EU ban of the major uses of nonylphenol (a higher tonnage chemical) might have a significant impact on background concentrations of 4-*tert*-octylphenol because it is present as an impurity in that substance. There could also be pressure to replace nonylphenol with 4-*tert*-octylphenol in such uses, although complete substitution could lead to a similar level of risk. Increased handling costs make this possibility unlikely, and some UK user industries have already agreed voluntarily not to use 4-*tert*-octylphenol or its ethoxylates as replacements for the nonylphenol equivalents that have been banned. Where long-chain alcohols cannot replace nonylphenol uses (e.g. in resin production), substitution by 4-*tert*-octylphenol would be expected to lead to similar risks.

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0 INTRODUCTION

In the mid-1990s, the chemical intermediate nonylphenol was nominated for a detailed risk assessment under the Existing Substances Regulation or 'ESR' by the United Kingdom because of concerns about its high aquatic toxicity. The report identified a large number of risks to the environment (as well as some specific risks to workers using speciality paints) (ECB, 1999). The subsequent risk reduction strategy proposed a ban for those uses that are the principal sources of environmental exposure (DETR, 1999). From 17 January 2005, European Union (EU) Member States are required to apply provisions necessary to comply with the resulting Directive 2003/53/EC (amending for the 26th time Council Directive 76/769/EEC, relating to restrictions on the marketing and use of certain dangerous substances and preparations (nonylphenol, nonylphenol ethoxylate (NPE) and cement)).

The main use of nonylphenol identified by the ESR assessment was for the manufacture of ethoxylate derivatives, and these substances are considered to be the main source of nonylphenol in the environment. Long-chain alcohol ethoxylates were identified as the main substitutes for nonylphenol ethoxylates in the risk reduction strategy. However, other alkylphenols (particularly octylphenol) were suggested as the only alternative to nonylphenol where it is used as an intermediate in the formation of derivatives other than ethoxylates (e.g., phenol–formaldehyde resins, phenolic oximes and plastic stabilisers). The strategy considered that it was inappropriate to recommend discontinuation of the use of nonylphenol as a chemical intermediate until further information on the comparative level of risks became available. In addition, although octylphenol ethoxylates (OPEs) were not identified as a likely substitute for NPEs (mainly because of cost factors), their similar chemical nature does not rule out the possibility.

Consequently, the Environment Agency commissioned a more detailed study in 2000 to prioritise other alkylphenols for risk assessment. The report has recently been published (EA, 2005), and 4-*tert*-octylphenol was identified as a high priority for assessment because it is the most likely immediate replacement for nonylphenol. It is also identified by the Oslo and Paris Convention for the Protection of the Marine Environment of the Northeast Atlantic (OSPAR) as a substance for priority action and has been included in the EU Water Framework Directive (2000/60/EC) list of priority substances.

The Environment Agency therefore commissioned this environment risk assessment as a pre-cursor to the development of a wider risk reduction strategy, to clarify the concentrations that might lead to environmental concern and identify those parts of the life-cycle at which risks might be occurring. It builds on an earlier assessment performed under the Organisation for Economic Cooperation and Development (OECD) Screening Initial Data Set (SIDS) programme[2] in the early 1990s (SIDS, 1994). As well as covering the current life-cycle of octylphenol in Europe, the report investigates whether its use as a potential nonylphenol substitute is likely to give rise

---

[2] An international programme that produces high-quality, peer-reviewed hazard assessments of major industrial chemicals, under the auspices of the Organisation for Economic Co-operation and Development (OECD). See http://oecd.org/.

to risks. The work began in 2001, and refinements have been made to reflect new risk assessment methodologies and scientific literature up to November 2004.

The layout follows the format of an ESR assessment with a few small modifications, so that readers familiar with such assessments (e.g., that for nonylphenol) can quickly find the information they are interested in. Note that the possibility of additive or synergistic effects with other alkylphenols or oestrogenic substances has not been considered in this report. Such an approach is more suited to site-specific assessments because of the differences in use pattern of the chemicals involved.

Note: The exposure assessment relies to a large extent on default assumptions, and thus may not be wholly realistic. It is also possible that some other uses exist that are not reflected in the use pattern provided by the main trade associations. Nevertheless, this report has been in preparation for some considerable time, and so, in the absence of better information, conclusions have had to be drawn based on current knowledge. However, it is emphasised that the information contained in this report should be read with care – to avoid possible misinterpretations or misuse of the findings, anyone wishing to cite or quote this report is advised to contact the Environment Agency beforehand.

1 GENERAL SUBSTANCE INFORMATION

The term 'octylphenol' represents a large number of isomeric compounds of general formula $C_6H_4(OH)C_8H_{17}$. The octyl group is a chain of eight carbon atoms, which may be branched or linear. This chain can also be located at the 2-, 3- or 4-position of the benzene ring. There are therefore many different potential octylphenol isomers.

European Industry representatives have identified '4-*tert*-octylphenol' (CAS No. 140-66-9) as the only isomer currently available commercially in Europe (CEPAD, 2000). It is a high production volume chemical (HPVC), that is, it was produced or imported in the EU by a company at 1000 tonnes per year or above at least once in 1990-1994. It therefore has a dataset in the latest version of the International Uniform Chemical Information Database (IUCLID). Other octylphenol isomers have IUCLID datasets, and these are used in this assessment where relevant (see Section 1.1 for further discussion of these).

Specific information has been sought for 4-*tert*-octylphenol. However, in a number of studies, especially those concerned with environmental monitoring, the general term 'octylphenol' has been used with no further description of the isomer(s) involved. Such data have been included where considered relevant. **Unless otherwise specified, the term 'octylphenol' is assumed to refer to the substance with CAS No. 140-66-9 for the purposes of this assessment.**

The data collection and peer review process is described in Appendix 7.

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS number: 140-66-9

EINECS number: 205-426-2

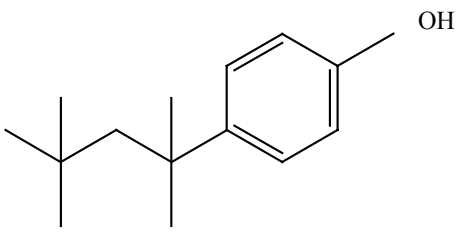
IUPAC name: 4-*tert(iary)*-Octylphenol

EINECS name: 4-(1,1,3,3-Tetramethylbutyl)phenol

Molecular formula: $C_{14}H_{22}O$

Structural formula: HO-C_6H_4-C_8H_{17}, where C_6H_4 is a benzene unit substituted at the 1,4-position.

Figure 1.1. Structure of 4-*tert*-octylphenol



SMILES code: Oc(ccc(c1)C(CC(C)(C)C)(C)C)c1

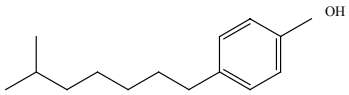
Synonyms (IUCLID, 2000; SIDS, 1994): octylphenol PT
para- (or p-)*tert*-octylphenol
p-(1,1,3,3-tetramethylbutyl)phenol

Molecular weight: 206.33 g/mole

Use of data from different isomers

Only one CAS number is used to describe the commercially available product, as indicated above (CEPAD, 2002). However, as is often the case with industrial chemicals, several other individual isomers (and mixtures of these) are called 'octylphenols' and have their own CAS numbers[3]. Among those listed in the European Inventory of Existing Commercial Chemical Substances (EINECS), the European Chemicals Bureau (ECB) website (http://ecb.jrc.it) only identifies one as a high production volume (HPV) substance:

iso-octylphenol CAS No. 11081-15-5



This isomer has been prioritised for action by OSPAR. However, it is no longer used commercially according to CEPAD (2002).

Other *para*-substituted octylphenols were also produced and marketed in the past, but marketing ceased several years ago (CEPAD, 2002). For example, four substances are listed on EINECS:

CAS No. 1806-26-4	4-octylphenol
CAS No. 27103-89-4	4-*iso*-octylphenol
CAS No. 27214-47-7	4-*sec*-octylphenol
CAS No. 99561-03-2	phenol, 4-octyl-, branched

None of these are listed as either high or low production volume (LPV, i.e., 10-1000 tonnes per year) substances by the ECB. CEPAD does not consider them to be commercially relevant in the EU. In particular, no linear octylphenol (CAS No. 1806-26-4) is known to be commercially available.

Four octylphenols with a different substitution pattern (i.e., the octyl group in the *ortho*- or the *meta*- position) are also included in EINECS:

CAS No. 949-13-3	2-octylphenol
CAS No. 3884-95-5	2-(1,1,3,3-tetramethylbutyl)phenol
CAS No. 26401-75-2	2-*sec*-octylphenol

[3] N.B. Two other commercially relevant substances have the same molecular formula as 4-*tert*-octylphenol. These are 2,4-di-*tert*-butylphenol (CAS No. 96-76-4) and 2,6-di-*tert*-butylphenol (CAS No. 128-39-2). Since they have a different substitution pattern, they are not considered further in this assessment.

CAS No. 27193-28-8 3-(1,1,3,3-tetramethylbutyl)phenol

Some other substances on EINECS also have no specified substitution pattern:

CAS No. 67554-50-1 octylphenol
CAS No. 93891-78-2 *sec*-octylphenol

At least one other CAS number is not listed on EINECS (*sec*-octylphenol, CAS No. 27895-70-2). Once again, none of these is listed as an HPV or LPV substance by the ECB.

In carrying out this risk assessment, the limitations of the available data set for CAS No. 140-66-9 have, in some cases, required careful use of data from some of these other isomers. This has only been done where the data could be considered to be representative and a justification is given in each case.

1.2 PURITY/IMPURITIES, ADDITIVES

1.2.1 Purity/impurities

Unlike nonylphenol, which is prepared from a nonene fraction that also contains octene and decene, 4-*tert*-octylphenol does not contain lower or higher carbon number homologues as impurities. The method of production also requires that the commercial substance consists of a single isomer. The purity of commercially produced 4-*tert*-octylphenol has been reported as 99.2% w/w (SIDS, 1994), with the major impurities reported as:

2-*tert*-octylphenol	(CAS no: 3884-95-5)	0.2% w/w
4-*tert*-butylphenol	(CAS no: 98-54-4)	0.1% w/w
unknown		0.2% w/w

Further information on purity and the impurities present in 4-*tert*-octylphenol is given in the confidential IUCLID dataset (1995). Purities in the range ≥75-96% w/w were reported. Reported impurities are typically in the range 1-6% w/w and include:

- 4-octylphenol isomers with different branching patterns,
  e.g., 4-(1,1,2,3-tetramethylbutyl)phenol; 4-(1,1,2-trimethylpentyl)phenol
- dioctylphenols ≤15% w/w,
  e.g., 2,4-bis(1,1,3,3-tetramethylbutyl)phenol (CAS no: 5806-72-4); dioctylphenol (CAS no: 29988-16-7); di-isooctylphenol (CAS no: 85958-96-9)
- alkene impurities,
  e.g., 2,4,4,6,6,8,8-heptamethyl-1-nonene (CAS no: 15796-04-0); trimethylpent-1-ene (CAS no: 107-39-1)
- starting materials, etc.,
  e.g. phenol (CAS no: 108-95-2); water

Various companies submitted the data and the purity and impurities appear to depend on whether the compound was produced in-house, and whether or not a high purity is

needed for the particular process in which the 4-*tert*-octyphenol is to be used. This purity range may now be historic, but care is needed in data interpretation as a consequence.

The substance is assumed to be essentially pure for the purposes of this report.

1.2.2 Additives

There are no reported additives used with 4-*tert*-octylphenol.

1.3 PHYSICO-CHEMICAL PROPERTIES

The following section provides a summary of the chemical and physical properties of 4-*tert*-octylphenol. A comparison of key properties with nonylphenol is given in Appendix 4.

1.3.1 Physical state (at n.t.p.)

Commercially produced 4-*tert*-octylphenol is a solid at 20°C and 101.3 kPa (SIDS, 1994) and exists as white or light pink flakes (EA, 2004c).

1.3.2 Melting point

The melting point has been reported to be in the range 79-82°C (no method was specified) (SIDS, 1994). An intermediate value of 80.5°C may be considered representative.

1.3.3 Boiling point

The boiling point range has been quoted as 277°C (Hüls AG cited in IUCLID, 2000) and 280-283°C (Sandoz Chemicals, cited in SIDS, 1994). Waern (2000) reported a wider range of 280-302°C, but the original data have not been reviewed.

No information is available on the methods used for any of these values. The boiling point range depends on the purity and origin of the material used for the test and the values quoted here can be considered to be typical of the commercially available material. An intermediate value of 281.5°C is considered representative.

1.3.4 Relative density

The relative density at 20°C has been quoted as 0.95 (Hüls AG cited in SIDS, 1994 and IUCLID, 2000), but no method was specified. Waern (2000) gives a value of 0.92, but no information on the method or temperature was provided and it was not possible to determine the original reference for this value.

1.3.5 Vapour pressure

A range of vapour pressure data (obtained at elevated temperatures) has been reported by Hüls AG (cited in IUCLID, 2000) and these are displayed in *Table 1.1*.

Table 1.1 Vapour pressure data for 4-*tert*-octylphenol (from IUCLID, 2000)

Temperature (°C)	Vapour Pressure (hPa)
150	11.3
180	43
200	93.7
220	189.3
250	483.4
276.9	1013

Using the equation: log (VP) = –3579(1/T) + 9.52527 (where T = temperature in Kelvin and VP = vapour pressure in hPa), Hüls AG estimated a vapour pressure of approximately 0.0021 hPa (0.21 Pa) at 20°C.

A value of 1 Pa (or 0.01 hPa) at 20°C has also been quoted (SIDS, 1994; IUCLID, 2000).

It has not been possible to obtain the original studies either to evaluate the data more fully or to determine the methods used. The value of 1 Pa quoted by the SIDS assessment is reasonably consistent with the value used for nonylphenol. However, that substance is a liquid and so might be expected to have a higher vapour pressure. Further support for this comes from a consideration of the Henry's law constant (HLC). For example, if the value of 1 Pa is used with the solubility value from Section 1.3.6 to estimate the HLC, the result is 10.86 $Pa.m^3$/mole. This is notably different from values obtained from both measurements and empirical calculation in Section 1.3.9.2 (recognising that the measurements were performed using seawater). The measured value for vapour pressure of 0.21 Pa gives a HLC of 2.3 $Pa.m^3$/mole, which is more consistent with the other estimates. The value of 0.21 Pa is therefore preferred in this assessment (slightly lower than the agreed value in SIDS, 1994).

1.3.6 Water solubility

4-*tert*-Octylphenol is described as being of very low solubility in water. A value of <0.1 g/L at 20°C produced by Hüls AG is quoted in IUCLID (2000). A value of 12.6 mg/L (± 0.5 mg/L) at 20°C is reported for 4-octylphenol (95% purity), using the generator column technique (Ahel and Giger, 1993b; also cited in SIDS, 1994).

Water solubilities of 17 and 19 mg/L at 22°C have been quoted for deionised and aquatic test water respectively (SIDS, 1994). Although the test method is unknown, the aquatic test water was presumably at equilibrium. The analytical method used was high pressure liquid chromatography (HPLC).

Waern (2000) reported a value of 0.01 g/L (10 mg/L) at 25°C. No further information was provided and the original reference could not be determined.

The value of 19 mg/L for aquatic test water cited in the SIDS assessment is preferred for the risk assessment, and is reasonably consistent with the other reported values.

1.3.7 n-Octanol–water partition coefficient

An n-octanol–water partition coefficient (log K_{ow}) of 4.12 at 20.5ºC has been reported using the shake flask method, to OECD guideline 107 (SIDS, 1994; IUCLID, 2000; Ahel and Giger, 1993a).

Other values reported by Hüls AG are 4.5 at 23°C (using the OECD guideline 107 method) and 5.3 (calculated using the MedChem-Programme version 1989, cited in IUCLID, 2000). Another estimated value, 5.28, has been cited by Gerritsen *et al.* (1998), and was calculated according to the log K_{ow} program produced by Syracuse Research Corporation (SRC; a more recent version of the program in EPISUITE (2004) gives the same value).

A value of 3.7 (no temperature indicated) was measured by HPLC (cited in IUCLID, 2000; McLeese *et al.*, 1981; SIDS, 1994; Waern, 2000), but no other information was available.

Ahel and Geiger (1993a) reported a log K_{hw} (partition coefficient between n-hexane and water) of 3.0 (carried out under OECD guidelines).

The log K_{ow} value of 4.12 produced using the OECD shake flask method and reported in the SIDS assessment is preferred for this risk assessment.

1.3.8 Hazardous physico-chemical properties

Flash points of 145°C (open cup) and 147°C (closed cup) have been assigned by Hüls AG when tested to guideline DIN 51376 and DIN 51758 (cited in IUCLID, 2000). An autoflammability value of 410°C (method unknown) has been quoted (cited in SIDS, 1994). The chemical structure of this compound does not suggest a likelihood of explosivity or oxidising properties.

1.3.9 Other relevant physico-chemical properties

1.3.9.1 Viscosity

No information could be located for this parameter.

1.3.9.2 Henry's Law constant

The water–air partition coefficient (known as the Henry's law constant or HLC) has been measured using a dynamic equilibrium system (Xie *et al.*, 2004). The water used was artificial seawater, and the concentrations of 4-*tert*-octylphenol were 0.08-0.10 mg/L. Experiments were conducted at three temperatures, and the results were (units are M/atm): 2008 ± 703 at 278 K; 1277 ± 447 at 287 K; and 195 ± 68 at 298 K. The value at 298 K corresponds to a HLC of 0.52 $Pa.m^3$/mole.

The HENRY program in EPISUITE (2004) provides predictions of the HLC of 0.46 and 0.70 $Pa.m^3$/mole, with the average being 0.58 $Pa.m^3$/mole.

The HLC can be calculated from the vapour pressure, molecular weight and water solubility of the substance. Using a vapour pressure of 0.21 Pa, a molecular weight of 206.33 g/mol and a water solubility of 19 mg/L gives a HLC for 4-*tert*-octylphenol of 2.3 $Pa.m^3$/mol.

The measurement of the water-air partition coefficient in seawater may not be directly applicable to freshwater, as the solubility of 4-*tert*-octylphenol may be different in seawater. There is no information on this, although in general terms the solubility of an organic compound would typically be lower in salt water (so the HLC would be higher). Ionisation of the phenol group could lead to increased solubility, but the pH of salt water is not high enough for significant ionisation of the substance to take place (see Section 1.3.9.3). The measured value is in very good agreement with that predicted using the Henry program. There are some uncertainties in the values for the solubility and vapour pressure as noted in previous sections, in terms of the available detail on the experiments. Therefore the measured value for the HLC is used in the assessment (0.52 $Pa.m^3$/mole at 25°C).

1.3.9.3 pKa

A calculated pKa value (Hammet method) of 10.33 at 25°C is reported in SIDS (1994). The pKa value for phenol is 9.9 and an alkyl-substituted phenol is expected to be slightly less acidic; the calculated pKa value fits this observation. 4-*tert*-Octylphenol would consequently be undissociated at typical environmental pH values.

1.3.10 Summary of physico-chemical properties

A summary of the physico-chemical data used for the risk assessment is given in *Table 1.2*.

Table 1.2 Physico-chemical properties

Property	Value and comment
Physical state at n.t.p.	White or light pink flakes
Molecular weight	206.33 g/mol
Vapour pressure	0.21 Pa at 20ºC (no method specified)
Water solubility	19 mg/L at 22ºC (for an aquatic test water)
n-Octanol–water partition coefficient (K_{ow})	log K_{ow} 4.12 at 20.5ºC (OECD 107 shake flask method)
Henry's Law constant	0.52 $Pa.m^3$/mol at 25°C (measured)
Acid dissociation constant (pKa)	>9.9 and <12.19

2 GENERAL INFORMATION ON EXPOSURE

The primary information summarised in this Section has been provided by CEPAD. References to the EU in this Section refer to the 15 Member States (EU-15) prior to expansion in May 2004. This assessment does not take account of any additional production or importation that might take place in the new Member States.

2.1 PRODUCTION

2.1.1 Production processes

The production processes used to produce 4-*tert*-octylphenol are analogous to those used to make nonylphenol (CEPAD, 2002). There are two alternative routes:

1. Phenol and *tert*-octene (di-isobutene) are reacted in the presence of an ion-exchange resin or boron trifluoride complex in a batch reactor. Neutralised and/or deactivated catalyst is disposed of via authorised waste facilities in accordance with regulations.

2. Phenol and *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 *tert*-octene is produced by dimerisation of isobutene, which ensures that the octene is branched rather than linear. The purity of isobutene means no other homologues are expected. The reaction with phenol leads predominantly to substitution by *tert*-octene in the 4- (para-) position.

SIDS (1994) reports some additional information as follows. The reaction takes place at temperatures of 80 to 100°C, with mean and maximum batch sizes of 4.5 t (presumed tonnes) and 7.2 t respectively. In a Swiss production plant, the substance is produced on about 30 days per year. Most of the octylphenol sold by Schenectady Pratteln AG, the only distributor in Switzerland, is delivered in a liquid state. The substance is directly transferred from the reactor to a reservoir via pipes and from there to heated railway tankers. Some 4-*tert*-octylphenol is converted to granulate and filled in sacks of 25 kg. In 1993 346 t (presumed tonnes) were shipped in a liquid state, with 76 t sold as granulate.

2.1.2 Production capacity

Information collated for Europe for the period 1992-1996 indicates production by five companies each producing in the range of 1000 to 5000 tonnes per annum (IUCLID, 2000). One of these companies ceased production in 1995. *Table 2.1* outlines production volumes, exports and imports of 4-*tert*-octylphenol within Europe for the five years from 1997 to 2001. No data are available for current global distribution or production.

Table 2.1 European production volume, exports and imports (CEPAD, 2002)

	Amount (tonnes/year)				
	1997	**1998**	**1999**	**2000**	**2001**
Production volume	17,520	18,259	19,626	22,215	22,633
Exports	234	104	6	0	150
Imports	1,035	1,337	1,240	1,308	375
Tonnage used	**18,051**	**19,492**	**20,928**	**23,523**	**22,858**
Captive use*	14,969	16,074	17,592	19,910	20,060

* Used on-site to produce other substances

Production was carried out at six sites in Europe in 2001 (identified as A–F – details are given in the confidential annex), in Germany, Belgium, Switzerland, France and the UK[4]. Additional producers or importers might have existed in the EU-15 that are not members of CEPAD. It has not been possible to identify such companies during this project, and the significance of their omission is unknown.

Older production data are available for Switzerland (SIDS, 1994). The tonnage declined from 887 t (presumed tonnes) in 1991 to 377 t in 1993.

2.2 USES

2.2.1 General information on uses

Information on the uses of 4-*tert*-octylphenol in Europe in 2001 has been obtained from CEPAD (2002). These data are summarised in *Table 2.2*.

Table 2.2 Main uses of 4-*tert*-octylphenol within Europe in 2001 (CEPAD)

Use	**Volume (tonnes)**	**Percentage (fraction of total use)**
Production of phenol–formaldehyde resins	22458	98
Production of octylphenol ethoxylates and subsequent derivatives	400	2
Total used in EU	22858	100

Note: these figures do not include the 20 tonnes of octylphenol imported into the EU as ethoxylate.

The following sections give details of these uses.

2.2.2 Phenolic resins

This is the major use reported by the manufacturers (98% of the use volume in 2001). Data on use pattern and releases (where relevant) were provided by CEPAD in collaboration with the EPRA. EPRA has confirmed that the description of the resin uses in this section is representative of the situation in Europe in 2001. These two organisations are believed to represent a large and representative sample of the resin manufacturing companies in Europe.

[4] The baseline year for this assessment is 2001, since this was when EU producers were asked to supply tonnage figures. It is known that there has been some restructuring within the Industry, and there appears to be only three companies producing 4-*tert*-octylphenol (at five sites, none of which are in the UK) in the EU-15 in 2005. No further general consultation on this aspect has taken place. Further details are given in the confidential annex.

2.2.2.1 Production process

Phenol–formaldehyde (Bakelite) resins are one of the oldest-known thermosetting and thermoplastic synthetic polymers. The initial stage of production is the base-induced reaction of the phenol and formaldehyde to give a hydroxybenzyl alcohol. With 4-*tert*-octylphenol the addition takes place at the ortho- position. The next step in the condensation is the formation of a dihydroxydiphenylmethane derivative with elimination of water. Continuation of these reactions leads to a two-dimensional polymer for 4-*tert*-octylphenol.

The phenol–formaldehyde resins may be made with 4-*tert*-octylphenol alone or in admixture with other phenols depending on the properties desired for the final resin. They are produced captively (i.e., at the same site as octylphenol production).

2.2.2.2 Types

There are two main types of phenolic resin:

- **Novolacs**, which are heat reactive, are made with a molecular excess of phenol over formaldehyde and are usually catalysed with acid (e.g., hydrochloric, sulphuric or oxalic acid);
- **Resoles**, which are heat reactive, are made with a molecular excess of formaldehyde over phenol and are usually catalysed with alkali (e.g., sodium hydroxide, ammonia, amines).

Phenol–formaldehyde resin manufacture is based almost exclusively on discontinuous batch processes using a traditional reactor or 'kettle'. Most of the 4-*tert*-octylphenol in the resins is chemically bound and cannot be released even on subsequent chemical or biological degradation, but the resins may also contain a small proportion (~3-4%) of unreacted 4-*tert*-octylphenol. This assessment assumes resins are made using 4-*tert*-octylphenol as the sole phenol (i.e., it is not mixed together with other phenols). This affects the estimation of how much resin is manufactured at a site, but not the emission estimates themselves. The majority of the octylphenol resins produced and used in the EU are of the novolac type.

2.2.2.3 Phenolic resin use

Resins are used non-captively (i.e., at a different location to their production) as follows.

2.2.2.3.1 Rubber compounding for tyres

This is the major use of octylphenol-based novolac resins (up to 98%) (CEPAD, 2002). The resins are an essential ingredient in rubber compounding for tyre manufacture. Their function is to increase the tackiness of the rubber and improve adhesion of the different layers during vulcanisation. The resins are added to rubber in amounts up to 1.5% of the rubber formulation (German UBA, 2001), though the maximum figure for the percentage of resin in rubber used for tyres is 10%. This results in a maximum concentration of free 4-*tert*-octylphenol in tyres of 0.3% (CEPAD, 2002). The impermeable nature of the rubber means the alkylphenol is not available to the aqueous environment except as a result of abrasion.

Tackifier resins were produced at four sites in Europe in 2001: two in France, one in Belgium and one in the UK. The volume of 4-*tert*-octylphenol used for this purpose was estimated to be 18,458 tonnes in 2001.

2.2.2.3.2 Electrical insulating varnishes

These are used for secondary insulation of electric windings (e.g. in motors and transformers) to improve insulation and to bond windings together. They are stoving enamels, which are fully cured to form a thermosetting polymer, which is insoluble in water. The demand for 4-*tert*-octylphenol for this specific use was estimated to be 2000 tonnes in 2001.

2.2.2.3.3 Printing inks

Novolac resins are essential components of modern printing inks. They make it possible to apply inks and coatings to paper, plastic, metal and other surfaces more quickly and accurately and with faster drying. Printing ink carriers are fluids and gels, which provide the ability for lithographic and letterpress printing inks to carry colour onto a variety of printing surfaces. They also make it possible for printing presses to run at higher speeds using inks that require less pigment, produce less waste and offer better performance characteristics such as gloss, brightness and rub or scratch resistance.

Octylphenol-based resins enable toxic aromatic solvents to be replaced by far less toxic aliphatic alternatives. The inks are manufactured in high-temperature processes in which the resins are reacted with other resins and oils, etc. (leaving no significant trace of free alkylphenol). They are then diluted in ink solvents and pigmented. The phenolic resin typically makes up around 7-8% of the ink formulation. The weight of ink as a percentage of a printed page is very small. No substitutes for alkylphenolic resins are currently available for this use. The volume of 4-*tert*-octylphenol used for this purpose was estimated to be 1000 tonnes in 2001.

2.2.2.3.4 Ethoxylated resins

These are used as emulsifiers to separate water from oil in oil recovery on offshore production platforms. They are added in very small amounts, often as low as a few parts per million, to oil–water emulsions produced during oil recovery and permit a high degree of separation. The residual 4-*tert*-octylphenol present in the ethoxylated resins is <0.01% (CEPAD, 2002). The demand for this specific use was estimated to be 200 tonnes in 2001.

2.2.2.3.5 Other uses of resins

Octylphenol–formaldehyde resins are described as being used in some other applications such as the foundry industry and in special paints used in marine applications because of the high resistance to saline waters that they provide. However, no further information is available on these uses at present. The demand for 4-*tert*-octylphenol for these other uses is estimated to be 800 tonnes/year (around 3% of the overall tonnage), although the actual amounts per application are not known. Use in

paper coatings has also been described, but this is not a use for these resins in the EU (see Section 2.2.2.3.6).

2.2.2.3.6 Comparison of resin uses with those for nonylphenol

Nonylphenol-based resins are used as tackifiers in rubber, carbonless copy paper (CCP) coatings, printing inks and electrical varnishes (ECB, 1999). Ethoxylated derivatives of the resins are also used in oil recovery. This suggests that there is some degree of interchangeability between nonylphenol and 4-*tert*-octylphenol in resins.

Kirk-Othmer (1996) indicates that the resins for both substances are predominantly used in CCP and in coatings; this was presumably the use pattern in the USA at the time. EPRA (2004) has confirmed that technology selection and resulting usage patterns vary substantially between the USA and Europe for CCP application. In Europe, resin-based CCP systems represent only 20% of the market; in the USA they represent closer to 80%. This makes the cross-reference to Kirk-Othmer (1996) somewhat unreliable in this context.

Additionally, while it is technically possible to switch between nonyl- and octylphenols (and this might have happened in the past in the USA), EPRA (2004) confirm that there is no record of any previous commercial use of 4-*tert*-octylphenol in the European CCP industry, which is currently based entirely on nonylphenol resin technology. The Industry has made a commitment under the UK Voluntary Agreement Framework (see Section 2.6) not to switch to octylphenol-based resins in future. Since this is a voluntary agreement, it is possible that some users in the UK or elsewhere could make a switch. Consequently, the implications of 4-*tert*-octylphenol being used in this application are considered in Appendix 6, since the information may be of use in other parts of the world.

2.2.3 Octylphenol ethoxylates

2.2.3.1 General information

SIDS (1994) states that octylphenol ethoxylates (OPEs) are manufactured by the addition of ethylene oxide to octylphenol under pressure. CEPAD member companies have provided additional information as follows.

OPE production is only a minor use of 4-*tert*-octylphenol in the EU at present (accounting for only 400 tonnes, or 2% of the total use volume, in 2001). There were about four or five producer companies in 2001. Production of OPEs is non-captive (i.e., it takes place at a different location to 4-*tert*-octylphenol production).

The residual content of free 4-*tert*-octylphenol in the ethoxylates decreases with increasing extent of ethoxylation. It ranges from 1% for OP3EO to 0.01% for OP10EO, with lower levels for greater degrees of ethoxylation (CEPAD, 2002). The majority of the ethoxylates on the market have 10 or more ethoxylate groups.

2.2.3.2 Octylphenol ethoxylate use

1050 tonnes of OPE were used in 2001 (CEPAD, 2002). The uses are described below.

2.2.3.2.1 Emulsion polymerisation

550 tonnes of OPE (equivalent to 220 tonnes of 4-*tert*-octylphenol) were used in 2001 as emulsifiers for emulsion polymerisation (e.g., to make styrene–butadiene polymers). This is the main use of OPEs. The end applications for the polymer dispersions include in paints, paper, inks, adhesives and carpet backings.

2.2.3.2.2 Textile and leather auxiliaries

OPEs are used in textile and leather auxiliaries (e.g., hot melts, textile printing, leather finishing). They generally act as emulsifiers in finishing agents, which are mainly styrene-butadiene copolymers. Finishing agents cover leather and textiles with a thin polymer film to make the material more resistant to water, dust and light. They also give leather a shiny appearance. The OPE is physically bound in the polymer matrix, which adheres to the substrate. Releases of OPE from this insoluble polymer structure are unlikely. 150 tonnes of OPE (equivalent to 60 tonnes of 4-*tert*-octylphenol) were used in 2001 for this application.

2.2.3.2.3 Pesticide formulations

There is a small use of OPEs in pesticide formulations (100 tonnes of OPE, equivalent to 40 tonnes of 4-*tert*-octylphenol). They act as emulsifiers and aid dispersion of the product over leaf surfaces.

The UK Pesticide Safety Directorate (2003) searched its internal database and only identified a handful of UK-approved plant-protection products containing OPEs (many more products contained NPEs). While this is probably a reasonable indication of the scale of use of the two groups, the database mainly records information related to active substances.

2.2.3.2.4 Veterinary medicine formulations

The UK Veterinary Medicines Directorate (VMD) (2003) confirmed that there were two products with a Marketing Authorisation in the UK that contained OPEs in 2003. Both of these are ectoparasiticides, one for livestock and the other for use on companion animals. An estimated 3.4 tonnes of OPEs are sold in veterinary medicines per year in the UK. This quantity is included in the 100 tonnes used for pesticide formulations. The VMD reports that many companies are in the process of reformulating their products to replace alkylphenol ethoxylates (APEs) with alcohol ethoxylates.

2.2.3.2.5 Water-based paints

In water-based paints, OPEs act as emulsifiers and dispersants, although the emulsifying properties are more dominant. 50 tonnes of OPE were used in this application in 2001 (equivalent to 20 tonnes of 4-*tert*-octylphenol).

2.2.3.2.6 Octylphenol ether sulphates

OPEs can be used to produce octylphenol ether sulphates (OPE-Ss). These are mainly used as emulsifiers in water-based paints. They can also be used as an emulsifier or

dispersant in pesticide or herbicide formulations. As a result of the chain length of the ethoxylate group (approximately 10 ethyl oxide units per molecule) the dispersant properties are more dominant. In practice, this means the OPE-Ss act to disperse the pesticide emulsion as a very thin layer on the leaves of the plants.

As for OPEs, the residual, unreacted 4-*tert*-octylphenol present in OPE-Ss decreases with increasing extent of ethoxylation, ranging from 1% for OP3EO-sulphate to 0.01% for OP10EO-sulphate. Lower levels occur with greater degrees of ethoxylation (CEPAD, 2002). The majority of the ether sulphates on the market have 10 or more ethoxylate groups.

The market for OPE-Ss is declining gradually, but their substitution for use in water-based paints is considered to be very difficult. CEPAD is not certain that the production of OPE-Ss from the ethoxylates still occurs in the EU. However, no information has been found to confirm this. For the purposes of this assessment, it is considered that there may be three or four small companies in Europe producing around 250 tonnes/year of OPE-Ss.

The amounts of OPE-Ss used in the two applications are assumed to be as follows, with the corresponding tonnage of 4-*tert*-octylphenol used to make them shown in brackets:

- Water-based paints = 200 tonnes OPE-S (64 tonnes 4-*tert*-octylphenol);
- Pesticide formulations = 50 tonnes OPE-S (16 tonnes 4-*tert*-octylphenol).

2.2.3.2.7 Comparison of ethoxylate uses with those for nonylphenol

In contrast to 4-*tert*-octylphenol, ethoxylate production is the major use of nonylphenol. NPEs have a very wide variety of surfactant-type uses, although many of these have been recently phased out in the EU (see Section 0). In purely technical terms it appears that OPEs could perform most of the functions of NPEs. However, the higher cost of production of 4-*tert*-octylphenol (and hence of the ethoxylates) means that cheaper surfactants are more likely to be used in place of NPEs (e.g., long-chain alcohol ethoxylates). The implications of NPE substitution by OPEs are considered in Appendix 4.

2.2.4 Information from product registers

A Norwegian survey of the use of alkylphenols and their ethoxylates in products in 1999 (Norwegian Pollution Control Authority, 2001) showed that no octylphenol and only about 4.4 tonnes of OPE were used (in 215 tonnes of products; 2.05%). The OPEs were used as follows:

- Interior and exterior paint 0.03 tonnes (in 29 tonnes of product; 0.1%)
- Other paint and/or varnish products 0.24 tonnes (in 61 tonnes of product; 0.4%)
- degreasing products 0.07 tonnes (in 3 tonnes of product; 2.3%)
- other products 3.6 tonnes (in 36 tonnes of products; 10%)

The Danish Product Register (Danish Environment Protection Agency, 2002) lists the following uses for 4-*tert*-octylphenol:

- Manufacture of paints, varnishes and similar coatings, printing inks and mastics;
- Manufacture of structural metal products;
- Building and repairing of ships and boats;
- Construction of buildings and civil engineering works involving special trades;
- Insulating work activities;
- Joinery installation.

Although some of these uses appear to be different from those described in the preceding sections, the total amount used for these purposes is stated to be less than one tonne. One further minor use for 4-*tert*-octylphenol mono-ethoxylate (octoxynol; CAS No. 9002-93-1) was reported in paints, lacquers and varnishes and for surface treatment at less than one tonne/year for each application. In addition, the Danish Product Register lists one further minor use for 4-n-octylphenol (CAS No. 1806-26-4) in the maintenance and repair of motor vehicles at below one tonne/year. Considering the comments made in Section 1, this might be a reporting error.

The Finnish product register includes 4-*tert*-octylphenol as a registered substance, but no products, production or import were notified in 2001 (Finnish Ministry of the Environment, 2002).

2.2.5 Other reported uses

Although the major uses of OPEs are addressed in this assessment, inevitably some minor uses are not. For example, the British Association for Chemical Specialities (BACS) has reported a small use in metal cleaning applications in the UK (personal communication, 2003). Similarly, the American Chemistry Council (personal communication, 2004) commented that some of their member companies use 4-*tert*-octylphenol as a lubricant additive. Consultation with a number of companies in Europe showed that none made use of the substance as such in lubricants, although a small quantity of OPE is present in a range of lubricant types. Although this was not a complete survey, the information received suggests that the scale of these uses in the EU is very limited. The tonnages for both of these applications are included in the confidential annex.

Further information on use pattern is available from older sources, and these are mentioned below. It is likely that some of these are now historical, in Europe at least. In addition, there are no data on tonnage or percentage use, so they cannot be considered in this assessment. If these uses still occur in Europe, they are probably only minor. It is also likely that some or all of them relate to the use of the derivatives rather than the parent substance (e.g., the ethoxylates are non-ionic surfactants). However, it would be useful to obtain definitive information from users about whether they are still relevant. This would also be helpful for assessing trends and changes in use patterns.

Information from SIDS (1994) indicates that octylphenol is used to make:

- Fuel oil stabilisers;
- Antioxidants and emulsifiers;
- Adhesives;
- Dyestuffs;
- Fungicides;
- Bactericides;
- Vulcanising agents for synthetic rubber (a sulphide complex).

Additional information from Kirk Othmer (1992):

- Resole resins are reacted with alkaline earth metal hydroxides to yield metal resinates, which are used as adhesives;
- Chain termination of polycarbonates used in injection moulding, for example to produce compact discs;
- Reacted with sulphur dichloride to produce thio-bisphenol derivatives used as ultraviolet (UV) stabilisers, for example in polypropylene fibres used in outdoor carpets.

A past use as a production chemical at offshore oil and gas installations has also been suggested (CEFAS, 1997). OSPAR contracting parties have recently confirmed use in this sector (DEFRA, 2002a). However, this use is no longer valid for the UK and may relate to the use of ethoxylated octylphenol-based resins that are used for oil recovery (see Section 2.2.2.3.4). The European Oilfield Speciality Chemicals Association (EOSCA) is conducting a study on the possible presence of octylphenol in alkylphenol-based resins used offshore, and this work is due to be reported in 2005 (OSPAR, 2004). The OSPAR information also suggests a use in cosmetics, which has not been confirmed to the Environment Agency by suppliers.

In the USA 2-5% of the octylphenol (presumed to be 4-*tert*-octylphenol) produced is used in fuel for aeroplanes (SIDS, 1994). Note that the use pattern in the USA is likely to be different than in the EU because 4-*tert*-octylphenol is less expensive there (the availability of octene in Europe is more limited) (CEPAD, 2002). CEPAD are unaware of fuel use in Europe.

2.3 OTHER SOURCES OF 4-*TERT*-OCTYLPHENOL

No known natural occurrence of 4-*tert*-octylphenol was reported in SIDS (1994). However, the Norwegian Pollution Control Authority (2003) reports that octylphenol is a natural part of petroleum oil, and will be found in produce water from the extraction of oil. The amounts are believed to be very small.

A potentially important additional source of 4-*tert*-octylphenol arises from the production of nonylphenol. This is because nonylphenol is produced by the reaction of a commercial nonene feedstock that may contain 1-5% octene (and also 1% butene and 1% pentene, as well as decene). The level of octene can be as high as 10%, although typically it is 3-5% (CEPAD, 2002). Consequently, a similar proportion of the 'nonylphenol' produced actually contains alkylphenols with an alkyl chain length of eight carbon atoms (i.e., octylphenol isomers). This information was not available at the time the ESR risk assessment report for nonylphenol was produced, and the phenols derived from these alkanes were not identified as impurities in that report (ECB, 1999). Sasol Germany GmbH (2004) has confirmed that around 25-30 different octylphenol isomers are present in very low concentrations in the nonylphenol that it produces, although the specific 4-*tert*-octylphenol isomer was not detected. This information has been accepted at face value and other suppliers have not been contacted for additional data.

Other alkylphenol products may also contain branched C_8-alkylphenols for similar reasons. For example, manufacturers of dodecylphenol (also known as tetrapropenylphenol) – a HPVC mainly used to make lubricant additives – have confirmed that it contains small (<1%) levels of C_8-alkylphenols (HERTG, 2004). Heptylphenol is also produced commercially in significant quantities globally (although the amount on the EU market appears to be very low), and might be another source.

It is recognised that these C_8-alkylphenols might not be identical to the substance being assessed in this report, for example in terms of the degree of branching. However, it could reasonably be expected that these substances behave similarly in the environment. Since nonylphenol has been the highest tonnage alkylphenol on the market – with the most dispersive use – in recent years, Appendix 5 provides an illustration of its possible significance in terms of overall octylphenol exposure.

2.4 LIFE-CYCLE FOR 4-*TERT*-OCTYLPHENOL

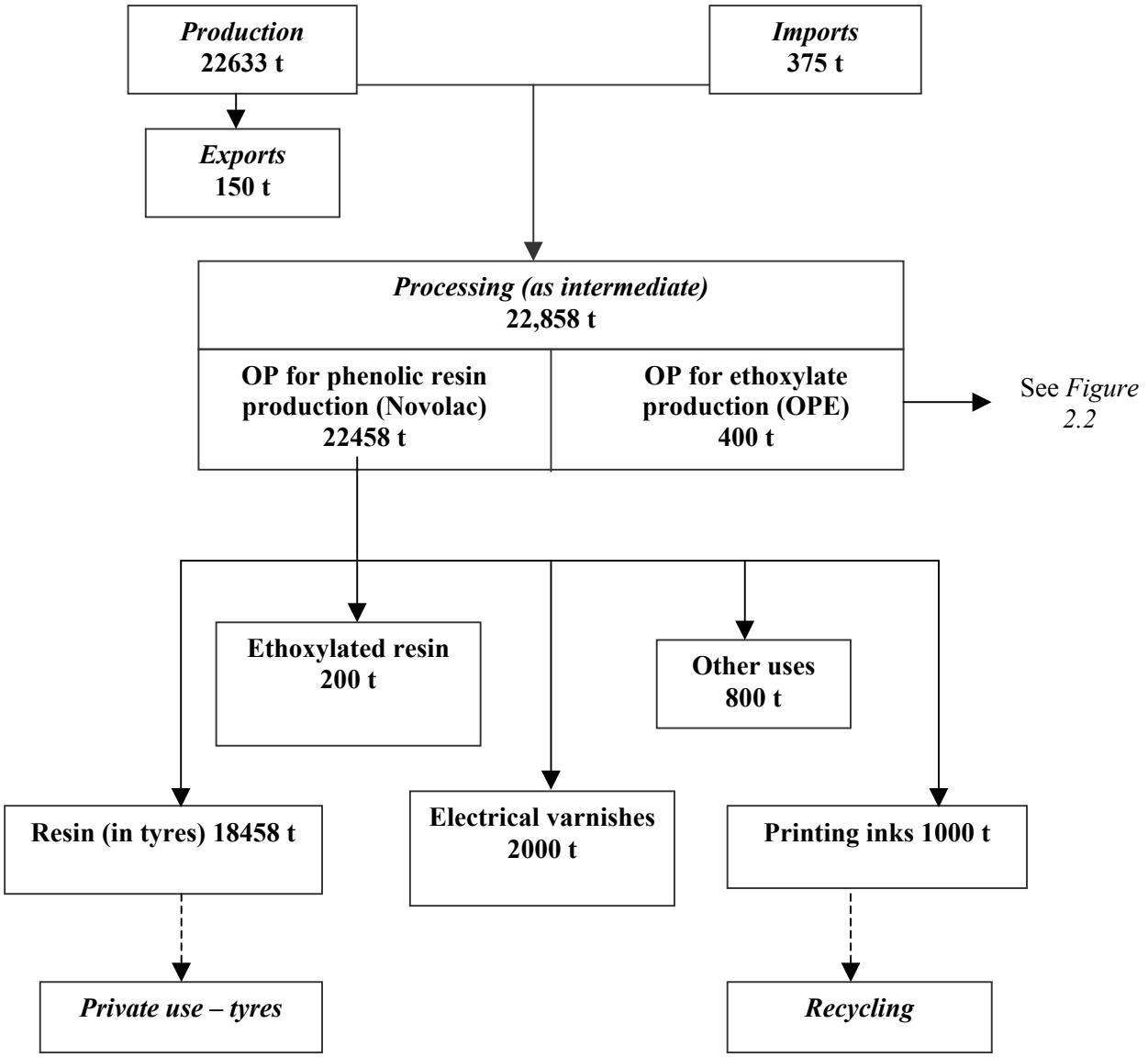
The life-cycle of 4-*tert*-octylphenol has been constructed from data supplied by the manufacturers. This life-cycle covers production or importation in the EU through to eventual releases into the environment. These come from either the processing of the octylphenol itself, or releases from processing and use, or from breakdown of the end products. The life-cycle considers that all of the substance is used as an intermediate in the production of other substances in the processing stage[5] of the life-cycle, these products being either phenol–formaldehyde resins produced captively (i.e., at the same site as octylphenol production) or OPEs. After processing, the resins are used for their intended purpose in products such as rubber for tyres (the major use), electrical insulating varnishes and printing inks, or are ethoxylated for use offshore. The OPEs are used for emulsion polymerisation, in agriculture, in textiles and paints, and to prepare ether sulphates (also used in paints and agriculture). The production of OPE and OPE-S takes place at a different location to octylphenol production.

There is also a possibility of releases following breakdown of OPEs in the environment (in a similar manner to NPEs), and this has been considered. Since 4-*tert*-octylphenol is present in nonylphenol (up to 10%) through impurities in the feedstock, OPEs may also be present in NPEs. These may be widely released, and so will act as a source of 4-*tert*-octylphenol in the environment. This non-deliberate (coincidental) release needs to be taken into account in the overall assessment of risks, although this aspect has not been included in the life-cycle of the substance since it is non-intentional. More details are provided in Section 3.

To aid clarity, the life-cycle has been split into two diagrams – *Figures 2.1* and *2.2* – each demonstrating one pathway of the 4-*tert*-octylphenol life-cycle. They connect together at the processing (as intermediate) stage.

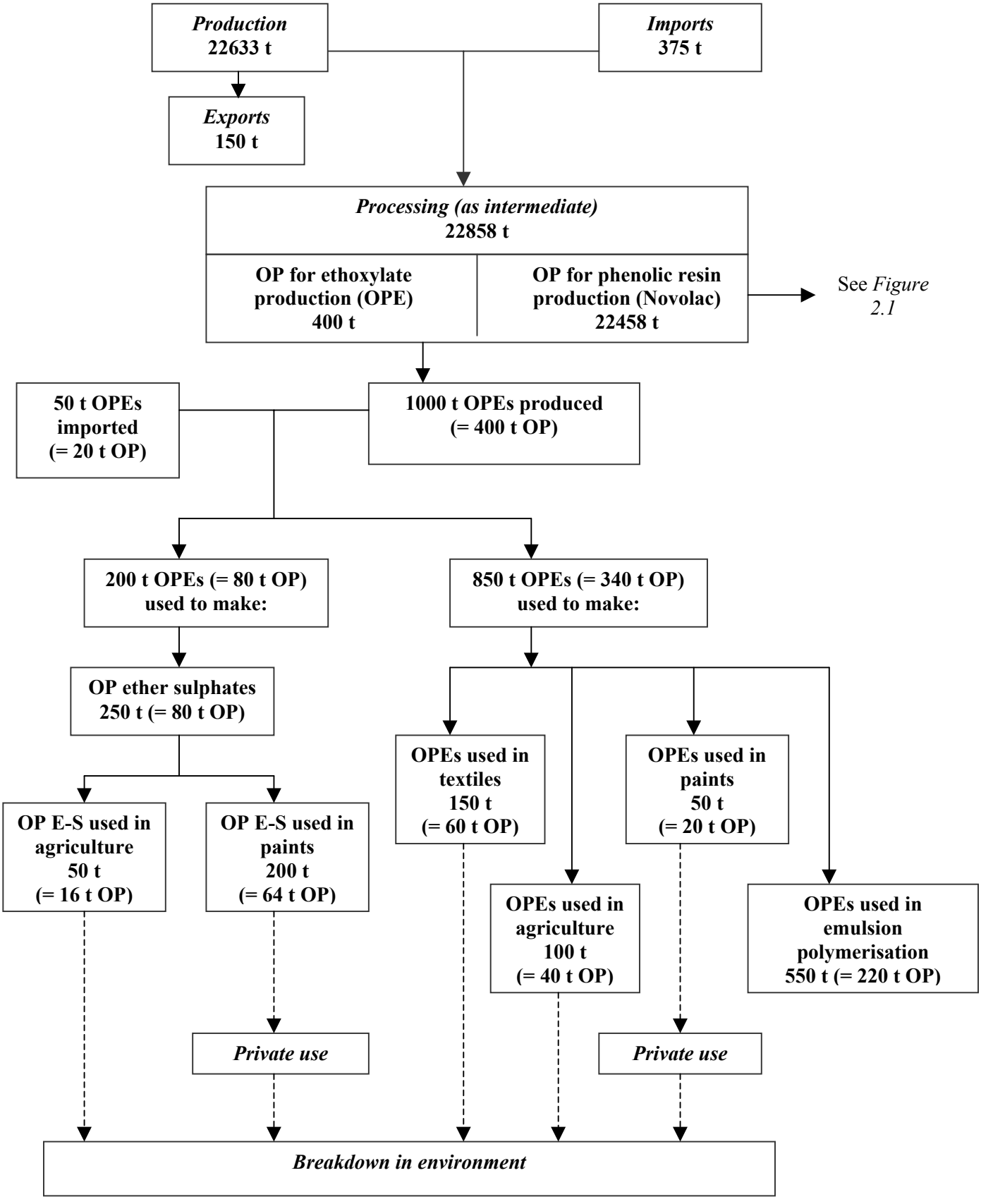
[5] There is no formulation since 4-*tert*-octylphenol is not incorporated into end products as the substance itself.

Figure 2.1 4-*tert*-Octylphenol (OP) life-cycle: phenolic resins



All of the tonnages for the five resin uses refer to the actual tonnage of 4-*tert*-octylphenol, rather than the tonnages of the products themselves.

Figure 2.2 4-*tert*-Octylphenol (OP) life-cycle: ethoxylates



2.5 TRENDS

The production data provided in Section 2.1.2 shows that in the 5 years from 1997 to 2001 there has been a steady growth in the production and use of 4-tert-octylphenol. The annual growth rate is approximately 1% and is expected to continue for 2002. No further tonnage data have been collected to allow a statement to be made on trends beyond that date.

2.6 REGULATORY INITIATIVES

No substance-specific legislative controls currently exist. Agreement on the classification of 4-*tert*-octylphenol as a 'dangerous substance' for Annex I of Directive 67/548/EEC was reached recently (September 2004), and it is now formally classified as Dangerous for the Environment (see Section 4.6). This has consequences for several other pieces of legislation (e.g., relating to hazardous waste).

As a consequence of its hazardous properties, a variety of activities have been initiated in an attempt to control this substance. For example, it was placed on the North Sea Action Plan as long ago as 1990 (NAP, 1990), and has been included on the OSPAR List of Chemicals for Priority Action (OSPAR, 2000). The OSPAR Commission objective is to prevent pollution of the maritime area by continuously reducing discharges, emissions and losses of hazardous substances with the ultimate aim of achieving concentrations in the marine environment near background values for naturally occurring substances and close to zero for man-made synthetic substances. OSPAR has published a background document on 4-*tert*-octylphenol that was based on an earlier draft of this risk assessment (OSPAR, 2001).

Regulation (EC) No 648/2004 on detergents may have consequences for the use of alkylphenol ethoxylates in detergents, as it requires consideration of possible recalcitrant breakdown products from surfactants, which may include octylphenol.

4-*tert*-Octylphenol has been considered in work for the development of the EC strategy on endocrine disrupters. The following information is taken from a recent working document[6]. An initial priority list of substances for further evaluation of their role in endocrine disruption was drawn up, identifying 118 substances (including 4-*tert*-octylphenol) with evidence of endocrine disruption (BKH, 2000). From this list, nine substances (including 4-*tert*-octylphenol) were identified as being neither restricted nor being addressed under current EC legislation. An in-depth evaluation of these substances has been carried out. It was concluded that 4-*tert*-octylphenol may present a risk to the aquatic compartment. Information on the potential consumer exposure for these substances is limited and targeted monitoring to provide these data is needed.

Companies regulated by the Environment Agency under the Integrated Pollution Control (IPC) and Integrated Pollution Prevention and Control Regulations (IPPC, due to have totally replaced IPC by the end of 2007) are required to report releases for a number of

[6] Commission Staff Working Document SEC (2004) 1372 on implementation of the Community Strategy for Endocrine Disrupters – a range of substances suspected of interfering with the hormone systems of humans and wildlife (COM(1999)706).

specified substances to the Pollution Inventory[7]. Octylphenols and their ethoxylates are on this list of substances with a reporting threshold of 100 kg/year to either sewer or to controlled waters.

IPPC does not impose any specific restrictions on the release of 4-*tert*-octylphenol or its ethoxylates to the environment. However, releases are restricted under the general principles of IPPC that all installations and mobile plant should be operated in such a way that:

(a) all the appropriate preventative measures are taken against pollution, in particular through application of the best available techniques;
(b) no significant pollution is caused[8].

In practice, if releases of 4-*tert*-octylphenol or its ethoxylates from an IPPC process were sufficiently high to cause concern then release limits would be imposed, or an improvement condition would be set requiring a reduction in releases over an agreed period of time.

The Environment Agency had also proposed an operational environmental quality standard (EQS) of 1 µg/L annual average and 2.5 µg/L maximum allowable concentration in both fresh and marine waters (EA, 2004c). This predates activity under the Water Framework Directive (see below).

Directive 2000/60/EC (the Water Framework Directive) established a framework for European Community (EC) action in the field of water policy and included strategies against water pollution. As part of the strategy, identification of priority hazardous substances was proposed, which included substances that may be hazardous to the marine environment. Consequently, 4-*tert*-octylphenol has also been included in the Water Framework Directive list of priority substances (EU, 2001). This places an obligation on Member States to take action for the progressive reduction of emissions of this substance via the aquatic environment, through setting quality standards and establishing emission control measures.

The substance meets the UK Government Chemicals Stakeholder Forum's persistence, bioaccumulation and toxicity criteria for substances of concern. The Forum has concluded that there is scope for early voluntary action to reduce emissions by the relevant producers and users in advance of any legislative action (Defra, 2001), including the replacement of octylphenol and its ethoxylates where possible and minimisation of discharges into the environment. Consequently, a number of industrial sectors have developed a voluntary agreement (CSI, 2004) to take action on these chemicals ahead of legislation and to phase out their use as soon as possible.

Most of the actions to be taken relate to nonylphenol and NPEs, but also include provisions in respect to not promoting octylphenol and OPEs as alternatives. The notes here relate only to the actions concerned with octylphenol:

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7 http://www.environment-agency.gov.uk/commondata/105385/pi_pages3_v031_636940.pdf.

8 Statutory Instrument 2000 No. 1973 The Pollution Prevention and Control (England and Wales) Regulations 2000 Part I (11).

- The chemical supply companies have agreed not to promote OPEs as substitutes for NPEs in applications subject to the intended marketing and use restrictions, and to introduce harmonised environmental classification and labelling for octylphenol and OPEs (see Section 4.6).
- Companies represented by the Cosmetic, Toiletry and Perfumery Association (CTPA) have agreed not to use octylphenol or OPEs as substitutes for nonylphenol and NPEs, to reformulate any products containing octylphenol or OPEs, and to stop the manufacture or import of such products by 31 December 2004.
- Companies represented by the British Association for Chemical Specialities (BACS) have agreed to a similar commitment.
- The Confederation of Paper Industries of Great Britain has agreed to phase out the use of any octylphenol or OPEs in existing formulations by 31 December 2004, and not to use these substances in any new or revised process after November 2003.
- The UK Cleaning Products Industry Association has agreed not to use OPEs as substitutes for nonylphenol or NPEs in cleaning or maintenance products, to reformulate any products containing octylphenol or OPEs and to stop manufacturing or importing such products by 31 December 2004.
- Companies represented by the Crop Protection Association have agreed to implement the redevelopment of formulations for plant protection products containing OPEs and to replace them by 31 December 2006. The European Adjuvants Association (EAA) has recently agreed to sign up to the voluntary agreement.
- The British Fragrance Association has agreed not to use octylphenol or OPEs as substitutes for nonylphenol or NPEs and to reformulate any products containing octylphenol or OPEs with completion by 31 December 2004.

In addition, there is an agreement to monitor and report on annual sales of OPE in the UK. As part of this commitment, the resin manufacturing industry is also intending to develop Good Practice Guidance on the handling of nonylphenol in resin manufacture (EPRA, 2004). This document would be equally applicable to 4-*tert*-octylphenol. A parallel set of guidelines is also under development for the CCP industry.

Finally, some EU countries have introduced national restrictions on either alkylphenol ethoxylates generally or more specifically against 4-*tert*-octylphenol. For example, the Norwegian Environment Ministry introduced the following restrictions as of 1 January 2002:

> It is prohibited to import, export, sell or use nonylphenol and octylphenol and their ethoxylates and preparations containing these substances. This prohibition does not apply to nonylphenol and octylphenol and their ethoxylates in or for use in paints and varnishes and in lubricating oils. (Norwegian Pollution Control Authority, 2002).

3 ENVIRONMENTAL EXPOSURE

This assessment has been prepared in accordance with the principles of the ESR and the methods laid down in Commission Regulation (EC) 1488/94[9], which is supported by a technical guidance document (TGD, 2003)[10]. The TGD models are implemented by the EUSES computer program[11] (version 2.0.1[12]). The assessment is generic in that it represents a *realistic worst-case approach* for a hypothetical environment that broadly reflects average European conditions. Further details can be found in the TGD. Given that tonnage data were supplied for the EU market, the following discussion is applicable across Europe.

3.1 ENVIRONMENTAL RELEASES

3.1.1 General introduction

In considering potential releases of 4-*tert*-octylphenol to the environment, all aspects of the life-cycle of both the substance and the main products of chemical processing need to be taken into account, as follows:

- Production and use of 4-*tert*-octylphenol itself;
- Releases of 4-*tert*-octylphenol present as residual monomer in resins;
- Releases of 4-*tert*-octylphenol from the breakdown of OPEs.

Information has been supplied by CEPAD on the amounts of 4-*tert*-octylphenol produced and used in Europe in 2001 (some of this is summarised in a confidential annex to this report). The data have been used along with the default emission scenarios given in Chapter 3 Appendix 1 (Tables A and B) of the TGD (2003) to give default releases for the different use patterns and life-cycle stages. EUSES has been used to perform the calculations, supplemented with manual calculations for specific use patterns where necessary (as outlined in the following sections).

Very few actual site-specific release data are available for any life-cycle step, and so many of the emissions are predicted based on TGD defaults and analogy with nonylphenol, which has some similar uses (ECB, 1999). *Table 3.1* provides some values for discharges of octylphenol that have been reported in the Environment Agency's Pollution Inventory (EA, 2004a).

[9] Official Journal No. L 161, 29/06/1994 p. 03–11.
[10] Technical Guidance Document, Second Edition, Parts I-IV, EUR 20418 EN/1-4.
[11] Available from the European Chemicals Bureau, http://ecb.ei.jrc.it/.
[12] The use of the EUSES 2 model means that there are some differences in calculation compared to the ESR risk assessment of nonylphenol. One of the main differences is that it is now assumed that 100% of the activities involving a substance take place in the region, unless there is a good reason to consider a more dispersed distribution. For many of the scenarios considered, there is insufficient information to assume a different distribution. One effect of assuming that the activity takes place wholly within the region is that the default amount calculated to be used at a local site is increased in comparison to the default estimates from EUSES 1. Although the numbers of days for use may also increase, the usual consequence is that the resulting PEC values are increased.

Table 3.1 Discharges of octylphenol from the Environment Agency's Pollution Inventory (EA, 2004a)

	Discharges to controlled waters (kg)						Discharges to sewer (kg)				
Year	1998	1999	2000	2001	2002	2003	1999	2000	2001	2002	2003
Uniqema, Middlesbrough	300	300	300	300	300	240	-	-	-	-	-
Rohm and Haas (UK) Ltd, Dewsbury	-	-	-	-	-	-	23	38	<1	-	-
Asahi Glass Fluoropolymers UK Ltd, Hillhouse International, Thornton-Cleveleys	<1	2.5	4	9.8	<100	-	<1	<1	-	-	-

The Uniqema site produces OPEs, which are typically used in the paint and polymer industry. The site reported discharges of OPEs to controlled waters of 175 kg in 2002 and 200 kg in 2003. The Rohm and Haas site does not manufacture 4-*tert*-octylphenol, but does produce a wide range of resins for surface coatings, laminating and paints and the 4-*tert*-octylphenol was almost certainly used for this purpose; no emissions of octylphenols or OPEs have been reported for this site in 2002 or 2003. The Hillhouse site manufactures PTFE resins, and it is assumed that OPEs are used in the emulsion polymerisation processes for the production of these resins. The site reported discharges of OPEs to controlled waters of 9282 kg in 2002 and 12130 kg in 2003, and to sewer of 500 kg in 2003. The discharges are predicted by each site, and are not necessarily measured data.

3.1.2 Releases from the production and direct use of 4-*tert*-octylphenol

3.1.2.1 Production of 4-*tert*-octylphenol

4-*tert*-Octylphenol was produced at six sites within Europe in 2001, described as A-F in this report for the purposes of confidentiality. Details of these sites with company and location are provided in a confidential annex. Data regarding releases to the environment have only been received for three of these sites in the EU (CEPAD, 2002), and are limited:

- At site A, 4-*tert*-octylphenol is produced and used in phenolic resin and ethoxylate production. Site-specific wastewater treatment plant (WWTP) effluent data for 1997 indicate a 4-*tert*-octylphenol level of <0.2 µg/L, with the same recorded in the receiving water.
- For site B, where all of the 4-*tert*-octylphenol produced is for captive use on site, WWTP influent values for 4-*tert*-octylphenol were recorded as 1, 41 and 47 µg/L, while the concentrations recorded in the WWTP effluent were all below 1 µg/L.
- For site E, which produces 4-*tert*-octylphenol for captive use, one concentration was provided for 2001 from the untreated effluent, 10790 µg/L. This effluent is known to be tankered off-site to a municipal treatment plant, where it is diluted and biologically treated.

In addition, SIDS (1994) reported that at the manufacturing plant in Switzerland, a total of 200 L wastewater (arising from water for the reaction, neutralisation of the catalyst, cleaning of the manufacturing equipment) results from the conversion of 30 t (presumed tonnes) of raw material. The joint sewage resulting from all processes in the plant (200-400 m^3 sewage weekly) were analysed regularly, with the measured octylphenol concentrations ranging from <0.1 mg/L to 0.6 mg/L. This was reported to give rise to

emissions of <2 kg/year in 1994. Air emissions were reported to be negligible (since the manufacturing operation was carried out in a totally closed system, and the contaminated air from the reactor was directly transferred to an incinerator).

The representivity of these data is uncertain, and it is not possible to estimate emission factors from them. However, the processes used to produce 4-*tert*-octylphenol are considered to be virtually the same as those used for nonylphenol. Therefore, information from the nonylphenol risk assessment can be used in the estimation of emissions. The highest level of release from a nonylphenol production site to surface water (i.e., after on-site wastewater treatment) was 2.15 kg/day. The quantity of 4-*tert*-octylphenol produced in the EU is only 30% that of nonylphenol (at the time the nonylphenol assessment was written), and six sites produce 4-*tert*-octylphenol compared to four sites for nonylphenol. These facts suggest that the amount of 4-*tert*-octylphenol produced at a site should be lower than that of nonylphenol, and hence the emissions would be lower. In the absence of specific information on this, however, the emissions from 4-*tert*-octylphenol production are assumed to be the same as for nonylphenol. Similarly, the highest release to air from nonylphenol production, 0.16 g/day, is used for air emissions from 4-*tert*-octylphenol production. These are the local release rates.

The total 4-*tert*-octylphenol emissions for the EU are assumed to be equal to the nonylphenol emissions (recognising that this is likely to be an overestimate). These are 53 g/year for air and 668 kg/year for surface water. Regional emissions are taken as the largest local emissions, and are 53 g/year to air and 645 kg/year to surface water. Continental emissions are zero to air and 23 kg/year to surface water.

3.1.2.2 Production of phenol–formaldehyde resins

In this use step, 4-*tert*-octylphenol is used as a monomer in a polymerisation process. Water is present in the process, both from the formaldehyde solution used in most cases and as formed during the reaction. There are 10-15 companies producing such resins in the EU, with the largest using around 9000 tonnes of 4-*tert*-octylphenol per year. The default emission factors from the TGD (2003, Table 3.10, Appendix 1, wet process) are 10^{-4} for emissions to both air and water.

There are no specific emission data for 4-*tert*-octylphenol. From the nonylphenol risk assessment, one site provided data to estimate a water emission factor of 8×10^{-6}, which was close to the default value for nonylphenol of 10^{-5}. 4-*tert*-Octylphenol has a higher factor as it is a little more water-soluble and has a higher vapour pressure. Although the increase in the default emission factor over that for nonylphenol is an order of magnitude, and may therefore be too conservative, there is currently not enough specific information to justify over-writing the defaults. Therefore the emissions are estimated using these factors. The number of production days is assumed to be 300.

For this assessment it has been assumed that the resins produced from 4-*tert*-octylphenol have this as the only phenol component. It is not clear to what extent mixed phenols are used, although Kirk-Othmer (1996) describes the resins in terms of single phenols. If mixed phenols are made this increases the amount of resin produced at the default site, but probably does not affect the amount of 4-*tert*-octylphenol used at the site to the same degree. Most octylphenol-based resins are of the novolac type, where the phenol is in excess, so 4-*tert*-octylphenol makes up at least 90% by weight of the resins.

It has therefore been assumed for the sake of simplifying the calculations that the resin consists only of 4-*tert*-octylphenol (i.e., the formaldehyde has been neglected).

The estimated emissions are:

Air: local 3 kg/day; regional 900 kg/year; continental 1,350 kg/year.
Wastewater: local 3 kg/day; regional 900 kg/year; continental 1,350 kg/year.

3.1.2.3 Production of octylphenol ethoxylates

This use is as an intermediate. The default TGD (2003) emission factors are zero to air and 0.007 to water (Table 3.3, Appendix 1, intermediate stored off-site). SIDS (1994) states that the explosivity and toxicity of ethylene oxide make it necessary that all operations and equipment are closed to the atmosphere, so that no release to the environment should occur during this process. No other specific information on the production of OPEs has been provided, but the production of NPEs is considered to be similar and information on these was provided for the nonylphenol assessment.

The total emissions of nonylphenol to air were estimated as 463 kg/year. For water, the total emission to surface water was estimated as 159 kg/day. The production of OPEs is 400 tonnes/year; at the time the nonylphenol assessment was written the production of NPEs was 47,000 tonnes/year. Hence the level of OPE production is less than 1% that of NPEs, which gave rise to the emissions above. As the production methods are the same, the overall emissions are taken as 1% of those for NPEs. For the local releases, it is assumed that 50% of the total occur at one site. These are also the regional emissions, and the continental emissions are the remaining 50%. The resulting emissions are:

Air: local 7.7g/day; regional 2.3 kg/year; continental 2.3 kg/year.
Surface water: local 1.22 kg/day; regional 235 kg/year; continental 235 kg/year.

In addition to the emissions of 4-*tert*-octylphenol, there are emissions of OPEs from the production step. Following the same assumptions as above, the estimated emissions are (in terms of OPEs, not as 4-*tert*-octylphenol):

Air: zero.
Wastewater: local 1.9 kg/day; regional 190 kg/year; continental 190 kg/year.

3.1.3 Releases from the use of products made from 4-*tert*-octylphenol

3.1.3.1 Releases from the use of phenol–formaldehyde resins

In the assessment of emissions from the use of the resins, it has been assumed that the content of residual 4-*tert*-octylphenol in the resin is 3% of the 4-*tert*-octylphenol used. The amount of 4-*tert*-octylphenol used in this application is 22,458 tonnes, which gives a total amount of residual 4-*tert*-octylphenol of 674 tonnes. This figure has been used as an ‘import’ tonnage in the EUSES calculations for this area of release (so that there is no production step).

3.1.3.1.1 Tyres

Rubber formulation

The majority of the resins produced from 4-*tert*-octylphenol are used in the production of tyres. The combination of the resin with other components of the rubber can be considered as a formulation step. There are no site-specific data for this process. Using the approach outlined in the Emission Scenario Document (ESD) for additives in the rubber industry (German UBA, 2001), an emission factor for the release of free 4-*tert*-octylphenol from the resins in the rubber to water has been derived as follows:

Emission factor = Weight % of substance in product x (1–F) x 0.01

The default value for F (i.e., fraction of substance remaining) from the ESD (Table 6 in German UBA, 2001) is 0.995 for processing aids (such as tackifiers). This gives an emission factor of 1.5×10^{-5} of free 4-*tert*-octylphenol to water.

The default emission factor to air is taken from the TGD (2003) and is 5×10^{-4} (Table A2.1, Appendix 1, dedicated equipment, little cleaning).

The amount used on a site has been estimated using the default method in the TGD. The local site accounts for 40% of the total EU use over 300 days. The estimated emissions from this are:

Air: local 0.369 kg/day; regional 277 kg/year.
Wastewater: local 0.011 kg/day; regional 8.3 kg/year.

Tyre production

The formulated rubber is then used to manufacture tyres. The ESD for rubber (German UBA, 2001) indicates that losses from this process are likely to be negligible. This step is therefore not considered further in this assessment.

Tyres in use

Losses from tyres in use need to be considered, through abrasive wear of the material rather than through leaching or volatilisation. Based on information from Schenectady International, Inc., one of the world's largest producers of octylphenol-based resin for tyre consumption, the resin is used in the interior portion of the tyre and therefore is not subject to abrasion and loss during use (APERC and CEPAD, 2005). Consequently, losses from tyres in use might be expected to be insignificant. However, some evidence is available to suggest the presence of the substance in road runoff (e.g., Aabøe *et al*., 2004, discussed below). In addition, tyre abrasion is of interest for other chemicals. The following discussion is therefore provided as an illustration of the potential significance of this source, *recognising that it might not be wholly realistic for 4*-tert-*octylphenol*.

Information from Australia suggests that an average new tyre weighs 10 kg and a used tyre weighs about 9 kg. The weight of rubber is 85% of the total tyre weight, so about 12% of the rubber is lost in use during the service life of the tyre (SA EPA, 2002). An Environment Agency report (EA, 1998) suggests losses of 10-20% of tyres by weight

over their lifetime, with a total loss in the UK of 53,000 tonnes of rubber per year. This total corresponds to 14% of the weight of tyres disposed of each year. An average value of 15% is used to estimate losses of rubber per year. Assuming that the new rubber processed into tyres goes to replace that withdrawn from use at the end of the tyre service life, the equivalent of 15% of new rubber is released each year. It is assumed that the resin and the unreacted 4-*tert*-octylphenol are lost at the same rate as the rubber. Hence the amount of resin abraded per year is 15% of 18,458 tonnes, or 2769 tonnes. The residual 4-*tert*-octylphenol is 3% of the resin, so the 4-*tert*-octylphenol release is 83 tonnes per year. This release is assumed to be split equally between surface water and industrial soil (e.g. roadside verges), with 10% release to the region. The emissions are:

Surface water: regional 4.15 tonnes/year; continental 37.4 tonnes/year.
Industrial soil: regional 4.15 tonnes/year; continental 37.4 tonnes/year.

Note that even if the abraded material contained 4-*tert*-octylphenol, not all of it might be available to the environment. Complete availability has been assumed for the remainder of this discussion as a worst case.

Tyre disposal

In contrast to the situation described above for tyres in use, there may be possible releases from the tyre rubber at the end of the service life as a tyre. The disposal of tyres into landfills is no longer allowed. Council Directive 1993/31/EC[13] (the EU Landfill Directive) banned the disposal of whole tyres to landfill in July 2003. The ban will be completed for all tyres (including tyre crumb) by July 2006. The use of whole tyres in landfill engineering applications will still be allowed.

A number of other civil engineering applications for used tyres have been investigated as a way to use up the waste material (e.g., as part flood defence measures, and surfacing for playgrounds, sports arenas and roads). Some studies were carried out on the possible leaching of components from the rubber in these new uses. Most of the studies were concerned with inorganic components (mainly zinc) and only one report was located that addresses the levels of 4-*tert*-octylphenol in such situations. Evidence for the possible extent of release of 4-*tert*-octylphenol from tyres in these uses is therefore largely indirect.

Collins (2002) reviewed the available information on leaching. From studies on artificial reefs made from tyres, the release of components from the tyres appears to be limited. Under the stable pH conditions in seawater, and away from UV degradation, leaching is confined to a surface micro-layer only a few micrometres thick. A comparison of the colonisation of such artificial reefs with ones made of concrete showed no significant differences. Also, the levels of zinc (used as a marker for leaching) found in organisms on the test reefs and on a nearby natural reef showed no indication of enhanced uptake from the tyres. The results do not demonstrate that leaching does not occur, but do suggest that the levels of leached substances are sufficiently low not to have harmful effects.

[13] Official Journal No. L 182, 16/07/1999 p. 01-19. More information can be obtained from http://www.tyredisposal.co.uk/Landfill.asp.

These studies relate to tyres in whole form. Collins (2002) also summarised work relating to the use of tyre chips used in bulk-fill applications in the USA. This work showed no evidence of increased levels of substances in water exposed to the tyre chips compared to substances present in natural groundwaters.

Measurements on 4-*tert*-octylphenol have been reported in relation to the use of shredded tyres in a noise barrier alongside a highway in Norway (Aabøe *et al*., 2004). 4-*tert*-Octylphenol was measured at concentrations of up to around 0.1 µg/L in infiltrated water from the barrier. The measurements from the site are considered in the risk characterisation section, but it is worth noting here that the concentrations measured in water from the barrier were less than those measured in road runoff. It is not possible to quantify the extent of removal of 4-*tert*-octylphenol from the tyre material from the available information.

Overall, the picture appears to be that 4-*tert*-octylphenol may be leached from tyre materials in civil engineering uses. Where tyres are used whole or baled, and especially where the use is underground or in seawater, the potential for losses appears to be low. There may be greater potential for losses in situations where shredded materials are used, but there is currently not enough information to make estimates of the potential for releases from this route. However, considering the relative concentrations measured in road runoff and in infiltration water, it is expected that the contribution from subsequent engineering use will be lower than that from tyre wear during the original lifetime. Therefore no additional estimates have been made for the civil engineering area in this assessment.

The same comments apply to any use of whole tyres in landfill engineering. The potential for releases of 4-*tert*-octylphenol from heat recovery (incineration) is also considered to be low. Re-use of tyre rubber in tyres is covered by the assessment of emissions from the original production processes. *Hence the overall contribution of tyre disposal, re-use or use in other areas to the overall emissions of 4-tert-octylphenol is considered to be negligible.*

3.1.3.1.2 Varnishes

The use of resins in this application involves heat curing of the resin. This requires further cross-linking and reaction, and so can be considered as a polymer-processing step with the resin acting as a cross-linking agent. The default TGD (2003) emission factors (Table A3.11, Type V) are 0.075 to air and 5×10^{-5} to water.

The default amount of resin used at a site is 300 tonnes per year, which is 15% of the total EU tonnage, and equates to 9 tonnes of 4-*tert*-octylphenol. The number of emission days is 120.

The estimated emissions are:

Air: local 5.62 kg/day; regional 4500 kg/year.
Wastewater: local 3.75×10^{-3} kg/day; regional 3 kg/year.

3.1.3.1.3 Inks

The life-cycle step in which resins are used to produce inks can be considered as a formulation step, as the resin is mixed with a number of other components. The resin makes up 7-8% of the ink concentrate, excluding the solvent. There is no specific information on this step. The default TGD (2003) emission factor for air is 2-5 x 10^{-3}, and for water is 0.003 (Table A2.1). The default amount of resin used at a site is 700 tonnes, containing 3% (21 tonnes) of residual 4-*tert*-octylphenol. This corresponds to 8750-10000 tonnes of ink containing 4-*tert*-octylphenol resins produced on the site each year. The number of emission days is 300. The estimated emissions are:

Air: local 0.175 kg/day; regional 75 kg/year.
Wastewater: local 0.21 kg/day; regional 90 kg/year.

The ink production process actually involves some reaction between the components. There are no significant traces of 4-*tert*-octylphenol left in the finished inks. Hence there will be no significant releases from the printing process, or from the recycling of paper printed with these inks. These two steps are not considered further in this assessment.

3.1.3.1.4 Ethoxylated resin production

This step involves the reaction of the resins with ethylene oxide and can be considered as a chemical synthesis. The default TGD emission factors are zero to air and 0.007 to water. The default amount of resin used at the site is estimated as 100 tonnes (3 tonnes of residual 4-*tert*-octylphenol). The number of emission days is 80. The estimated emissions are:

Wastewater: local 0.26 kg/day; regional 42 kg/year.

After two reaction processes, the residual amount of 4-*tert*-octylphenol in the ethoxylated resins is expected to be negligible, and so releases from the further use of these resins are not considered in this assessment.

3.1.3.1.5 Other resin uses

Other uses account for a total of 800 tonnes of 4-*tert*-octylphenol. These involve the foundry industry and marine paints. Marine paints appear to be the most likely use to lead to emissions, so as a worst case the tonnage is allocated to this use.

Marine paint

The resins are used as binders in marine coatings. The content of resin in the paints is 25%, although not all of this may be 4-*tert*-octylphenol. Assuming that all of the tonnage for other resin uses (800 tonnes) is used in this application, there are 800 tonnes of resin (neglecting the contribution of the formaldehyde) and so 3200 tonnes of paints containing the substance. The emissions from this use have been estimated using the draft ESD on coatings (EA, 2004b).

Table B2.3 from the TGD (2003) indicates one formulator for a tonnage of 3200 tonnes, operating over 300 days. Hence the daily formulation of paint is 10.7 tonnes, containing

2.7 tonnes of resin and 80 kg of residual 4-*tert*-octylphenol. This tonnage of paint fits into the larger batch sizes in the ESD, and so the large batch emission factors are used. It is assumed that the paints are organic solvent-based coatings. The relevant emission factors are 0.005% to air (as dust), 0.002% to wastewater (from floor washing) and 1.24% to waste. These give emissions of 4-*tert*-octylphenol of 4.0 g/day to air, 1.6 g/day to wastewater and 1 kg/day to waste. The annual regional emissions are 300 times these, and as there is only one formulator there are no continental emissions. The emissions from formulation are therefore:

Air: local 4 g/day; regional 1.2 kg/year.
Wastewater: local 1.6 g/day; 0.48 kg/year.
Waste: local 1 kg/day; regional 300 kg/year.

For the application of the paints, use in an industrial situation is assumed, rather than use by the public. For a quantity of paint of 3200 tonnes, the B table in the TGD (2003; B3.13) indicates a main fraction of 0.15. The amount of paint assumed to be used on the site is therefore 480 tonnes per year, which over 300 days is 1.6 tonnes applied per day. This daily amount contains 400 kg of resin at 25%, and 12 kg of 4-*tert*-octylphenol.

The ESD assumes a transfer efficiency of 65% for the application of paint; of the remaining 35%, 90% is captured for disposal and the remainder is lost to land and to water, with equal amounts assumed to each receiving compartment. Hence the local releases are 0.22 kg/day to water and to industrial soil. The overall emissions are 432 kg/year to each of water and industrial soil, all considered to be within one region. The emissions from application of the paint are therefore:

Wastewater: local 0.22 kg/day; regional 432 kg/year.
Industrial soil: local 0.22 kg/day; regional 432 kg/year.

Losses can also occur during the service life of the paint. The ESD estimates this as 1% loss to water over the lifetime of the paint, which corresponds to 240 kg for the annual use. As marine paints, the losses are most likely to be to marine waters. This is not an emission route included in the EUSES model; instead this emission has been added to the diffuse emissions to surface water in the land environment. This has been split 90:10 between the continent and region. The emissions from service life are therefore:

Surface water: regional 24 kg/year; continental 216 kg/year.

The ESD also considers possible losses when the paint is removed at the end of its service life. Most of the coating removed is assumed to go to waste, but losses are considered possible to water and to soil, and a factor of 3.2% is assumed for each of these. It is also assumed that the old paint is removed at a similar rate to the application of new paint. The emission factor relates to the amount of paint initially applied, hence to 800 tonnes of resin containing 24 tonnes of substance. The overall emissions are therefore 768 kg/year to water and to soil. It is assumed that the activities of paint removal and paint application are related, so the same fraction of main source (0.15) and number of days (300) are used as for paint application. The resulting emissions are:

Wastewater: local 0.38 kg/day; regional 768 kg/year.
Industrial soil: local 0.38 kg/day; regional 768 kg/year.

3.1.3.2 Releases from the use of octylphenol ethoxylates

As described in Appendix 1, OPEs are degraded in sewage treatment systems and in the environment. A proportion of the ethoxylates are degraded back to 4-*tert*-octylphenol. Hence the assessment needs to take account of the possible releases of 4-*tert*-octylphenol to the environment from the use of the ethoxylates. From Appendix 1, the amount of 4-*tert*-octylphenol released in the effluent from a WWTP is estimated as 2.5% of the OPE entering the plant, and the amount leaving the plant absorbed to sludge is 19.5%.

To estimate the releases of 4-*tert*-octylphenol from the use of OPEs, the releases of the ethoxylates have to be estimated. The percentages given above can then be used to convert these into releases of 4-*tert*-octylphenol.

Note: One of the uses of OPEs is to make ether sulphates. These products are used for similar purposes to the ethoxylates (i.e., in paints and in pesticide formulations). To simplify the calculations, the amounts of ether sulphates used for these purposes are treated as the amount of OPE used to make them. For paints, 200 tonnes of ether sulphates are used, made from 160 tonnes of OPEs. There are also 50 tonnes of OPE used directly, giving a total of 210 tonnes of OPE used in this application. Similarly, for agricultural use the amounts are 100 tonnes of OPE and 50 tonnes of ether sulphates made from 40 tonnes of OPE, giving a total of 140 tonnes of OPE. Production of the ether sulphates is, however, included as a life-cycle step.

3.1.3.2.1 Formulation

In some applications (e.g., paints, textiles, agrochemicals), OPEs are formulated with other components before use. This is likely to take place at different locations to the production of OPEs and to the use of the formulated products. Some specific information on the formulation of paints that contain NPEs was obtained for the nonylphenol assessment, and this is used in the appropriate section below. For formulation in other areas, specific information gathered for the nonylphenol assessment supported the use of the default emission factors for this step. These are 0.0025 to air and 0.003 to wastewater.

The total amount of OPEs formulated for agrochemicals and textiles is 290 tonnes. This is very much smaller than the amount of NPEs formulated, which was 74,000 tonnes. Hence the emission estimates for larger sites that were made for the nonylphenol assessment are not relevant here (these were 1000 tonnes and 250 tonnes formulated at one site). Instead the estimate for a small site for NPEs can be used; this used 10 tonnes per year, over 30 working days. The estimated emissions are:

Air: local 0.83 kg/day; regional 725 kg/year.
Wastewater local 1 kg/day; regional 870 kg/year.

3.1.3.2.2 Emulsion polymerisation

The European Polymer Dispersion and Latex Association (EPDLA)[14] supplied the following information for the nonylphenol risk assessment, which is also assumed to be relevant to this assessment.

Many polymer dispersions contain alkylphenol ethoxylates as surfactants used in the manufacturing process. The end applications for polymer dispersions include in paints, paper, inks, adhesives, and carpet backings. At manufacturing sites, the amount used varies between 3 and 2000 tonnes/year. This is based upon a survey of seven manufacturing sites. Production typically occurs for 300 days a year or more. Releases to air are reported as zero. Releases to water during manufacture are reported as being very low and a conservative estimate of the amount released is 0.1 kg/tonne produced. The level of NPEs in the final product is between 0 and 5%. From the survey of manufacturers, wastewater is reported as typically being treated on-site or is totally enclosed (no liquid effluent stream).

For NPEs, the amount used on site was assumed to be 3000 tonnes. This is much larger than the amount of OPEs used, which is only 550 tonnes for the whole of the EU. For this assessment the amount used on site is assumed to be 100 tonnes over 300 days. From the emissions factors above, the releases are estimated as:

Wastewater: local 33 g/day; regional 10 kg/year; continental 45 kg/year.

In contrast to this estimate, a site assumed to be using OPEs in emulsion polymerisation has reported much higher discharges of ~12 tonnes of ethoxylates to surface waters (possibly coastal waters) for one year, and 500 kg to sewer (data from the Environment Agency's Pollution Inventory – see Section 3.1.1). This is clearly different to the scenario described above, but the reasons are not clear. The discharge from the site may be an estimate itself, or the amount of OPE used in this area may have increased significantly since the usage figures were obtained. Alternatively, the OPEs may not be retained in the particular polymers produced at the site to the same degree as assumed in the calculation (the site produces fluorinated resins). For now, the generic calculated values are retained, but a further local calculation for the release via sewer at this site is included. It is not possible to make a meaningful estimate of the local release of 4-*tert*-octylphenol from the emission of OPEs to surface water.

Emissions may occur from the subsequent use of the polymeric materials produced in this way. To take these into account in this assessment, it is assumed that use in paints is representative. Accordingly, the tonnage of OPEs used in emulsion polymerisation is added to the tonnage of OPEs used directly in paint (see Section 3.1.3.2.4).

3.1.3.2.3 Textiles

OPEs are used as emulsifiers in finishing agents for textiles. As such they are bound in to the polymer film on the surface of the material. No specific information on releases from this process is available. The nonylphenol assessment does not provide a suitable model for this, as NPEs are used for different purposes (as scouring agents, fibre

[14] A CEFIC Sector Group representing the European manufacturers of polymer dispersions and latex.

lubricants and dye levellers). Therefore the default emission factors are used. The nature of the use means that the OPE remains in or on the surface of the material, so that the process is closer to printing than other processes. Peters (2003) measured a concentration of 99 mg/kg of OPE in a sample of textiles (children's pyjamas). Table A3.14 in the TGD (2003, Appendix 1) has emission factors for low solubility colourants used in printing, which are 0.14 to wastewater and 0.0025 to air.

There are a large number of potential textile sites. For this assessment, it is assumed that 1 tonne of OPE is used at one site, over 60 days. The emissions are:

Air: local 0.042 kg/day; regional 37.5 kg/year; continental 338 kg/year.
Wastewater: local 2.33 kg/day; regional 2.1 tonnes/year; continental 18.9 tonnes/year.

No specific information on the release of OPE from textiles in use is available, and as already noted the use of NPEs in textiles is for a different purpose. However, releases from the polymer structure in the coatings are considered unlikely (Section 2.2.3.2.2) and so are not considered further in this assessment.

3.1.3.2.4 Paints

The information used here is based on that for the related use of NPEs in the risk assessment for nonylphenol; this is preferred to the use of emission scenarios or TGD default values.

Formulation (production of paint)

Information on the formulation of paints with NPEs gave an emission to wastewater of 5 kg/tonne of ethoxylate used. This is considered to include emissions from emulsion polymer materials as well as ethoxylate used directly in the formulation process. Emissions to air were negligible. The amount of NPE used in paints is much larger than that of OPE, so the amount of ethoxylate formulated at a site from the nonylphenol assessment (200 tonnes) is too large. For this assessment, it is assumed that 25 tonnes are used at one site. In the nonylphenol assessment, production of paint was taken to occur over 250 days at both large and small sites. For this assessment the slightly shorter period of 200 days is used. The estimated releases are:

Wastewater: local 0.625 kg/day; regional 125 kg/year; continental 925 kg/year.

Use of paint

It is assumed that all of the paint is used industrially. From Section 3.1.3.2, 210 tonnes of OPE are used in this area. For this assessment, it is assumed that the emulsion polymers produced using OPE are also used in this area. This adds a further 550 tonnes of OPE to the amount used in paints, giving a total of 760 tonnes. The nonylphenol assessment uses factors of zero to air and 0.005 to wastewater, figures considered to be realistic by the industry. Local emissions are based on a site using 0.5 tonnes per year, over 240 days. The same figures are used for this assessment. The estimated releases are:

Wastewater: local 10 g/day; regional 380 kg/year; continental 3420 kg/year.

3.1.3.2.5 Pesticides

The formulation of pesticides was dealt with under general formulation above. In use, all of the OPE in the formulation will be released to the environment. Based on information provided for the nonylphenol risk assessment, it is assumed in the calculation of local concentrations that 4% is sprayed onto water with the rest to soil (Sections 3.3.1.1 and 3.3.2.1). This distribution is applied to the regional and continental emissions. The small amount of ethoxylates used in veterinary medicines is included in these estimates. A separate calculation is made for the local concentrations that arise from this use. The total amount of OPE used in this application is 140 tonnes, which includes 40 tonnes of ethoxylates from ether sulphate use in pesticides. The estimated releases are:

Surface water: regional 0.56 tonnes/year; continental 5.04 tonnes/year.
Agricultural soil: regional 13.44 tonnes/year; continental 121 tonnes/year.

3.1.3.2.6 Ether sulphate production

The production of ether sulphates involves the use of the ethoxylates as intermediates. No specific information on this step is available, and so the default TGD emission factors are used. These are zero to air and 0.007 to wastewater. This step uses 200 tonnes of ethoxylates. It is assumed that this is a relatively specialist process, and so 50% of the total is assumed to be used at one site, over 150 days. The estimated releases are:

Wastewater: local 4.67 kg/day; regional 1.4 tonnes/year.

Releases from the use of ether sulphates are considered under textiles and pesticides above.

3.1.4 Summary of emission estimates

The emissions estimated above are summarised in *Tables 3.2* and *3.3*. *Table 3.2* covers the emissions of 4-*tert*-octylphenol as a substance, and *Table 3.3* covers the emissions of OPE, presented as OPE.

The emissions of ethoxylates in *Table 3.3* are converted into emissions of 4-*tert*-octylphenol, as described in Section 3.1.3.2 for releases via wastewater (with 2.5% to water and 19.5% to sludge). Regional and continental emissions to surface water and to soil are also considered to give rise to 2.5% of 4-*tert*-octylphenol. Some different assumptions are made for local estimates in calculating the predicted environment concentrations (PECs), and these are noted in the relevant sections.

Table 3.2 Emissions of 4-*tert*-octylphenol

Life-cycle stage	Compartment	Local (kg/day)	Regional (kg/year)	Continental (kg/year)
OP production	Air	1.6×10^{-4}	0.053	0
	Surface water	2.15	645	23
OP resin production	Air	3	900	1346
	Wastewater	3	900	1346
OPE production	Air	7.7×10^{-3}	2.3	2.3
	Surface water	1.22	235	235
Resin use:				
Tyres – rubber formulation	Air	0.369	277	
	Wastewater	0.011	8.3	
Tyres – in use	Surface water		4150	37400
	Industrial soil		4150	37400
Varnish production	Air	5.62	4500	
	Wastewater	3.75×10^{-3}	3	
Ink production	Air	0.175	75	
	Wastewater	0.21	90	
Ethoxylated resin production	Wastewater	0.26	42	
Marine paint formulation	Air	4×10^{-3}	1.2	
	Wastewater	1.6×10^{-3}	0.48	
Marine paint application	Wastewater	0.22	432	
	Industrial soil	0.22	432	
Marine paint service life	Surface water		24	216
Marine paint disposal	Wastewater	0.384	769	
	Industrial soil	0.384	769	

Table 3.3 Summary of OPE emissions (all as OPE)

Life-cycle stage	Compartment	Local (kg/day)	Regional (kg/year)	Continental (kg/year)
OPE production	Wastewater	1.9	190	190
General formulation (pesticides and textiles)	Air	0.83	725	
	Wastewater	1	870	
Emulsion polymerisation	Wastewater	0.033	10	45
Textiles	Air	0.042	37.5	338
	Wastewater	2.33	2,100	18,900
Paint formulation	Wastewater	0.625	125	925
Paint use	Wastewater	0.010	380	3,420
Pesticide use	Surface water	-	560	5,040
	Agricultural soil	-	13,440	121,000
Ether sulphate production	Wastewater	4.67	1,400	

Emissions of OPE to air also have to be converted. Assuming a typical OPE chain length to be 10 ethoxylate units, the 4-*tert*-octylphenol part would make up 32% of the weight. This factor has been used to convert the air emissions in *Table 3.3* into 4-*tert*-octylphenol emissions.

3.1.5 Unintentional releases

As described in Section 2.1.1, 4-*tert*-octylphenol can also be released to the environment as an impurity in products derived from nonylphenol. The scenarios used for the risk assessment of nonylphenol (ECB, 1999) have been used to provide release estimates assuming a typical 4-*tert*-octylphenol impurity level of 5%. Details are given in Appendix 5.

3.2 ENVIRONMENTAL FATE AND DISTRIBUTION

3.2.1 Atmospheric degradation

No measured data are available. 4-*tert*-Octylphenol released to the atmosphere is likely to be rapidly degraded by reaction with hydroxyl radicals. The rate constant for this fate process has been estimated using the AOP program (v1.53) as 45 x 10^{-12} cm^3/(molec.s), assuming a hydroxyl radical concentration of 500,000 molecule.cm^{-3} (IUCLID, 2000). The rate constant has been re-estimated using the latest version of the AOP program (v1.91, in EPISUITE, 2004) as 42 x 10^{-12} cm^3/(molec.s), assuming the same hydroxyl radical concentration. The slight difference in these values probably results from updating of some of the fragment and reaction values, which has occurred in versions 1.53 to 1.91 of the AOP program.

From this rate constant the estimated half-life for the reaction of hydroxyl radicals with 4-*tert*-octylphenol in the atmosphere is calculated as being 0.25 days (assuming 12 hours of daylight and 1.5 million hydroxyl radicals per cm^3). The reaction rate for this process is such that 4-*tert*-octylphenol is unlikely to be transported far from its emission source before being degraded.

3.2.2 Aquatic degradation

3.2.2.1 Abiotic degradation

Hydrolysis would not be expected in view of the chemical structure. Measured data cited in SIDS (1994) suggest that in the surface layer of natural waters, 30% of 4-*tert*-octylphenol can be degraded within one day, and the half-life in a shallow (20-25 cm depth) creek on a sunny day was reported as 13.9 hours, which suggests that photodegradation and/or hydrolysis may occur. However, the rate of photolysis can be significantly reduced by the presence of organic matter, and in general terms photolysis would not be expected to be an important removal mechanism in the environment (although there could be some circumstances where it is). In addition, based upon the stability of 4-*tert*-octylphenol during storage and lack of degradation in controls in biodegradation studies, it is likely that abiotic degradation is a negligible removal process.

3.2.2.2 Biodegradation

A limited number of biodegradation studies are available and are summarised below. Information on the degradation of OPEs is contained in Appendix 1.

In a study carried out under OECD Guideline 302C (inherent biodegradability, modified MITI test), using a mixed population of non-adapted micro-organisms from activated sewage sludge, no degradation of 30 mg/L 4-*tert*-octylphenol (equivalent to 100 mg/L dry weight (dw)) was seen after 28 days. The purity of the test substance was not specified. This result suggests that the substance is not inherently biodegradable. The study was not carried out to good laboratory practice (GLP) (SIDS, 1994; IUCLID, 2000).

Another study, carried out to GLP using non-adapted aerobic micro-organisms from activated sludge, showed 20% biodegradation of 27.5-27.6 mg/L 4-*tert*-octylphenol

(purity given as 95%) after 28 days. The method used was the BODIS test (ISO 10708) and the results suggest that biodegradation is slow (data from Hüls AG, 1991; also cited in SIDS, 1994 and IUCLID, 2000).

A further study (Gledhill, 1999) was carried out to OECD Guideline 301B and GLP on the biodegradation of 4-*tert*-octylphenol (purity of 99.64%) using a domestic activated sludge from a WWTP where a high (>600 µg/L) concentration of NPE had been found. A mean net carbon dioxide value of 69.9% of the theoretical amount (based on titration measurements) was measured following 35 days exposure. Although 4-*tert*-octylphenol could not be characterised as 'readily biodegradable' (which under OECD definition requires 60% or greater carbon dioxide within 28 days, with this value being achieved within a 10 day window) based on these results, it was extensively mineralised (>60% CO_2) during the 35-day study. In addition, carbon dioxide and dissolved organic carbon concentration confirmed extensive removal (>90%) of the initial test substance organic carbon by mineralisation, adsorption and biomass incorporation. The results from the Gledhill (1999) study suggest that micro-organisms may need a period of adaptation before degradation of 4-*tert*-octylphenol can occur.

Johnson *et al.* (2000) investigated the potential for 4-*tert*-octylphenol to biodegrade in some English rivers. Using laboratory microcosms, half-lives of 7 to 50 days were obtained for the water samples. Shorter half-lives were generally seen in more urban and industrialised rather than upland and rural areas. However, even then, half-lives varied within the river for samples of river water taken at different times, although a similar degradation rate was noted for a range of concentrations from 0.3 to 100 µg/L. Hence this work demonstrates that 4-*tert*-octylphenol could be degraded in river samples taken from a range of urban and rural reaches (with the possible exception being a sample taken from an upland stream). No degradation was observed over 83 days when bed sediments were spiked with 4-*tert*-octylphenol and incubated under anaerobic conditions (in anaerobic jars with oxygen removal after spiking of sediment with 4-*tert*-octylphenol).

Ying and Kookana (2003) studied the degradation of 4-*tert*-octylphenol in seawater samples taken from a jetty near to a beach near Adelaide, Australia. The starting concentration of 4-*tert*-octylphenol in water was 5 µg/L, with incubation at 20 ± 3°C, and concentrations were measured at regular intervals up to 56 days. The solutions were aerated by bubbling air through them. Rapid initial losses were seen in the exposures and in sterile controls, indicating that these losses resulted from abiotic processes – the authors considered these to be mainly volatilisation with some sorption. After this initial removal, the concentration in the non-sterile solutions continued to decrease steadily, and the concentration had dropped to 0.03 µg/L after 42 days (concentrations in the sterile controls decreased more slowly in the later period). Experiments were also conducted without bubbling air through the solutions. These showed a slower rate of removal, with a half-life of ~30 days. The article quotes a half-life of 60 days for this experiment, but the plot in the report clearly shows a half-life (i.e., time to reach 50% of the starting level) of around 30 days and complete removal by ~50 days.

Studies were also carried out on marine sediments, collected from close to the same beach. A slurry was made using 5 g of sediment with 5 mL of seawater, and 4-*tert*-octylphenol was added at 1 µg/g (four other substances, including 4-*n*-nonylphenol, were also added). Sufficient headspace was provided to maintain aerobic conditions

throughout the experiment, which was continued for 70 days with weekly monitoring of the concentrations. Complete degradation of 4-*tert*-octylphenol was seen within 70 days. There was an acclimation period of around 3 weeks, with a concentration of 0.84 µg/g remaining after 21 days, but then a decrease to 0.09 µg/g within a further week. The half-life is given in the article as >20 days; it is true that less than half had degraded after this time, but the effective half-life (i.e., time to reach 50% of the starting level) can be estimated as 8.4 days. A further experiment was conducted in which sediments were allowed to become anaerobic in chambers under nitrogen, after which they were spiked with the substances. No degradation was seen in this study.

3.2.3 Degradation in soil

No experimental data are available on the degradation of 4-*tert*-octylphenol in soil.

3.2.4 Evaluation of environmental degradation data

In the atmospheric compartment, degradation of 4-*tert*-octylphenol through reactions with hydroxyl radicals is rapid, with an estimated half-life of 0.25 days. Abiotic degradation processes in water are likely to be negligible. Older studies suggest that biodegradation under aerobic conditions would only occur slowly, if at all. However, a more recent biodegradation study carried out to GLP and OECD test guidelines together with a laboratory microcosm study suggest that 4-*tert*-octylphenol is inherently degradable, although micro-organisms may need a period of adaptation. A study on degradation in river waters and sediments suggested that biodegradation half-lives were generally shorter in more urban and industrialised areas, which suggests that some form of acclimation may occur. A similar pattern was seen in a study using seawater, which also showed degradation in aerobic salt-water sediments. Both studies showed that there was no degradation in anaerobic bed sediments.

No information was located on the degradation of 4-*tert*-octylphenol in the terrestrial environment.

Much more information on degradation is available for nonylphenol, for both water and soil (ECB, 1999). Nonylphenol is not considered to be readily biodegradable. However, significant biodegradation was seen in ready biodegradability tests with adapted micro-organisms. The widespread use and distribution of nonylphenol and its ethoxylates makes some degree of acclimation more likely. Therefore nonylphenol is considered to be inherently biodegradable in the environment. Although the release pattern of 4-*tert*-octylphenol is generally less widespread than that of nonylphenol, its similar chemical structure combined with the fact that it can be a significant impurity in nonylphenol probably means that some degree of general acclimation of microbial populations has occurred. In general terms, 4-*tert*-octylphenol is therefore considered to be inherently biodegradable, fulfilling the criteria. This effectively equates to a half-life of 150 days for water and 300 days for soil, in accordance with the recommendations in the TGD (2003).

The situation is more complex for WWTP. There are no positive results in inherent tests, although the ready degradability test using (probably) acclimated sludge showed significant removal. Other studies also show degradation, but with significant lag periods. On the basis of this evidence, the substance can not clearly be considered to be inherently biodegradable fulfilling the criteria in line with the recommendations of the

TGD (2003). It must therefore be assumed that 4-*tert*-octylphenol is inherently biodegradable, not fulfilling the criteria for the purposes of the assessment of WWTP. There is evidence for a greater degree of removal in treatment plants than is estimated by sorption only (see Section 3.2.5 for this estimate), which suggests that some removal by degradation is possible. The effect of assuming inherent degradability meeting the criteria is therefore considered further in the risk characterisation.

The information on the behaviour of the ethoxylates in WWTP given in Appendix 1 is used for the modelling of 4-*tert*-octylphenol concentrations from the use of the ethoxylates.

3.2.5 Environmental partitioning

According to calculations using a Mackay level III fugacity model, 4-*tert*-octylphenol will, after release to a specific environmental compartment, distribute in the environment as in *Table 3.4* (SIDS, 1994).

Table 3.4 Environmental distribution of 4-*tert*-octylphenol

Compartment	Mass %		
	Release to air	Release to water	Release to soil
Air	26	1.2	<0.1
Water	5.1	77.9	0.3
Soil	67.7	3.1	99.6
Sediment	1.2	17.8	0.1

The following partition coefficients have been calculated using EUSES from the log K_{ow} value of 4.12:

K_{oc}	2740 L/kg	Partition coefficient for organic carbon–water
Kp_{susp}	274 L/kg	Partition coefficient for solids–water in suspended matter
Kp_{sed}	137 L/kg	Partition coefficient for solids–water in sediment
Kp_{soil}	54.8 L/kg	Partition coefficient for solids–water in soil
$K_{soil\text{-}water}$	82.4	Soil–water partitioning coefficient
$K_{susp\text{-}water}$	69.4	Suspended matter–water partitioning coefficient
$K_{sed\text{-}water}$	69.3	Sediment–water partitioning coefficient

The WWTP model used in EUSES estimates the fraction of a substance entering the works that will be directed to air, water and sludge. For 4-*tert*-octylphenol the fractions are 0.007 to air, 0.74 to water and 0.25 to sludge. For information, the fractions if the substance were considered to be inherently biodegradable meeting the criteria would be 0.005 to air, 0.46 to water and 0.22 to sludge, with 0.31 degraded.

In addition, a partition coefficient between soil and/or sediment and water (K_d) of 197.27 (log K_d 2.29) has also been calculated with FUGMOD V1.0 (SIDS, 1994).

3.2.6 Adsorption

Based on a log K_{ow} of 4.12, the organic carbon–water partition coefficient (K_{oc}) for 4-*tert*-octylphenol can be estimated as 2740 l/kg. Johnson *et al.* (1998) used laboratory batch techniques to study the sorptive behaviour of 4-*tert*-octylphenol to sediment from three

English rivers of contrasting water quality. The results showed that given either sufficient time or mixing a large proportion of the 4-*tert*-octylphenol in solution will sorb to bed sediments with distribution coefficients (K_d) of 6-700 L/kg and organic carbon normalisation partition coefficients (K_{oc}) of 3500-18000 L/kg. The sediments that sorbed the highest quantities of 4-*tert*-octylphenol had higher total organic carbon levels and a greater proportion of clay and silt particles. Johnson *et al.* (1998) stated that the work predicted that suspended sediments might also play a key role in the fate of 4-*tert*-octylphenol in industrialised areas. In rural areas a higher proportion of 4-*tert*-octylphenol might be predicted to remain free in solution.

Thus, experimental data and calculated partition coefficients suggest that 4-*tert*-octylphenol are strongly adsorbed to soils, sludges and sediments.

Given that 4-*tert*-octylphenol is a weak acid, pH might also have an effect on its adsorptive behaviour. However, the pKa is thought to be around 10, meaning that in most situations encountered in the environment the substance will be present in the undissociated and hence more hydrophobic form.

3.2.7 Volatilisation

The volatilisation of 4-*tert*-octylphenol from surface water to air may be estimated from the HLC. This has been measured as 0.52 $Pa.m^3/mol$ (see Section 1.3.9.2). An air–water partitioning coefficient ($K_{air\text{-}water}$) may be derived from the HLC and is calculated as 2.1×10^{-4} m^3/m^3. The $K_{air\text{-}water}$ and HLC are low and suggest that volatilisation is unlikely to be a significant removal mechanism for 4-*tert*-octylphenol from water systems.

Based on the HLC of 0.52 Pa m^3/mol, the volatilisation half-life from a model river (1 m deep flowing at 1 m/sec with a wind velocity of 3 m/sec) can be estimated to be about 250 hours (EPISUITE, 2004).

3.2.8 Precipitation

Based upon the reaction with hydroxy radicals, 4-*tert*-octylphenol is expected to be relatively short-lived in the atmospheric environment. It is not very volatile and so it is unlikely to enter the atmosphere in large amounts. The fraction that adsorbs to aerosol particles is also likely to be low, based on the behaviour of the related substance nonylphenol. Therefore the potential for transport of 4-*tert*-octylphenol in the atmospheric environment by this route is likely to be low.

Removal of 4-*tert*-octylphenol from the atmosphere is therefore likely to be negligible, with resulting rainwater concentrations being low. As the lifetime of 4-*tert*-octylphenol in the atmosphere is relatively short it is unlikely to be transported a long distance from its point of emission and therefore concentrations due to precipitation of 4-*tert*-octylphenol from the atmosphere are likely to be greatest near the point of emission.

3.2.9 Bioaccumulation and metabolism

3.2.9.1 Measured data

One set of laboratory bioaccumulation test results is available and some field bioconcentration factors (BCFs) have been estimated from tissue samples collected from wild organisms. The latter indicate that accumulation may be relatively low. These studies are outlined below.

Tsuda *et al.* (2000) performed surveys of nonylphenol and 4-*tert*-octylphenol in water and fish samples obtained from eight rivers flowing into Lake Biwa (Japan) every 2 months from April/May 1998 to March 1999. 4-*tert*-Octylphenol was detected in water at concentrations ranging between 'not detected' (limit of detection was 0.01 ng/mL) to approximately 0.09 ng/mL. Six fish species (Pale chub, Ayu sweetfish, Dark chub, Bluegill sunfish, Crucian carp and large-mouth bass) were tested for 4-*tert*-octylphenol tissue concentrations. Average field BCFs calculated for the different types of fish collected were found to range from 46 to 297. The analysis of tissue and water samples appears to have been carried out satisfactorily.

Another Tsuda *et al.* (2001) study reported carrying out laboratory bioaccumulation and excretion experiments on 4-nonylphenols and 4-*tert*-octylphenol using killifish (*Oryzias latipes*). For 4-*tert*-octylphenol, BCFs determined in whole fish (wet weight, wwt) were 261±62 (n = 4), and the biological half-life in whole fish was 7.7 hours. Parallel field studies were carried out for waters and aya fish *(Plecoglossus altivelis*) from three rivers flowing into Lake Biwa. Field BCF values (wwt) in the aya fish were recorded as 297±194 (n = 3) for 4-*tert*-octylphenol. These BCF studies do not appear to follow specific regulatory protocols. Although the methods appear to be generally appropriate, there are some significant issues that affect the overall confidence in the results (e.g., the test fish for the laboratory experiments were purchased from a local pet shop rather than an approved supplier).

Blackburn *et al.* (1999) analysed muscle and liver samples from a number of wild fish from the River Aire, Yorkshire, for 4-*tert*-octylphenol. In all tissue samples 4-*tert*-octylphenol concentrations were <0.1 µg/g (water concentrations were <0.5 µg/L).

Several alkylphenol compounds have been used as production chemicals at offshore installations and are released into the open sea as part of the production of discharge water. To ensure that these chemicals do not reach the food supply chain, a number of herring, haddock and dab taken from around North Sea offshore installations were analysed for 4-*tert*-octylphenol as part of a preliminary Food Quality Assurance monitoring programme. The results of this study are reported in CEFAS (1997). This study showed that concentrations of 4-*tert*-octylphenol in liver and muscle were below the limits of detection (i.e. <0.1-0.004 mg/kg depending on the species and tissue-type tested).

Lye *et al.* (1999) measured the accumulation of 4-*tert*-octylphenol (and nonylphenol and nonylphenol monoethoxylate) in juvenile and mature male flounder (*Platichthys flesus*) collected from the Tees and Tyne estuaries. 4-*tert*-Octylphenol (17 ng/g wwt) was detected in homogenised tissue of the single fish sample collected from the Tees estuary, but was not detected in tissue from fish from the Tyne estuary (limit of detection not

quoted directly, but inferred to be below 17 ng/g wwt). 4-*tert*-Octylphenol was not detected in water samples.

Bennett and Metcalfe (2000) investigated the concentration of degradation products of alkylphenol ethoxylates such as 4-*tert*-octylphenol in sediments and water downstream of a WWTP in the USA and also their potential to bioaccumulate in mussels deployed at sites at or downstream of the WWTP. The highest concentrations were detected in mussels deployed directly in the WWTP discharges. 4-*tert*-Octylphenol concentrations ranged from 'not detected' (limit of detection was 0.001 µg/g) to 0.01 µg/g wwt.

Ferreira-Leach and Hill (2001) investigated the biotransformation, bioconcentration and tissue distribution of 4-*tert*-octylphenol in juvenile rainbow trout (*Oncorhynchus mykiss*) using radiolabelled substance. 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. BCFs were measured in the study, with a value for whole fish of 471 after 10 days exposure. Some individual tissues had higher factors, with BCFs of 800-1200 measured in fat, intestine, liver and pyloric caeca. BCFs were below 300 in other tissues. These values are for soluble residues, and so may include contributions from metabolites as well as from the parent compound.

Madsen *et al.* (2002) exposed flounder (*P. flesus*) to 4-*tert*-octylphenol through their diet, at dose levels of 10, 50 and 100 mg substance per kg body weight to look for effects on vitellogenin (VTG) levels. Fish were force fed through a tube and syringe, and treatment occurred every 2 days for a period of 11 days. There was a significant accumulation of 4-*tert*-octylphenol in liver and muscle tissue, and the concentrations were correlated with the VTG levels. The authors did not estimate accumulation factors for 4-*tert*-octylphenol. From the graphs presented in the article, the highest levels of 4-*tert*-octylphenol were 50,000 ng/g in liver, and 12,000 ng/g in muscle. Thus the highest level measured, equivalent to 50 mg/kg in liver, is of the same order as the concentrations in food, which indicates little biomagnification in this experiment.

It has been reported that this substance is unlikely to bioaccumulate in mammalian species (oral bioavailability is low and it is rapidly metabolised and eliminated) (Certa *et al*., 1996).

3.2.9.2 Calculated BCFs

A BCF of 634 was calculated using EUSES with the log K_{ow} of 4.12 and the following TGD equation for substances with a log K_{ow} of 2-6 (this equation is based on a linear relationship in fish developed by Veith *et al.*, 1979):

$$\log BCF_{fish} = 0.85 \log K_{ow} - 0.70 - 3$$

Using a different equation (log BCF = 0.61 x log K_{ow} + 0.26), a BCF of 3291 has been estimated by McLeese *et al.* (1981; also cited in SIDS, 1994[15] and Waern, 2000). This value was estimated based on tests with a group of alkylphenols and suggests that bioaccumulation of 4-*tert*-octylphenol in aquatic organisms would occur to a significant

[15] Note that the BCF of 331 calculated in SIDS (1994) is based on a different log K_{ow} value.

extent. However, this BCF is not considered to be as reliable as that calculated using EUSES. This is because of inadequacies in the study design (e.g., it was noted that the concentrations within the tissue samples varied over a period of time within the study and there was some doubt as to their reliability).

For comparison, the mean of reliable measured BCFs (on a fresh weight basis) for nonylphenol was 741. The BCF calculated from the log Kow of 4.48 (1280) was used in the ESR risk assessment (ECB, 1999). 4-*tert*-Octylphenol has a lower log Kow value, and so would also be expected to have a lower BCF.

3.2.9.3 Summary of bioaccumulation

The limited measured data from fish studies suggest that the potential for bioaccumulation in aquatic organisms will be low to moderate. The calculated fish BCF of 634 is used in the risk assessment as a reasonable worst case. Measured levels in biota are reported in Section 3.3.4.

3.2.10 Summary of environmental fate and distribution

The available data indicate that 4-*tert*-octylphenol is of low volatility and low water solubility, and will sorb strongly to organic matter in soils, sediments and sludges. Degradation processes within these media (biotic and abiotic) are predicted to be relatively slow. If released directly to the atmosphere, degradation occurs rapidly through hydroxyl radical attack. The potential for bioaccumulation in aquatic organisms is expected to be low to moderate.

3.3 ENVIRONMENTAL CONCENTRATIONS

3.3.1 Aquatic compartment (surface water, sediment and wastewater treatment plant)

3.3.1.1 Estimated aquatic environmental concentrations

The PECs for water local to the point of release (PEC_{local}) are calculated using the environmental releases detailed in Section 3.1 and the equations set out in Chapter 3 of the TGD.

The local PEC is made up of a local water concentration (C_{local}) resulting from the relevant process emission, and a background concentration that results from emissions in the regional environment ($PEC_{regional}$). This regional PEC is itself a result of direct emissions, and indirect emissions as a consequence of biodegradation of end products such as OPEs. In the absence of specific information, sewage effluent is assumed to be diluted in river water by a factor of 10.

The PEC for sediment can be derived from the PEC_{local} for surface water using the suspended matter–water partitioning coefficient, assuming equilibrium partitioning.

PECs for WWTP have been calculated for uses that result in direct release of 4-*tert*-octylphenol. The formation of 4-*tert*-octylphenol from the ethoxylates takes place in sludge digestion, which occurs after the activated sludge processes. Although water from the sludge digestion may be discharged from the treatment plant along with that from the activated sludge plant, the WWTP micro-organisms will not be exposed to this water.

The results are presented in *Tables 3.5* and *3.6*.

Table 3.5 Local aquatic PECs

Life-cycle stage		PECs for surface water (µg/L)	PECs for sediment (mg/kg wwt)	PECs for WWTP organisms (mg/L)
Production of 4-*tert*-octylphenol		5.4	0.33	0.215
Phenol–formaldehyde resins	Resin manufacture	5.6	0.34	0.22
	Resins in rubber formulation	0.49	0.03	0.004
	Resins in varnishes	0.22	0.013	0.001
	Resins in printing inks	7.85	0.47	0.078
	Ethoxylated resin production	9.8	0.59	0.098
	Marine paint formulation	0.14	0.009	5.9×10^{-4}
	Marine paint application	8.07	0.49	0.08
	Marine paint disposal	14.3	0.86	0.14
Octylphenol ethoxylates	Production	3.2	0.20	0.39
	Formulation (textile, pesticide)	1.33	0.08	na
	Emulsion polymerisation	0.125	0.008	na
	Textiles	3.0	0.18	na
	Paint formulation	0.86	0.052	na
	Paint application	0.096	0.006	na
	Pesticide application*	0.34	0.02	na
	Veterinary medicine*	0.18	0.011	na
	Ether sulphate production	5.9	0.36	na

* Intermittent release; na – not applicable.

Table 3.6 Overall regional and continental aquatic PECs

Scale	PECs for surface water (µg/L)			PECs for sediment (mg/kg wwt)		
	Direct	Indirect	Total	Direct	Indirect	Total
Regional	0.0825	1.8×10^{-3}	0.0843	8.2×10^{-3}	1.8×10^{-4}	8.4×10^{-3}
Continental	7.2×10^{-3}	1.4×10^{-4}	7.3×10^{-3}	7.2×10^{-4}	1.4×10^{-5}	7.3×10^{-4}

Note: Indirect PECs represent the releases of 4-*tert*-octylphenol resulting from the breakdown of ethoxylates (and ether sulphates).

Note that for production of OPEs the concentrations are the combination of contributions from the release of 4-*tert*-octylphenol itself and of OPEs (with the exception of the WWTP, where only the 4-*tert*-octylphenol releases are relevant).

In Section 3.1.2.1, information is presented on the concentrations at three specific production sites (out of six). The measured values relate to effluent waters, and so are before any dilution in surface water. For site A, the concentration in surface water was also measured, but was below the same detection limit as in the wastewater and so the measurement does not provide any further information. The measurement at one site relates only to untreated effluent, which is taken for treatment off-site. The levels

measured for the other two sites were <0.2 and <1.0 µg/L (i.e., 4-*tert*-octylphenol was not detected in the effluent at either site). If the standard dilution of 40 for production sites is assumed for surface water, the local concentrations estimated would be <0.005 and <0.025 µg/L. Adding the regional PEC of 0.084 µg/L (see *Table 3.6*) leads to PEC values of <0.089 and <0.11 µg/L. These are much lower than the default value calculated for production (5.4 µg/L). The database of measurements for these sites is limited, and it would be useful to confirm these results with more extensive measurements.

A calculation based on the reported releases of OPEs from a UK site carrying out emulsion polymerisation (see Sections 3.1.1 and 3.1.3.2.2) gives a PEC value of 2.2 µg/L. Site-specific factors (e.g. the available dilution) are not taken into account in this estimate.

The concentration in wastewater from specific production sites can be used as PEC values for WWTP. The effluent concentrations are <0.2 and <1.0 µg/L, which are much lower than the calculated default values for production.

3.3.1.1.1 Pesticide application

A different approach has been used to estimate possible concentrations of 4-*tert*-octylphenol in water as a result of pesticide application. This is based on the calculations in the nonylphenol risk assessment, which made use of information supplied by Zeneca Agrochemicals in 1997 (ECB, 1999). This information is reproduced here, with references to nonylphenol replaced by 4-*tert*-octylphenol. The same assumptions are used to allow a comparison to be made, although it is recognised that more sophisticated models may be available.

As a first tier of aquatic exposure assessment, spray drift entry of pesticides, or in this case OPE, into surface waters is considered. Runoff from the soil surface and leaching are not significant sources of water contamination because OPEs and 4-*tert*-octylphenol are strongly bound to soil (see Section 3.2.6). When sprayed, some proportion of the material may drift downwind and deposit on an adjacent surface water. The conventional assumption is that application occurs from a tractor-mounted boom sprayer, and the surface water onto which drift deposits is 1 metre downwind. The drift rate is equivalent to 4% of the intended application rate in the treated field. The amounts of OPE applied to crops are typically equivalent to 50-200 g/ha, the higher rates being used as wetters, and the lower rates as emulsifiers. In a 1 metre deep water body the resulting concentration range for the ethoxylates is given by:

C_{local} for OPEs = Applied rate × (Percent drift/Water depth) = 0.2-0.8 µg/L

The OPE reaching the surface water is readily broken down by microbial activity. Assuming instant break down to 4-*tert*-octylphenol (a worst-case scenario), the PEC range for 4-*tert*-octylphenol is given by the following:

C_{local} for OP = PEC for OPE × (Mol wt of OP/Mol wt of OPE)

Taking an average ethoxylate side-chain length of 10 ethylene oxide units, 4-*tert*-octylphenol makes up 32% of the weight. Therefore:

C_{local} for OP = 0.064-0.26 µg/L

Adding the regional background concentration of 0.0825 µg/L, the PEC range is 0.15-0.34 µg/L. This estimate of the PEC is very conservative because it assumes:

- Presence of surface water 1 metre downwind of the treated area of the field;
- Instantaneous 100% conversion of the ethoxylates to 4-*tert*-octylphenol;
- The concentration is appropriate to that at the edge of a water body (for a water body of any width the average drift entry rate is less than the 4% assumed here);
- No account is taken of dilution effects in flowing water bodies.

3.3.1.1.2 Veterinary medicine use

The following calculation was provided by the UK VMD, as an initial approach to such a calculation and with a number of untested assumptions (VMD, personal communication 2003). OPEs are used in a pour-on treatment for sheep to prevent blow fly strike. The treatment is used mainly in the summer months, and has an intermittent use pattern as one dose provides protection for 10 weeks. The scenario considers runoff from the area where the sheep are treated. Information on the treatment is as follows:

Amount used per sheep	50 mL
Content of OPE in treatment	2% w/v
Number of sheep treated	100
Hard standing required	100 m^2
Fraction of treatment reaching ground at treatment site	5%

Other assumptions relating to the site of treatment are as follows. The treatment that does not remain in the sheep falls to the hard standing and dries there, where the components remain until rainfall. The rainfall producing runoff is 15 mm, the first 0.5 mm of which wets the surface but does not produce runoff (hence a net rainfall of 14.5 mm is considered). It is assumed that 80% of the runoff goes to surface water. The runoff initially goes into a ditch, where it is diluted by 5000 times. The ditch flows into the receiving surface water, where it is diluted three times.

The calculation is as follows:

Amount of OPE in treatment of 1 sheep = 50 x 0.02 = 1 g
Amount of OPE used at site = 1 x 100 = 100 g
Loss to surface = 100 x 0.05 = 5 g
Volume of runoff to ditch = 100 m^2 x 14.5 mm x 80% = 1160 L
Concentration of OPE in runoff = 5/1160 = 4.31 mg/L
Concentration of OPE in ditch = 4.31/5000 = 0.86 µg/L
Concentration in surface water = 0.86/3 = 0.29 µg/L.

This is the concentration of OPE. As a worst-case assumption, complete conversion to 4-*tert*-octylphenol, is assumed. For a 10-unit ethoxylate chain the conversion factor is 0.32, and hence the concentration of 4-*tert*-octylphenol is 0.093 µg/L. Adding the regional background concentration of 0.0825 µg/L gives a PEC of 0.18 µg/L. This is an intermittent release.

3.3.1.2 Measured aquatic environmental concentrations

Information on measured concentrations in various environmental media in European and other countries is presented below. For some studies the actual 4-*tert*-octylphenol isomer measured has not been stated. Where this is the case, the general term 'octylphenol' is used.

A variety of extraction techniques and quantification methods may be used in determining concentrations of octylphenol, the techniques used being dependent upon the type of sample being analysed. The most frequently used extraction technique for environmental samples appears to be that used for nonylphenol – steam distillation; other techniques employed are likely to include using hexane and methylene chloride as extraction solvents. Quantification of samples is usually by HPLC or gas chromatography (GC) using either UV or mass spectrometer (MS) detectors.

When analysing samples of octylphenol, the solubility in water needs to be taken into account. As with nonylphenol, octylphenol may also be adsorbed onto the surface of glassware, thereby reducing the concentration measured in solution.

3.3.1.2.1 Freshwaters, wastewater treatment and industrial effluents

United Kingdom

The Environment Agency's pollution inventory (EA, 2004a) shows three sites that are discharging octylphenol. The predicted amounts discharged from each site to controlled waters and sewer are summarised in *Table 3.1*, but no further monitoring data are available. *Table 3.7* summarises available data for England and these are discussed below.

Table 3.7 Measured concentrations of octylphenol in England, UK

Area	Year	Concentration (µg/L)		References
		Effluent	Surface water	
Yorkshire (WWTP)	1994	0.5	-	Blackburn and Waldock (1995)
South East (River Lea)	1994	-	0.4	
North East (Tees Estuary)	1994	-	13	
Thames (various rivers)	1994	<0.02-2.32	<0.02-0.43	Britnell (1995) Survey of alkylphenols in Thames Region Rivers and Sewage effluents, cited in EA (2004c)
North West	no dates	0.01-0.07	no data	Personal communication (1996) Chris Jarvis (Environment Agency), cited in EA (2004c)
South West (River Dart)	1994	<0.02-0.36	<0.02-0.12	Warhurst (1995), cited in EA (2004c)
North East (Tees and Tyne Estuaries)	1997	no data	no data	Lye *et al.* (1999)

In a survey of levels of octylphenol in the UK aquatic environment, six rivers in England and Wales were sampled at five or six sites each, together with 15 WWTP effluents and six estuaries (Blackburn and Waldock 1995). Concentrations of octylphenol were below 1 µg/L at all river sites. In this study, the highest concentration recorded in a sewage effluent was 0.5 µg/L total extractable octylphenol at a WWTP receiving wastewaters from a textile-based industrial area in Yorkshire. The highest concentrations in surface water were 0.4 µg/L in the River Lea at Leyton and 13 µg/L in the outer Tees estuary, the

only estuary in which levels were reported above the limit of detection (<0.1 µg/L). Note that at the time of the study on the Tees a manufacturing plant for OPEs was operating on the shore of the estuary. These data may represent high local concentrations resulting from 4-*tert*-octylphenol and/or OPEs discharged in effluent from that plant. Lye *et al.* (1999) measured 4-*tert*-octylphenol (and nonylphenol and nonylphenol mono-ethoxylate) concentrations in effluents from two WWTP discharging to the Tees and Tyne estuaries. Octylphenol was not detected in the effluent or receiving water samples (no information given on the limit of detection), though detectable levels of octylphenols were measured in sediment samples (see below). EA (2004c) reported that the highest level of octylphenol in effluents is 2.32 µg/L based on monitoring data in the Thames region in the UK, although levels were generally less than 1 µg/L.

The Environment Agency analysed levels of octylphenol at an outfall from a sewage works into the River Dart in Devon (UK) in the summer of 1994. This sewage works, which has secondary treatment, receives trade effluent from a wool-processing factory that used alkylphenol polyethoxylate (APE) surfactants. Levels of octylphenol in the sewage outfall were found to be up to 0.36 µg/L. The maximum concentration found in the mixing zone of the river downstream from the sewage outfall was 0.12 µg/L octylphenol. Since this survey the use of APE at the factory has been discontinued (Warhurst (1995) cited in EA (2004c)). The Environment Agency North West region (NCEDS, 1999) carried out monitoring for octylphenol, over a period of several months, on the trade effluent from a production plant (which has subsequently closed), the effluent from a WWTP and receiving waters on the Wyre peninsula. In all of the surface water samples (n = 108) from the Manchester Ship Canal the concentration of 4-*tert*-octylphenol was below the limit of detection (0.2 µg/L) except one where it was measured at 0.38 µg/L. In all of the WWTP effluent samples (n = 21) it was below the limit of detection (0.2 or 1.0 µg/L). In all of the Wyre estuary samples (n = 6) except one, 4-*tert*-octylphenol was below the limit of detection (0.2 µg/L) and was only marginally above at 0.22 µg/L when present. In the trade effluent samples (n = 19), the concentration ranged from limit of detection (4-40 µg/L) to 1690 µg/L.

The Scottish Environmental Protection Agency (SEPA, 1997) found that levels of 4-*tert*-octylphenol being discharged in effluents from seven WWTP into rivers were generally <3.3 µg/L, while those of nonylphenols ranged between <0.4 and 12.9 µg/L.

A single sample of a trade effluent collected from an octylphenol production site in the UK in 2002 has been analysed (personal communication, Schenectady, 2002) for a series of alkylphenols including octylphenol. The method used was acidification of the sample followed by solvent extraction with di-isopropyl ether and quantification by capillary GCMS. The concentration of 4-*tert*-octylphenol found in the trade effluent was 10.8 mg/L. The octylphenol is used on-site to produce phenol–formaldehyde resins and the trade effluent is tankered off site to a WWTP.

Blackburn *et al.* (1999) investigated concentrations of degradation products of alkylphenol polyethoxylates (including octylphenol) in samples of British waters collected in 1994 and 1995. In addition a series of samples of tissues of wild fish from the River Aire, Yorkshire, and from laboratory dosing experiments were analysed for alkylphenols to assess the degree of bioaccumulation of these compounds. In rivers octylphenol concentrations were <0.5 µg/L at all sites.

As part of a scoping exercise for a larger monitoring project, the Environment Agency analysed water samples taken from a number of locations for OPEs in 2003. A separate report is in preparation that will summarise the results and their implication in terms of possible risk.

Germany

Samples of trade effluents collected from an alkylphenol production site in Germany and samples of the receiving river were analysed for nonylphenol in 1997 (ECB, 1999) and showed that concentrations were below the limit of detection (0.2 µg/L). The site also manufactures 4-*tert*-octylphenol and, although 4-*tert*-octylphenol was not specifically determined, the analysis method (steam distillation followed by GCMS) was able to detect 4-*tert*-octylphenol. The limit of detection was probably similar to that for nonylphenol and there was no evidence of 4-*tert*-octylphenol in any of the samples analysed (CEPAD, 2002).

Several studies determined levels of octylphenol in Germany (*Table 3.8*). A large number of samples taken from WWTP in Berlin over the 1996/1997 period (Fromme, 1998) were analysed for octylphenol. In most of the influent samples, octylphenol was below the detection limit of 0.05 µg/L, but in the effluent samples concentrations ranged from <0.03 to 0.43 µg/L, almost certainly as the result of biological degradation of OPEs present in the influent water of the WWTP.

Table 3.8a Concentration of octylphenol in Berlin WWTP samples (water)

Sample type	Year	Limit of detection (LOD, µg/L)	No. of samples	No. below LOD	Concentration, µg/L	
					Median	Maximum
Influent	1996	0.03	84	74	-	-
Influent	1997	0.05	65	56	-	0.272
Effluent	1996/1997	0.03	18	4	0.088	0.112
Effluent	1997	0.05	12	4	0.08	0.43

Table 3.8b Concentration of octylphenol in Berlin WWTP samples (suspended sediment)

Year	Limit of detection (LOD, mg/kg)	No. of samples	No. below LOD
1996	0.01	59	59
1997	0.01	23	23

An extensive study of concentrations of 4-*tert*-octylphenol in the River Elbe and selected tributaries and in the German Bight have also been reported (Working Group for Cleanliness of the Elbe, 2000). The concentration of alkylphenols in the water samples studied (excluding communal and industrial discharges) ranged from the detection threshold to 0.22 µg/L. The average concentration for 4-*tert*-octylphenol was 0.0033 µg/L. No further details were available.

The distribution of 4-*tert*-octylphenol in the River Elbe was investigated by Stachel *et al*. (2003). Samples were taken from 10 sites along the course of the river in July 2000, from the border with the Czech Republic downstream. Samples were also taken from the mouths of tributaries to the Elbe. 4-*tert*-Octylphenol was found in all but one of the water samples from the Elbe, at concentrations from <0.5 ng/L to 5 ng/L. Concentrations

were generally higher toward the upstream end of the river, and were higher in the tributaries.

A study has been undertaken on the River Weser (Working Group for the Cleanliness of the River Weser, 2000) and other German rivers (*Table 3.9*). The concentrations of 4-*tert*-octylphenol in the river waters ranged from less than 0.0019 µg/L to 0.101 µg/L, but in most samples the concentration was below 0.025 µg/L.

Samples from two rivers in Germany – the Lockwitzbach and the Korsch – were analysed for octylphenol (Nagel, 2000). Concentrations of 0.008 and 0.105 µg/L were reported but no further information is available.

Table 3.9 Concentrations of octylphenol in German rivers*

River	Location	Year	Limit of detection (LOD, ng/L)	No. of samples	Concentration, ng/L		
					Min.	Median	Max.
Elbe		1998	0.05	10	0.4	0.9	1.3
Saale		1998	0.05	1		2	
Mulde		1998	0.05	1		2	
Schwarze Elster		1998	0.05	1		1	
Weiße Elster†		1998	0.05	6	1.5	3.2	6
Werra	Gerstungen	1999		4	6.9		13
Fulda	Wahnhausen	1999		4	<5		19
Weser	Hemeln	1999		4	<1.9		15
Weser	Porta	1999		4	<1.9		22
Weser	Verden	1999		4	1.9		101
Weser	Hemelingen	1999		4	2.4		51.5

* UBA data from the Working groups on the Cleanliness of the River Elbe and Weser.
† Saalenebenfluß.

Kuch and Ballschmiter (2001) sampled effluents from three municipal WWTP in Southern Germany in June to October 2000. Samples were also taken from the watercourses receiving the effluents from these WWTP, and from waters not receiving any wastewater input (run-off from the Alps). Streams flowing into a drinking water reservoir were also sampled. Only summary results were reported. The mean concentration in WWTP effluent was 22 ng/L (range 2.2-73). The mean concentration in surface waters was 7.2 ng/L (range 0.8-54). The authors also sampled drinking water from surface water and groundwater sources, the mean level being 2.0 ng/L (range 0.2-4.9). The authors reported that they were able to detect 4-*tert*-octylphenol in the bi-distilled or reverse osmosis water that they used, at a level of 200-400 pg/L.

Austria

An Austrian monitoring study for 4-*tert*-octylphenol in surface water (Austrian Umweltbundesamt, 2001) analysed 30 samples in 1998. 4-*tert*-Octylphenol was below the detection limit in all but one sample, in which a concentration of 240 ng/L was reported. A monitoring study of influent and effluent waters of a WWTP in Vienna in 2000 was also reported (Hohenblum *et al*., 2000). This study showed median concentrations of <50-106 ng/L in filtered and 440 ng/L in unfiltered influent samples, and 91-196 ng/L in filtered and 49 ng/L in unfiltered effluent samples.

Hohenblum *et al*. (2004) measured the levels of substances in water samples taken from various locations in Austria. Surface water samples were taken from 27 selected locations on between six and 12 occasions; 59 groundwater sites were sampled up to three times each; and eight springs were sampled twice. In total, 414 samples were taken in 2001, spread across the country. All sites were close to populated, industrial or agricultural areas. Octylphenol was included in the analyses, described as CAS No. 27193-28-8 (the structure included in the article does not indicate the detailed structure of the octyl group in the 4-position). Also included were the OPEs with one and two ethoxylate groups (OP1EO and OP2EO, respectively). Octylphenol was detected in only one of the 261 surface water samples, at 41 ng/L; OP1EO was present in two samples at 20 ng/L and OP2EO in one sample at 11 ng/L. (For comparison, nonylphenol was present in around half of the samples.)

Belgium

The 4-*tert*-octylphenol production site in Belgium uses activated carbon to remove organics from the wastewater prior to discharge to a municipal WWTP using biological treatment. In 1996, samples of the production plant wastewater from the influent to and effluent from activated carbon were taken on three occasions and analysed for 4-*tert*-octylphenol. The results are given in *Table 3.10*.

Table 3.10 Measured levels at Belgian production site

Date	Influent of octylphenol into activated carbon WWTP (µg/L)	Discharge of octylphenol into municipal sewer (µg/L)
Week 1 March 1996	41	<1
Week 2 March 1996	47	<1
Week 3 March 1996	<1	<1

On all three occasions, the concentration of 4-*tert*-octylphenol in the plant effluent after activated carbon treatment was less than the limit of detection of the analytical method.

France

Fenet *et al*. (2003) measured 4-*tert*-octylphenol (and nonylphenol) at four sites in the Seine basin in France downstream of Paris. The four sites were of different types: predominantly agricultural, heavily industrial, influenced by WWTPs from an urban area and a rural area. Samples of surface water and sediment were taken. The quantification limit (LQ) in water was 0.001 µg/L. Water samples were taken in September and November 1999 and in February 2000. Levels at the agricultural site were 0.002-0.004 µg/L, at the industrial site <LQ-0.077 µg/L, at the urban site 0.012-0.070 µg/L and at the rural site <LQ-0.007 µg/L.

The Netherlands

Data are available from a study called 'Endocrine-disrupting compounds in water systems: A pilot study of the occurrence of oestrogenic compounds in surface and wastewaters in the Netherlands' that was carried out during 1997/1998 (RIZA/RIKZ, 1999). OPEs and octylphenol were measured in sewage sludge from municipal WWTPs at concentrations up to 28 µg/L and up to 2 µg/L, respectively. The corresponding levels for NPEs and nonylphenols were 0.7-880 µg/L and up to 125 µg/L, respectively, in

sewage sludge. OPEs and octylphenol concentration in sewage sludge in industrial WWTPs in the Netherlands were measured in concentrations up to 50 µg/L and up to 24 µg/L, respectively. The corresponding levels for NPEs and nonylphenols were 2400 µg/L and up to 2500 µg/L, respectively, in sewage sludge.

A subsequent, more elaborate measurement programme showed a generally (much) lower presence of 4-*tert*-octylphenol and OPE (RIZA/RIKZ, 2002). The highest 4-*tert*-octylphenol concentration found in municipal effluent was 1.3 µg/L. OPE was not found at all (detection limit 0.7 µg/L). In sewage sludge neither 4-*tert*-octylphenol nor OPE were detected, with one exception (a 4-*tert*-octylphenol concentration of 1.5 mg/kg dw). In the investigated surface waters and sediments neither 4-*tert*-octylphenol nor OPE were detected.

Denmark

Information from the Danish Ministry of the Environment (1997) indicated that surface water concentrations of alkylphenols were <0.2 µg/L. No more data were available regarding location or source.

Spain

Petrovic *et al.* (2002a) collected samples of water from coastal locations around the coast of Spain. The sites were chosen as potential 'hot-spots' for the discharge of untreated wastewater or sewage. The analysis involved solid phase extraction of samples, followed by liquid chromatography and MS (LCMS). A total of 14 water samples were analysed for a range of substances, including 4-*tert*-octylphenol. This substance was not detected (detection limit 0.15 µg/L). The locations were not related to specific sources by the authors of the article.

Petrovic *et al.* (2002b) measured the concentration of 4-*tert*-octylphenol and OPEs in samples from four WWTPs in Spain. Two of the plants (STP 1 and 3) had low level industrial wastewater input, site STP 2 received a combination of textile and tannery wastewater and municipal water, and STP 4 received domestic wastewater and wastewater from the rubber industry. The analytical method was the same as that used in Petrovic *et al.* (2002a).

4-*tert*-Octylphenol was not detected in either the influent or the effluent of sites STP 1 and 3 (detection limit 0.1 µg/L). At site STP 2, the concentration in the influent was 1-4.3 µg/L, that in the effluent was 2.4-22 µg/L, while the sludge contained 0.7-0.8 mg/kg. At site STP 4 the influent contained <0.1-2.8 µg/L, while the effluent contained <0.1µg/L, and the sludge contained 5.8-8.0 mg/kg. No specific measurements were made for 4-*tert*-octylphenol in receiving waters.

Ethoxylates with ethoxylate chains of two to 15 ethoxy units were included in the reported concentrations. These were: STP 1, in 24-45 µg/L, out <0.05 µg/L; STP 2, in 60-84 µg/L, out <0.05 µg/L; STP 3, in 7.5-10 µg/L, out <0.05 µg/L; STP 4, in 20-32 µg/L, out <0.05-1.5 µg/L.

Other European countries

The Pristine Project (which is concerned with the investigation of priority surfactants and their toxic metabolites in waste effluent discharges) has produced some information on 4-*tert*-octylphenol concentrations in surface waters and effluents in a number of European countries (Pristine, 2000). 4-*tert*-Octylphenol concentrations in surface waters and effluents from municipal and industrial wastewaters were generally below the limit of detection, but in certain effluents concentrations of 0.2 µg/L and below were reported.

Espejo *et al.* (2002) analysed samples of municipal wastewater from Geneva for nineteen alkylphenol substances. The samples were taken from one WWTP after completed primary treatment, and sampling (15 samples) took place from October to December 2001. The average concentration of 4-*tert*-octylphenol was 0.204 µg/L, with the range of concentrations 0.167-0.301 µg/L.

Summary

Information on the presence of octylphenols is limited. Reported concentrations of octylphenols (and 4-*tert*-octylphenol specifically) in surface waters are generally lower than those for nonylphenol. Based on the available data, it appears that levels of octylphenols in surface waters are typically less than 1 µg/L. Higher values have been detected on a few occasions (e.g., 13 µg/L in the Tees Estuary in the UK). The causes of these higher values are unknown, but they may be a consequence of high local discharges.

3.3.1.2.2 Sewage sludge

Results from the Pristine Project have shown octylphenol concentrations in the sewage sludge of various municipal wastewaters in a number of European countries to range from below the limit of detection (not provided) to 2 mg/kg dw. The range in industrial wastewaters was from below the limit of detection (not provided) to 24 mg/kg dw (Pristine, 2000).

As part of a study on the effects of sludge application to soils, Rhind *et al.* (2002) measured the concentration of octylphenol (structure not specified) in liquid sludge. The mean concentration found was 0.277 ± 0.14 mg/kg. The source of the sludge was not described in the article.

3.3.1.2.3 Marine waters

There are no reported measured concentrations in marine or estuarine waters (except one value for the Tees estuary (UK) and a study in the German Bight, reported above).

3.3.1.2.4 Groundwater

Hohenblum *et al*. (2004) measured the levels of substances in spring water and groundwater samples taken from various locations in Austria (see Section 3.3.1.2.1 for more details of the study). Eight springs were sampled twice and 59 groundwater sites were sampled up to three times each. Octylphenol was detected in five groundwater

samples from the total of 112, with a maximum concentration of 42 ng/L. The two ethoxylates were not detected.

Latorre *et al.* (2003) took samples of surface and groundwater from locations in an agricultural area of northeastern Spain, with intensive crop production (corn and wheat) and with high industrial activity in the surrounding area. Two surface water and twelve groundwater samples were taken in autumn 2000. The groundwater samples were taken from wells and related to two different aquifers. All samples (with the exception of one groundwater sample) contained detectable levels of 4-*tert*-octylphenol; all concentrations were less than 0.1 µg/L with the exception of one result of 1.5 µg/L in groundwater.

3.3.1.2.5 Sediment

Results from the Pristine Project have shown 4-*tert*-octylphenol concentrations in sediments and suspended matter in surface waters for a number of European countries to be below the limit of detection (not provided) (Pristine 2000).

UK

Lye *et al.* (1999) measured 4-*tert-octyl*phenol (and nonylphenol and nonylphenol monoethoxylate) concentrations in sediments taken from a series of locations from the Tees and Tyne estuaries which were impacted to varying degrees by industrial and WWTP discharges. 4-*tert*-Octylphenol was detected in the range 0.03-0.34 mg/kg dw in the Tees estuary and 0.002-0.02 mg/kg in the Tyne estuary.

Archived samples of sediment (n = 50) taken from sites around the UK (originally collected for a project examining releases of chlorinated paraffins) were analysed for total branched 4-octylphenol and total branched 4-nonylphenol using solvent extraction and LCMS (Defra, 2002b). Limits of detection, ranged from 0.2 to 1 parts per billion (ppb) wwt. Total branched 4-octylphenol was not detected in any of the samples, although total branched 4-nonylphenol was detected in 56% of the samples with a concentration range of 0.66-3430 ppb dw with a mean of 191.13 ppb. Since the sample collection was not specifically linked to octylphenol emissions, the absence of total branched 4-octylphenol may be of limited significance.

Germany

The concentrations of octylphenol in suspended and bed sediments of German rivers have been reported (German UBA, 2002; see *Table 3.11*). These ranged from below the limit of detection (2 µg/kg dry matter) to 294 µg/kg dry matter, but most samples were <10 µg/kg dry matter.

Table 3.11 Concentrations of octylphenol in German river bed and suspended sediments

River	Location	Year	No. of samples	Concentration, µg/kg dry matter	
				Minimum	Maximum
Werra	Gerstungen	1999	4	<2	294
Fulda	Wahnhausen	1999	4	<2	6
Weser	Hemeln	1999	4	<2	17
Weser	Porta	1999	4	<2	<10
Weser	Verden	1999	4	<2	<10
Weser	Hemelingen	1999	4	<2	<10

In their investigation of the levels of 4-*tert*-octylphenol in the River Elbe in Germany, Stachel *et al*. (2003) took composite samples of freshly deposited sediment from the same locations as the water samples (see Section 3.3.1.2.1). Samples were taken from 10 sites along the course of the river in July 2000, from the border with the Czech Republic downstream. 4-*tert*-Octylphenol was found in all of the sediment samples, with the highest level being 62 µg/kg dm (at the same location as the highest water level). As with the water concentrations, the concentrations were higher at the upstream end of the river.

Spain

Petrovic *et al.* (2002a) collected samples of sediment from coastal locations around the coast of Spain. The sites were chosen as potential 'hot-spots' for the discharge of untreated wastewater or sewage. The analysis involved solid phase extraction of samples, followed by LCMS. A total of 26 sediment samples were analysed for a range of substances, including 4-*tert*-octylphenol. This substance was only detected in three of the samples, at concentrations of 145, 21 and 17 µg/kg (detection limit 10 µg/kg). The locations were not related to specific sources by the authors of the article.

France

Fenet *et al*. (2003) measured 4-*tert*-octylphenol (and nonylphenol) at four sites in the Seine basin in France downstream of Paris. The four sites were of different types: predominantly agricultural, heavily industrial, influenced by WWTPs from an urban area and a rural area. Samples of surface water and sediment were taken. The LQ in sediment was 0.001 µg/g. Sediment samples were taken in November 1999. The levels in sediment were rural site 0.012 µg/L, agricultural site 0.001 µg/g, industrial site 0.005 µg/g and urban site 0.491 µg/g.

3.3.1.2.6 Measurements outside Europe

Measured concentrations have been located for the USA, Canada and Japan.

North America

Two octylphenol isomers were identified at a level of 5000 µg/kg (wwt) in a river sediment sample downstream of a chemical manufacturing plant on an unidentified US river. The isomers were present in the wastewater of the plant at between 1-75 µg/L, but not in the downstream water (Jungclaus *et al.*, 1978).

Bennett and Metcalfe (1998) found mean concentrations of 4--*tert*-octylphenol ranged from non-detectable to up to 23.7 mg/kg (dry weight) in sediments with the highest concentrations found near WWTP and industrial wastewater discharges in the Great Lakes. However, they concluded that concentrations of 4-*tert*-octylphenol greater than 1 mg/kg (dry weight) were not widespread throughout the lower Great Lakes. The study also reported concentrations of up to 21 mg/kg (dry weight) in sewage sludge.

Bennett and Metcalfe (2000) investigated the concentration of degradation products of alkylphenol ethoxylates such as octylphenol in sediments and water downstream of

WWTP in the USA and also their potential to bioaccumulate in mussels deployed at sites at or downstream of the WWTP. The analytical data for sediment samples indicated that concentrations of octylphenol varied between 0.005 and 1.70 mg/kg dw, with the concentration declining as the distance from the outflows of the WWTP increased. For deployed mussels, octylphenol concentration ranged from non-detectable to 0.01 mg/kg wwt. The highest octylphenol concentrations were found at sites located directly in the WWTP discharges.

The study showed that concentration of the degradation products of APEs such as 4-*tert*-octylphenol declined to near background levels (presumed to be µg/kg, although not stated) in sediments, the water column and biota at stations about 1 km downstream of the discharge.

Hale *et al.* (2000) reported an investigation of nonylphenol in sediments and effluents associated with diverse wastewater outfalls in the USA. They reported that mixtures of octylphenol and decylphenols were generally found associated with nonylphenols, although their contributions were minor. 4-*tert*-Octylphenol was detected in sediment near a WWTP that had ceased working in the 1970s at a concentration of 8220 µg/kg.

La Guardia *et al.* (2001) analysed freeze-dried samples of sewage sludge (biosolids) samples from eleven US WWTP for octylphenol by accelerated solvent extraction, size exclusion and silica gel chromatographic clean-up and GCMS analysis. Concentrations of octylphenol ranged from below the limit of detection (0.5 mg/kg) to 12.6 mg/kg. In the same samples, nonylphenols ranged from 5.4 to 887 mg/kg.

The occurrence of 4-*tert*-octylphenol has been investigated in samples related to a drinking water treatment facility in the USA (Stackelberg *et al*., 2004). The facility is in a heavily populated, heavily urbanised basin, with over 50 WWTP discharging effluent to the two streams from which the plant draws its raw water (or to their tributaries). The treatment plant treats 62 million gallons (~235,000 m^3) per day. The water is treated by coarse filtering, addition of powdered activated carbon, pH adjustment, coagulation, primary disinfection with sodium hypochlorite, sedimentation, filtration, secondary disinfection with sodium hypochlorite to give a chlorine residual and addition of caustic to give a pH in the range 7.8 to 8.2. Unfiltered water samples were taken from within the plant at various locations. The timing of the sampling did not completely allow for the expected retention times in the various sections. Samples were taken under low-flow conditions. Twelve samples of stream and raw water were taken, and 4-*tert*-octylphenol was detected in only one, the others being below the detection limit of 1 µg/L. The substance was not detected in finished water, at the same detection limit. Mono- and di-ethoxylates of 4-*tert*-octylphenol were not detected in any samples (same detection limit).

Industrial wastewater samples from the Toronto area were analysed for a range of compounds including octylphenol (Lee *et al.*, 2002). Analysis was by GCMS; samples were filtered, followed by solid-phase extraction at pH 3, and derivatisation was with pentafluoropropionic acid anhydride (PFPA). 4-*n*-Octylphenol was used as a surrogate standard. The wastewater samples were taken from 40 facilities, divided into 10 industry classes. Levels were generally low, with the exception of samples collected from the chemical and chemical products industries. The results of the study are presented in *Table 3.12*.

Table 3.12 Levels of octylphenol in industry wastewater samples from Toronto

Industry	Minimum (µg/L)	Mean (µg/L)	Maximum (µg/L)	No of samples	Number below LOD (0.01 µg/L)
Chemical and chemical products	<0.01	0.72	195.1	37	2
Commercial laundries	0.06	0.41	1.53	17	0
Textile products/clothing	0.08	0.11	0.47	10	0
Fabricated metal products	<0.01	0.09	4.54	11	2
Paper and allied products	0.09	0.28	39.05	8	0
Plastics processing	<0.01	0.04	0.18	9	3

Note: LOD = limit of detection.

Lee and Peart (1995, cited in EA, 2004c) measured 4-*tert*-octylphenol in four WWTP effluents in Canada. Concentrations ranged from 0.12-1.7 µg/L with the majority of samples (10 of 13) containing concentrations below 1 µg/L. Octylphenol was also measured in nine sludge samples taken from two Canadian WWTP. Levels of 9200 µg/kg and 12,100 µg/kg were reported. In the same study, 4-*tert*-octylphenol sediment levels were <5, 6, 28, 70 and 400 µg/kg. In one sludge–sediment mix, levels of 910 µg/kg were reported.

Lee and Peart (2002) investigated the levels of 4-*tert*-octylphenol in sewage sludges collected from cities across Canada. Samples were taken from primary sedimentation tanks and from secondary clarifiers (raw and digested sludge, respectively). Samples were extracted by supercritical carbon dioxide, phenols were converted into their acetyl derivatives and determined by GCMS. 4-*n*-Octylphenol was used as a surrogate internal standard. 4-*tert*-Octylphenol was detected in all 35 of the sludge samples analysed, at concentrations ranging from 0.8 to 43.9 mg/kg with a mean level of 7.2 mg/kg. These levels were much lower than those of nonylphenol in the same samples, with the nonylphenol:octylphenol ratio being >10 in most samples. The authors commented that the probable sources of the 4-*tert*-octylphenol were the anaerobic degradation of OPEs, which are present as minor components in NPEs, or wastewater from a few speciality chemical or chemical product industries. They noted that the level of nonylphenol increased from the raw to the digested sludge where samples were taken at the same time, and commented that this was consistent with degradation of the NPEs to nonylphenol. They made no comment on the octylphenol data, but a comparison of the data shows a similar increase between raw and digested sludges.

Lee *et al*. (2004) measured the levels of 4-*tert*-octylphenol in samples of influent and effluent waters from Toronto. Samples were taken from four WWTP in the city of Toronto area, using auto samplers to collect hourly samples and then combining them in proportion to the flow rate to give 24-hour composite samples (15 in total, taken in 1999-2000). All four sites have primary and secondary treatment, all chlorinate their effluent and all but one have anaerobic sludge digesters. The median concentrations with ranges were influent 1.05 µg/L (range 0.34-2.84) and effluent 0.14 µg/L (range 0.04-0.68). Concentrations were also measured in the raw and digested sludges; the median raw sludge level was 3.7 µg/g (range 0.78-13.8) and the median digested sludge level was 6.7 µg/g (range 1.9-13.4). Concentrations of the carboxylic acid breakdown product from OPEs (OP1EC, see Appendix 1 for explanation of the code) were also measured in the influent and effluent; median concentrations were 0.14 µg/L and 1.91 µg/L, respectively.

Japan

Tsuda *et al.* (2000) performed surveys of nonylphenol and 4-*tert*-octylphenol in water samples obtained from eight rivers flowing into Lake Biwa every 2 months from April 1998 to March 1999. 4-*tert*-Octylphenol was detected at lower concentrations than nonylphenol (not detected – limit of detection = 0.01 µg/L – to approximately 0.09 µg/L) and at lower frequency than nonylphenol (i.e., 23 of 48 samples for 4-*tert*-octylphenol compared to 48 of 48 for nonylphenol).

Isobe and Takada (2004) measured the concentrations of 4-*tert*-octylphenol at two WWTP in the Tokyo area. Samples were taken as time weighted 24-hour samples, in the influent, primary effluent, secondary effluent and final effluent. The concentrations in the influent were 0.088 and 0.03 µg/L, in the primary effluent 0.068 and 0.024 µg/L, in the secondary effluent both 0.004 µg/L and the same in the final effluent (close to the quantitation limit of 0.003 µg/L). The removal of 4-*tert*-octylphenol was 94% and 85% at the two sites.

Isobe and Takada (2004) also measured the concentrations of 4-*tert*-octylphenol at 17 sites in the Tokyo area. One site had a concentration of 0.118 µg/L, one had 0.035 µg/L, six sites had levels that ranged from 0.003 to 0.005 µg/L and the remaining nine were below the detection limit of 0.003 µg/L. Surface sediments at five sites had levels from 0.005 to 0.025 µg/g dw, the substance was not detected at two others.

3.3.1.2.7 Comparison between European and non-European data

While a number of studies have investigated the concentration of 4-*tert*-octylphenol in the surface water of various EU countries, fewer data are available for river sediments and sewage sludge. In addition, most of these studies are still in progress (e.g., Pristine) and final data or full study details are not yet currently available. Those that are available indicate that concentrations in surface waters are typically less than 0.1 µg/L and either below the limit of detection or in the low µg/kg range for sediments and sewage. However, in most cases it is not known whether the studies have been targeted at known discharges of 4-*tert*-octylphenol.

Information is available on 4-*tert*-octylphenol concentrations in sediment and sewage sludge for countries outside the EU, particularly the USA and Canada. A number of studies have reported very high concentrations, but these appear to have targeted areas where, because of previous industry (e.g., chemical manufacture), high levels could be expected. More general studies show that concentrations in effluents and sediments are in a range similar to that reported in EU countries.

Note that the use of OPEs might be more widespread in North America because of the lower price of the feedstock compared to Europe.

One study (Bennett and Metcalfe, 1998) implied that differences in sewage treatment processes between the USA and the EU may mean that sewage treatment in the USA may be more efficient than that in Europe at removing alkylphenols. However, no further data were located to confirm this assumption and the discussion in Appendix 1 on the formation of 4-*tert*-octylphenol from OPEs in WWTP uses all of the available data to derive values for the fate of OPEs.

3.3.1.2.8 Comparison of measured and estimated aquatic concentrations

The monitoring database is relatively small. In general, it is not possible to relate the reported measured environmental concentrations to specific uses of 4-*tert*-octylphenol. It is therefore difficult to make a valid comparison with the aquatic PECs that have been estimated for individual life-cycle stages. In addition, some of the measurements refer to octylphenol without specifying the isomer(s) involved. It is possible that some of these relate to substances that are impurities in other alkylphenols (especially nonylphenol – see Section 2.3).

The calculated regional concentration (0.084 µg/L) is of a similar order to the maximum measured values in most of the surface waters sampled. The majority of the measured values are, however, significantly lower than those predicted using modelling. The large difference might suggest that 4-*tert*-octylphenol is not as persistent as assumed in this assessment, or that emissions are lower than predicted. As a consequence of the limited scope of the monitoring data, the modelled PECs (based on a number of assumptions) are used for risk characterisation. It is recognised that they are likely to be conservative – and possibly unrealistic – which is considered further in the risk characterisation (Section 5).

3.3.2 Terrestrial compartment

3.3.2.1 Estimated soil concentrations

The TGD method takes into account direct releases to soil, application of sewage sludge containing the chemical and atmospheric deposition. For 4-*tert*-octylphenol no direct releases to soil are expected. Concentrations caused by atmospheric deposition are expected to be negligible because of the small amounts released to air and the atmospheric behaviour of 4-*tert*-octylphenol. The calculated soil concentrations therefore result principally from the application of sewage sludge (this is to be expected when the behaviour of 4-*tert*-octylphenol in WWTP is considered – see Section 3.2.5).

For the use of OPEs, a concentration of 4-*tert*-octylphenol in sewage sludge resulting from the breakdown of the ethoxylates in the WWTP is calculated according to Appendix 1, and this is used to estimate concentrations of 4-*tert*-octylphenol in soil. There are two exceptions to this, where direct release of OPEs to soil is possible.

For the use of OPEs in pesticide formulations, the scenario in Section 3.3.1.1.1 is used, with 96% of the applied formulation reaching the soil. This is treated in the same way as a single application of sludge in the standard TGD method. As a worst case, complete conversion of the applied OPE into 4-*tert*-octylphenol is assumed.

The second exception relates to the use in veterinary medicines. The treatment of sheep with a veterinary medicine that contains OPE is described in Section 3.3.1.1.2 Following this treatment, the sheep may go back into the fields to graze. The components of the treatment may be absorbed into the animals or washed from the surface (or possibly degraded). As a worst case, it is assumed that all of the OPE applied to the sheep in the treatment is lost to soil. The receiving soil is taken as grassland, as in the TGD. From Section 3.3.1.1.2, the amount applied per sheep is 1 g, of which 5% is lost at treatment,

and thus each sheep has 0.95 g of OPE. The grazing density for lowland sheep is 15 sheep per hectare (VMD, personal communication, 2003). Treating the loss from sheep as an application of substance to soil, the application rate would be 14.25 g/ha (1.4 mg/m^2). Using the TGD methods gives an initial concentration of 8.4 µg/kg wwt (considering the worst case of all of the substance being lost to soil at one time). The concentration averaged over 30 days is 8.1 µg/kg. This is as OPE; assuming complete conversion to 4-*tert*-octylphenol gives a concentration of 2.6 µg/kg wwt. Values for use in the secondary poisoning assessment have also been calculated and are included in *Table 3.14*,

Three different soil PECs are calculated depending on the protection goal: soil (PEC_{soil}), agricultural soil ($PEC_{agr,soil}$) and grassland ($PEC_{grassland}$). These vary in terms of the depth of soil considered and the duration and/or route of exposure. The 30-day average for soil represents the PECs for soil organisms, while the 180-day averages for agricultural and grassland are used to estimate exposure of animals and humans through the food chain.

At the regional level the soil concentration in unpolluted or 'natural' soil must be used as the background concentration, to avoid double counting of application through sludge. Regional and continental PECs for 4-*tert*-octylphenol are provided in *Table 3.13*. The PECs for the terrestrial compartment are given in *Table 3.14*.

Table 3.13 Overall regional and continental terrestrial PECs ('indirect' PECs arise from the breakdown of ethoxylates)

Scale	Soil type	PECs (mg/kg wwt)		
		Direct	Indirect	Total
Regional	Natural	1.1×10^{-5}	4.22×10^{-7}	1.14×10^{-5}
Continental	Natural	4.3×10^{-7}	1.4×10^{-8}	4.4×10^{-7}

Table 3.14 Local terrestrial PECs

Life-cycle stage		PEC for soil (mg/kg wwt)	PECs agricultural soil (180 days) (mg/kg wwt)	PECs grassland (180 days) (mg/kg wwt)	PEC porewater (agricultural soil) (µg/L)	PEC porewater (grassland) (µg/L)
Production of 4-*tert*-octylphenol		0.45	0.38	0.15	7.85	3.1
Phenol–formaldehyde resins	Resin manufacture	0.47	0.40	0.16	8.15	3.22
	Resins in rubber formulation	0.009	0.007	0.003	0.15	0.07
	Resins in varnishes	0.004	0.003	0.003	0.07	0.06
	Resins in printing inks	0.16	0.14	0.055	2.85	1.13
	Ethoxylated resin production	0.20	0.17	0.067	3.56	1.41
	Marine paint formulation	0.001	0.001	0.0004	0.022	0.009
	Marine paint application	0.17	0.14	0.056	2.93	1.16
	Marine paint disposal	0.30	0.25	0.10	5.22	2.06
Octylphenol ethoxylates	Production	0.26	0.22	0.085	4.45	1.76
	Formulation (textile, pesticide)	0.23	0.20	0.077	4.03	1.59
	Emulsion polymerisation	0.02	0.017	0.007	0.35	0.14
	Textiles	1.43	1.21	0.48	24.9	9.82
	Paint formulation	0.38	0.32	0.13	6.63	2.62
	Paint application	0.006	0.005	0.002	0.11	0.042
	Pesticide application	0.029	0.024	-	4.9×10^{-4}	-
	Veterinary medicine	0.003	-	0.002	-	0.045
	Ether sulphate production	2.84	2.4	0.95	49.5	19.5

3.3.2.2 Measured soil environmental concentrations

Rhind *et al.* (2002) reported a study in which liquid sewage sludge was applied to pasture soil. Sludge was applied to three plots, in spring and in summer over a period of 2.5 years (with three summer applications and two spring applications). The application rate was 2.25 tonnes dry matter per hectare. Control plots were treated with inorganic fertiliser containing an equivalent amount of nitrogen to that applied to the test plots. At between 69 and 91 days after each application, 15 soil samples were collected from one-half of each plot. Sludge samples were analysed on delivery of the sludge. Extraction for both soils and sludge was by dichloromethane, with recovery of 86 ± 7% for soil, and analysis was by GCMS.

The mean concentration of octylphenol (no specific structure indicated) in the sludge was 0.277 mg/kg. (For comparison, the calculated levels in sludge from EUSES range from 0.6 to 1430 mg/kg.) The source of the sludge was not described. Levels in soil samples were generally below the limit of detection (0.01 mg/kg). The study also looked at nonylphenol. The authors concluded that the application of sludge to soil does not increase the soil concentrations of alkylphenols.

The limited amount of measured data for soils means it is not possible to compare the estimated PECs with measured concentrations.

3.3.3 Atmospheric compartment

Considering the low vapour pressure of 4-*tert*-octylphenol, its rapid reaction with hydroxyl radicals and its tendency to adsorb to soils and sediments, it can be expected that atmospheric concentrations will be low. PECs for the air compartment have been estimated for each use pattern using EUSES and are in the order of µg/m^3 at most (most scenarios give concentrations substantially lower than this). Indirect releases from WWTP that treat octylphenol-containing wastes are virtually zero.

One report of levels of 4-*tert*-octylphenol in air has been located. Saito *et al*. (2004) investigated the levels of 4-*tert*-octylphenol in air in Tokyo. Air samples were taken from 90 rooms in 45 houses, from 38 offices in 19 buildings and from 33 outdoor locations in Tokyo between July 2001 and March 2002. The outdoor locations were near to the buildings where the indoor samples were taken. A quartz-fibre filter and solid-phase extraction disc were used in series, with extracts from these being combined. The detection limit for 4-*tert*-octylphenol in air was 3.1 ng/m^3. The results of the analyses are provided in *Table 3.15*.

Table 3.15 Levels of 4-*tert*-octylphenol in indoor and outdoor air in Tokyo

Sample type	Mean ± SD (ng/m^3)	Median (ng/m^3)	Range (ng/m^3)	Percentage detection
Houses	7.6 ± 9.6	3.2	ND-45.7	52
Offices	5.7 ± 5.3	4.2	ND-30.5	71
Outdoor	ND	ND	ND-5.3	6

These measurements are in line with the calculated values. The study also looked for the linear isomer 4-*n*-octylphenol; this was not detected in any of the samples.

3.3.4 Food chain exposure

3.3.4.1 Estimated environmental concentrations

If a substance accumulates in the food chain, it might reach a concentration in food that could cause toxic effects in a predator that eats that food. This is known as secondary poisoning. PECs for secondary poisoning from eating fish have been calculated with EUSES using the BCF value of 634 calculated from the log K_{ow} along with the estimated PECs for the aquatic compartment. For the terrestrial food chain, the PEC values in worms have been estimated using the method in the TGD and the PEC values for soil and pore water. These PECs are shown in *Table 3.16* for a simple aquatic food chain and a terrestrial one. The calculations follow the procedures outlined in the TGD, and include a biomagnification factor for the aquatic food chain; for 4-*tert*-octylphenol this is 1 (log K_{ow} <4.5, BCF <2000).

Table 3.16 PECs for secondary poisoning

Life-cycle stage		PECs for fish eaten by predators (mg/kg wwt)	PECs for worms eaten by predators (mg/kg wwt)
Production of 4-*tert*-octylphenol		1.45	0.58
Phenol–formaldehyde resins	Resin manufacture	1.5	0.60
	Resins in rubber formulation	0.16	0.012
	Resins in varnishes	0.068	0.006
	Resins in printing inks	2.08	0.21
	Ethoxylated resin production	0.73	0.26
	Marine paint formulation	0.069	0.002
	Marine paint application	2.13	0.22
	Marine paint disposal	3.76	0.39
Octylphenol ethoxylates	Production	0.19	0.33
	Formulation (textile, pesticide)	0.086	0.78
	Emulsion polymerisation	0.064	0.027
	Textiles	0.21	1.84
	Paint formulation	0.19	0.49
	Paint application	0.056	0.008
	Pesticide application	0.054	0.078
	Veterinary medicine	0.054	0.007
	Ether sulphate production	0.81	3.66

A contribution of approximately 50% is made to each PEC value by both the regional and local concentrations. This is done because foraging for food can occur over a wide area for some species (i.e., half of the dietary intake for both aquatic and terrestrial food chains is assumed to come from local and half from regional sources). In the case of 4-*tert*-octylphenol, the local aquatic surface water and soil concentrations provide a significantly higher percentage of the overall value than do the regional concentrations.

A further possible route of exposure for higher animals that might be considered is the consumption of plants that have been sprayed with pesticide formulations containing OPEs.

An application rate of 20 mg/m^2 (see Section 3.3.1.1) to leaves of 2 mm thickness gives 20 mg to 2 x 10^{-3} m^3 leaf, giving a concentration of 10^4 mg/m^3. Taking the density of plant material as 700 kg/m^3 from the TGD gives a concentration of 14 mg/kg. This is in terms of OPE; assuming this is converted entirely into 4-*tert*-octylphenol gives a concentration of 4.5 mg/kg plant. This is likely to be a significant overestimate, since it

combines a number of worst-case assumptions (e.g., complete breakdown to 4-*tert*-octylphenol, animals eat only from contaminated leaves, etc.).

3.3.4.2 Measured environmental concentrations in biota

The most extensive European dataset available has been provided by the German UBA (1999). Studies of the concentration of 4-*tert*-octylphenol and octylphenol monoethoxylate (OP1EO) in both freshwater and marine biota were performed. The samples were solvent extracted and derivatised (to trimethylsilyl ethers) before determination by GCMSMS (gas chromatography coupled to tandem mass spectrometry). In the marine samples, mussels had higher concentrations than the other biota. The concentrations in freshwater biota were higher than those in the marine biota. The UBA study also reported concentrations of nonylphenol, which were always higher than the concentrations of 4-*tert*-octylphenol in the same biota. This study is discussed in more detail below. Results from this work have also been published by Wenzel *et al*. (2004).

3.3.4.2.1 Freshwater biota

Concentrations of 4-*tert*-octylphenol were reported in two types of freshwater fish for samples collected between 1992 and 1997 on several rivers (*Table 3.17*). Measured concentrations were generally above the limit of detection of 0.2 µg/kg wwt with the highest reported level being 5.5 µg/kg wwt.

Table 3.17 Concentrations of 4-*tert*-octylphenol in freshwater aquatic biota in Germany (µg/kg wwt)

Year	River	Location	*Abramis brama* (carp)	*Dreissena polymorpha* (zebra mussel)
1995			-	1.8
1995			-	2.3
1996	Elbe	Prossen	0.4	-
1996		Zehren	0.2	-
1996		Barby	0.3	-
1996		Cumlosen	0.3	-
1996		Blankenese	0.3	0.7
1996	Rhein	Weil	0.8	1.0
1996		Iffezheim	0.9	0.9
1996		Koblenz	0.4	0.8
1996		Bimmen	0.2	1.1
1996	Saale	Wettin	0.8	-
1998			0.5	-
1996	Mulde	Dessau	0.3	-
1998			0.3	-
1992	Saar	Güdingen	4.3	-
1994			5.5	-
1995			2.0	-
1996			3.7	-
1997			2.1	-
1998			1.2	-
1992		Rehlingen	5.5	-
1994			3.3	-
1995			2.3	-
1996			2.8	-
1997			1.8	-
1998			3.2	-
1992	Belauer See	Bornhöved	<LOD*	-
1997			<LOD*	-

*Limit of detection (LOD) = 0.2 µg/kg wwt

3.3.4.2.2 Marine biota

Concentrations were reported in a marine alga (*Fucus vesiculosus*), a marine invertebrate (*Mytilus edulis*) a marine fish and a marine bird from Germany (German UBA, 1999) for samples collected between 1985 and 1996 at several locations (*Table 3.18*). Measured concentrations in the aquatic organisms were generally below or slightly above the limit of detection of 0.2 µg/kg wwt with the highest reported level being 0.8 µg/kg. Levels in Herring gull (*Larus argentatus*) samples from every year and site were below the detection limit (1 µg/kg wwt), with the exception of Heuwiese in 1991 when a level of 1.1 µg/kg was measured.

Archived cormorant livers (n = 28) and porpoise blubber (n = 25) taken from birds and animals found dead around the UK coastline were analysed for total branched 4-octylphenol and total branched 4-nonylphenol using solvent extraction and LCMS (Defra, 2002b). Neither alkylphenol was detected in any of the cormorant or porpoise samples (limit of detection estimated at 0.2 to 1 ppb), except for 4-nonylphenol in a single cormorant liver sample at a concentration of 2.6 ppb wwt. However, the absence of 4-*tert*-octylphenol may not be significant since these samples were not necessarily taken from areas that would be expected to receive inputs of the substance.

Several alkylphenolic compounds have been used as production chemicals at offshore installations and are released into the open sea as part of the production of discharge water. To ensure that these chemicals are not reaching the food supply chain a number of herring, haddock and dab taken from around North Sea offshore installations were analysed for 4-*tert*-octylphenol as part of a preliminary Food Quality Assurance monitoring programme. The results of this study are reported in CEFAS (1997). This study showed that concentrations of 4-*tert*-octylphenol in liver and muscle were below the limits of detection (i.e., <0.1-0.004 mg/kg depending on the species and tissue type tested).

Table 3.18 Concentrations of 4-*tert*-octylphenol in marine aquatic biota in Germany (µg/kg wwt)

Year	Sample location	*Fucus vesiculosus* (bladderwrack)	*Mytilus edulis* (Common mussel)	*Zoarces viviparus* (eelpout)
1994	Jadebusen	-	-	0.5
1995		-	-	0.5
1996		-	-	0.8
1985	Eckwarderhörne	0.5	0.5	-
1986		-	0.3	-
1987		0.3	-	-
1988		-	0.3	-
1989		0.2	-	-
1990		-	0.3	-
1991		0.2	-	-
1992		-	0.3	-
1993		0.4	0.3	-
1994		0.2	0.2	-
1995		0.2	<LOD	-
1996		0.2	0.3	-
1985	List/südl. Hafen	<LOD	-	-
1986		-	0.3	-
1987		<LOD	-	-
1988		-	0.2	-
1989		<LOD	-	-
1990		-	<LOD	-
1991		<LOD	-	-
1992		-	0.2	-
1992	List/Königshafen	0.3	0.2	-
1993		<LOD	0.2	-
1994		<LOD	0.2	-
1995		<LOD	<LOD	-
1996		<LOD	<LOD	-
1992	Darßer Ort	-	0.3	-
1993		-	<LOD	-
1994		-	0.2	-
1995		-	<LOD	-
1996		-	<LOD	-

Limit of detection (LOD) = 0.2 µg/kg wwt

3.3.4.3 Comparison of predicted and measured environmental concentrations

The only measurements in biota that can be compared with the calculated values are those for freshwater fish. The calculated values are much higher than the measured levels. There is no information on the locations from which the fish were taken, so they may not be from locations close to sources of 4-*tert*-octylphenol. The calculated values are influenced by local emissions. Combining the regional concentration in water with the BCF gives a concentration in fish of 0.054 mg/kg, which is closer to the measured levels, but is an order of magnitude greater than most of them.

3.3.5 Potential exposures arising from the use of nonylphenol

The preceding sections summarise the PECs that arise from the intentional use of 4-*tert*-octylphenol. Exposure can also arise from releases during the production and use of nonylphenol, since 4-*tert*-octylphenol can be present as an impurity in that substance. To investigate the significance of this source, PECs have been calculated using EUSES assuming a typical impurity level of 5%, and the production and use scenarios from the original nonylphenol risk assessment report (ECB, 1999). The results are presented in Appendix 5. *These PECs are not added to the other PECs that arise from intentional use*, because:

- The nonylphenol tonnage is for 1997, while that for 4-*tert*-octylphenol is for 2001;
- The PECs are related to the uses of nonylphenol, which are changing because of the proposed restrictions in marketing and use.

4 EFFECTS ASSESSMENT

Studies are considered valid if they are relevant, fully describe the test material used, the test organism and the test method and conditions in detail. Measured concentrations of 4-*tert*-octylphenol can be markedly different from nominal levels, so analytical confirmation of exposure concentrations is usually necessary for the studies to be considered valid[16]. Where only some of these criteria are described the tests may be classed as 'use with care' or 'not valid'. Studies marked as 'use with care' can be used to support other studies and are clearly indicated in the text.

It has not been possible to obtain reports for every unpublished industry study. However, information in the peer-reviewed OECD SIDS Initial Assessment Report (SIAR) in the SIDS dossier (SIDS, 1994) is automatically considered to be valid (unless contrary data have become available since the report was published). In addition, studies that are cited in sufficient detail so as to provide the information outlined above are also classed as valid. A 'lack of full study' marking is given where full studies are unavailable.

4.1 AQUATIC COMPARTMENT (INCLUDING SEDIMENT)

All available data are summarised in *Tables 4.1-4.3*, with the most relevant and sensitive data outlined further in the text (studies marked 'use with care' are discussed where important issues – such as the timing and duration of exposure – are addressed). While toxicity data are available for three taxonomic groups in freshwater (i.e., algae, invertebrates and fish), the current saltwater dataset is more limited. Alkylphenols are known to affect endocrine systems, and relevant data are described in detail in Section 4.1.6. The data have been used to derive predicted no-effect concentrations (PNECs) for both freshwater and saltwater environments (the derivation of the latter is provided in Appendix 2 on marine risk assessment).

4.1.1 Toxicity to fish

All results are summarised in *Table 4.1*.

4.1.1.1 Acute toxicity

4.1.1.1.1 Freshwater species

An acute 96-hour LC_{50} value of 290 µg/L has been reported for fathead minnow (*Pimephales promelas*) in a flow-through test in which concentrations were measured and the study was carried out according to GLP. A 96-hour no observed effect concentration (NOEC) of 77 µg/L for lethality was also obtained (IUCLID, 2000).

[16] For example, studies by Gray *et al.* (1999a,b) have shown that the exposure concentrations of 4-*tert*-octylphenol in static renewal systems may decline by 30-50% over a 48-72 hour period. This probably results in part from sorption of the substance onto the surfaces of the test aquaria.

A similar 96-hour LC_{50} value of 260 µg/L 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 (SIDS, 1994; IUCLID, 2000). In the same study an LC_0 of 210 µg/L and an LC_{100} of 390 µg/L were also obtained. A higher 48-hour LC_{50} of 600 µg/L was found for the same species in another (use with care) study based on nominal concentrations and not carried out to GLP (SIDS, 1994; IUCLID, 2000).

A 14-day LC_{50} of 120 µg/L and NOEC of 84 µg/L (for lethality) were found in rainbow trout *(On. mykiss*) when tested under flow-through conditions. The concentrations were measured and the test was carried out to GLP. A 6-day LC_{50} of 170 µg/L was also obtained in this study (SIDS, 1994; 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.

4.1.1.1.2 Saltwater species

Kelly and Di Giulio (2000) investigated the toxicity of 4-*tert*-octylphenol (and nonylphenol) to embryos and larvae of the estuarine killifish or mummichog (*Fundulus heteroclitus*). In the (use with care) study, 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/L larvae were monitored daily for mortalities, structural abnormalities (such as craniofacial, cardiac, torso/abdominal, tail effects) and behavioural abnormalities (such as lethargy and lack of feeding). The 96-hour LC_{50} for the mortality of embryos was 3900 µg/L. At concentrations of 2063 µg/L 4-*tert*-octylphenol caused significant sub-lethal abnormalities, most often in the torso/abdomen and the tails of embryos. While these embryos survived to hatching age, they were often unable to hatch successfully. If the embryos did hatch the larvae were often observed to lack complete tail fins, have spinal curvature not apparent in embryos, show lethargic swimming behaviour and be unable to feed normally, often dying as a result.

For the newly hatched, 2-week-old and 4-week-old larvae the 96-hour LC_{50} values were 290, 280 and 340 µg/L, respectively. 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 by the authors that developmental toxicity may occur, at least in part, through the disruption of oestrogen-based signals (see also Section 4.1.6).

The results for nonylphenol (technical grade 85-90% purity) on embryo and larval survival were similar to those for 4-*tert*-octylphenol. The 96-hour LC_{50} value for embryos was 5442 µg/L, whereas for the newly hatched, 2- and 4-week-old larvae the 96-hour LC_{50} values were 213, 209 and 260 µg/L, respectively.

Karels *et al*. (2003) obtained a 96-hour LC_{50} of 720 µg/L for the sheepshead minnow *Cyprinodon variegatus* (this study should be used with care because there was no information on exposure levels).

4.1.1.2 Chronic toxicity

4.1.1.2.1 Freshwater species

A 60-day post-hatch early life stage toxicity study with rainbow trout (*On. mykiss*) has also been carried out to an American Society for Testing and Materials (ASTM) procedure under flow-through conditions 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 based on measured concentrations. The NOEC and lowest observed effect concentration (LOEC) values for the growth of fry (as measured by wwt after 60 days exposure) were 6.1 and 11 µg/L, respectively. Hatchability of the rainbow trout eggs after 20 days of continuous exposure to 4-*tert*-octylphenol was not significantly affected at the highest test concentration 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-*tert*-octylphenol concentrations greater than 22 µg/L. It should be recognised that the effects on growth observed could have been mediated through an endocrine-disruption mechanism.

Ashfield *et al. (*1998), in a use with care study, investigated the effects of exposure of 4-*tert*-octylphenol (and nonylphenol) on the growth of female juvenile rainbow trout. Two flow-through exposure regimes were used for the critical window of sensitivity between hatching and the swim-up fry stage:

1. Groups of fish were exposed to nominal concentrations of 1, 10 and 50 µg/L for a period of 22 days immediately following hatching 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 were exposed to nominal concentrations of 1, 10 and 30 µg/L for a period of 35 days immediately following hatching 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 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 4-*tert*-octylphenol (10 and 30 µg/L) causing a significant reduction in weight, relative to that of control fish. Fish exposed to 1 µg/L displayed body weights significantly higher than the controls at this time. Fish exposed to 10 µg/L 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 was no longer significantly different from control fish values. However, the body weight of fish exposed to 1 µg/L was still significantly lower than that of the controls. Length was significantly reduced in fish exposed to 10 µg/L 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 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.

Gray and Metcalfe (1999) carried out embryo–toxicity tests on the Japanese medaka (*O. latipes*), in a use with care study. 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 (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.

4.1.1.2.2 Saltwater species

No chronic toxicity data were identified for saltwater species.

4.1.1.3 Summary of toxicity data for fish

The lowest reliable toxicity data reported for freshwater fish are:

- An acute 96-hour LC_{50} value of 290 µg/L for fathead minnow (*Pi. promelas*);
- A 6-day LC_{50} value of 170 µg/L for rainbow trout (*On. mykiss*);
- A 60-day post-hatch early life stage toxicity study with rainbow trout that reported NOEC and LOEC values of 6.1 µg/L and 11 µg/L, respectively.

No fully valid acute or chronic toxicity data were available for saltwater species. For acute toxicity a ‘use with care’ study on the estuarine fish *F. heteroclitus* provided 96-hour LC_{50} values for larvae of 290-340 µg/L based on nominal concentrations. The data that are available for saltwater species suggest that the acute toxicity of 4-*tert*-octylphenol to freshwater and saltwater fish is comparable.

Table 4.1 Toxicity of 4-*tert*-octylphenol to freshwater and saltwater fish (ND = no data)

Species	Chemical tested	Age/Size	Static/ Flow	Temp. (°C)	Dissolved oxygen (mg/L)	Hardness (mg $CaCO_3$/L) or salinity (‰)	pH	Endpoint	Concentration (mg/L)	Reference	Validity
Freshwater											
Pimephales promelas (Fathead minnow)	4-*tert*-octylphenol (99.3% purity)	ND	Flow through	22	ND	ND	8.0-8.2	24-hour LC_{50} 48-hour LC_{50} 72-hour LC_{50} 96-hour LC_{50} 96-hour NOEC	0.29 0.25 0.25 0.29 0.077	Unpublished report from Analytical Bio-chemistry laboratory 1984 cited in SIDS (1994) and IUCLID (2000)	Valid (lack of full study; US EPA method, measured concentration data and carried out to GLP)
	4-*tert*-octylphenol	ND	ND	ND	ND	ND	ND	24-hour NOEC	0.15	ABC, 1984a (cited in Servos, 1999)	Use with care (lack of full study; no information on test conditions)
	4-*tert*-octylphenol	ND	ND	ND	ND	ND	ND	96-hour LC_{50}	0.25	Cited in Bennie (1999)	Use with care (lack of full study; no information on test conditions)
Leuciscus idus (Golden orfe)	4-*tert*-octylphenol	6 ± 2 cm	Semi-Static	20 ± 1	ND	ND	7.5-7.9	96-hour LC_0 96-hour LC_{50} 96-hour LC_{100}	0.21 0.26 0.39	Data from Hüls AG (1984) cited in IUCLID (2000)	Valid (OECD 203, measured concentration data and carried out to GLP)
	4-*tert*-octylphenol	ND	ND	ND	ND	ND	ND	48-hour LC_{50}	0.60	Data from Hüls AG cited in IUCLID (2000) and SIDS (1994)	Use with care; (carried out to DIN 38412, but nominal concentration data)
	4-*tert*-octylphenol (95% purity)	ND	ND	ND	ND	ND	ND	96-hour LC_{50} 96-hour NOEC	1.5-2.2 1.0	Data from BASF cited in IUCLID, 2000	Use with care (lack of full study; carried out to DIN 38412, not to GLP and nominal concentration data)

Table 4.1 Continued (ND = no data)

Species	Chemical tested	Age/Size	Static/ Flow	Temp. (°C)	Dissolved oxygen (mg/L)	Hardness (mg $CaCO_3$/L) or salinity (‰)	pH	Endpoint	Concentration (mg/L)	Reference	Validity
Oncorhynchus mykiss (Rainbow trout)	4-*tert*-octylphenol	ND	ND	ND	ND	ND	ND	24-hour LC_{50} 24-hour NOEC	0.45 0.17	ABC, 1984b cited in Servos (1999)	Use with care (lack of full study; no information on test conditions)
	4-*tert*-octylphenol (99.3% purity)	ND	Flow	12 ± 1	ND	ND	8.0-8.2	14-day LC_{50} 14-day NOEC	0.12 0.084	Unpublished report from Analytical Bio-chemistry laboratory 1984 cited in SIDS (1994)	Valid (lack of full study details but carried out to GLP and measured concentration data)
	4-*tert*-octylphenol (99.22% purity)	Post-hatch	Flow	ND	ND	ND	ND	60-day NOEC (effect on growth of fry as wwt) 60-day LOEC (effect on growth of fry as wwt)	0.0061 0.011	Unpublished report from Analytical Bio-chemistry laboratory 1986 (cited in SIDS, 1994)	Valid (lack of full study details, but carried out to GLP and measured concentration data)
	4-octylphenol	Post-hatch	Flow	7-13	ND	12.5	6.5	108-day LOEC (effect on body weight) 466-day NOEC (effect on body weight)	0.001 0.03	Ashfield *et al.*, (1998)	Use with care (information on study conditions but nominal concentration data)
Oryzias latipes (Japanese medaka)	4-*tert*-octylphenol	Fertilisation to swimup (day 0 to day 17) (embryo/ larvae)	Static	25	ND	ND	ND	17-day LC_{50}	0.45-0.94	Gray and Metcalfe (1999)	Use with care (nominal concentration data)

Table 4.1 Continued (ND = no data)

Species	Chemical tested	Age/Size	Static/ Flow	Temp. (°C)	Dissolved oxygen (mg/L)	Hardness (mg $CaCO_3$/L) or salinity (‰)	pH	Endpoint	Concentration (mg/L)	Reference	Validity
Saltwater											
Fundulus heteroclitus (Killifish or mummichog)	4-*tert*-octylphenol	Embryos Newly hatched larvae 2-week-old larvae 4-week-old larvae	Static	ND	ND	20‰	ND	96-hour LC_{50} 96-hour LC_{50} 96-hour LC_{50} 96-hour LC_{50}	3.9 0.29 0.28 0.34	Kelly and Di Giulio (2000)	Use with care (information on study conditions but nominal concentration data)
Cyprinodon variegatus (Sheepshead minnow)	4-*tert*-octylphenol	8-9 months old	Static renewal	27°C	ND	14-16‰	ND	72-hour LC_{50}	0.72	Karels *et al*. (2003)	Use with care (limited details, no information on exposure levels)

4.1.2 Toxicity to aquatic invertebrates

All results are summarised in *Table 4.2*.

4.1.2.1 Acute toxicity

4.1.2.1.1 Freshwater species

Only limited toxicity data are available for freshwater invertebrates, mostly for the water flea *Daphnia magna.* 24- and 48-hour LC_{50} values of 260 and 270 µg/L were reported for *D. magna* in a flow-through test carried out to GLP and with measured exposure concentrations. The corresponding NOEC was 110 µg/L (SIDS, 1994; IUCLID, 2000).

In two other studies using the same species, a 24-hour EC_{50} (immobilisation) of 170 µg/L and a 48-hour LC_{50} of 90 µg/L have been reported (IUCLID, 2000 and Zou and Fingermann, 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 article. 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 and a corresponding 96-hour LC_{50} of 19.6 µg/L (Sims and Whitehouse, 1998). The experiment involved a semi-static design with analytical confirmation of test solutions. There was also a clear time-dependence of toxicity, although toxicity appeared to begin to plateau by 96 hours.

Tominaga *et al*. (2003) exposed larvae of the nematode worm *Caernorhabditis elegans* to 4-*tert*-octylphenol in water for 5 hours. Effects on mobility of the larvae were noted. The ED_{50} for 4-*tert*-octylphenol was 50 µM (10.3 mg/L). For comparison, the ED_{50} values for 4-*n*-octylphenol, 4-*n*-nonylphenol and technical grade nonylphenol were 50 µM, 6 µM and 60 µM, respectively. The short exposure time means that the result is not directly useful for the risk assessment (the organism is also a soil dweller).

4.1.2.1.2 Saltwater species

A series of acute toxicity experiments for the mysid shrimp *Mysidopsis bahia*[17] provides 96-hour LC_{50} values ranging from 53.4 to 113.1 µg/L (Cripe *et al*., 1989). However, these data were taken from a study that investigated the effects of different feeding regimes on the acute toxicity of four substances to *Mysidopsis*, and were not carried out to any standard guidelines. There were thus a number of different variables that 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 selected endpoints, and well reported, there are 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 *G. pulex*. However, the range of LC_{50} values observed is broadly similar to the LC_{50} for the freshwater amphipod.

[17] *Mysidopsis bahia* has recently been renamed *Americamysis bahia.*

Andersen *et al.* (2001) investigated the effects of 4-*tert*-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-*tert*-octylphenol concentrations over a period of 48 hours. The exposure concentrations were confirmed analytically. The 48-hour LC_{10} and LC_{50} values for 4-*tert*-octylphenol were 230 and 420 µg/L, respectively.

4.1.2.2 Chronic toxicity

4.1.2.2.1 Freshwater species

A 21-day life-cycle toxicity study was carried out with *D. magna* according to a US EPA procedure under flow-through conditions and according to GLP (Hüls AG, cited in SIDS, 1994 and IUCLID, 2000). Exposure levels were 37, 62, 120, 230 and 510 µg/L based on measured concentrations. Statistical analysis of survival for *D. magna* after a 21-day exposure period indicated that adult survival was significantly different from that of the controls at the mean measured concentration of 510 µg/L. All of 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. Mean young produced per surviving adult per day after 21 days were significantly affected at the exposure levels of 120 and 230 µg/L, while no effects (relative to the controls) were evident at a NOEC of 62 µg/L. The mean adult lengths at concentrations of 62, 120 and 230 µg/L were significantly different from those of the controls. However, since the mean adult length of the 62 µg/L exposure concentration was only 2.6% less than that of 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 which would be the same value as the NOEC for juvenile reproduction.

In another 21-day *D. magna r*eproduction study, a 21-day LOEC of 100 µg/L and a NOEC of 30 µg/L were 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.

The effects observed in *D. magna* reproduction tests are probably not caused by direct oestrogenic effects, since other studies have shown an absence of reproductive impairment at 387 µg/L when animals are exposed to the synthetic steroid 17α-ethinyl estradiol (EE2; Schweinfurth *et al.,* 1986).

4.1.2.2.2 Saltwater species

No chronic toxicity data were identified for saltwater species.

Table 4.2 Toxicity of 4-*tert*-octylphenol to freshwater and saltwater invertebrates (ND = no data)

Species	Chemical tested	Age/Size	Static/ Flow	Temp. (°C)	Dissolved oxygen (mg/L)	Hardness (mg $CaCO_3$/L) or salinity (‰)	pH	Endpoint	Concentration (mg/L)	Reference	Validity
Freshwater											
Daphnia magna (water flea)	4-*tert*-octylphenol	ND	ND	ND	ND	ND	ND	24-hour EC_{50}	0.17	IUCLID (2000)	Use with care (lack of full study; nominal, concentrations not carried out to GLP)
	4-*tert*-octylphenol (99.3% purity)	ND	Flow	20 ± 2	ND	ND	8.3-8.4	24-hour LC_{50} 48-hour LC_{50} 48-hour NOEC	0.26 0.27 0.11	Unpublished report from Analytical Bio-chemistry laboratory 1984, cited in SIDS (1994)	Valid (lack of full study; US EPA method, carried out to GLP measured concentration data)
	4-octylphenol	Neonates	S	20-22	ND	ND	7.0-7.2	48-hour LC_{50}	0.09	Zou and Fingermann (1997)	Use with care (no information on study conditions)
	4-*tert*-octylphenol	ND	ND	ND	ND	ND	ND	21-day NOEC (effects on reproduction rate) 21-day LOEC (effects on reproduction rate)	0.030 0.100 (N.B. both values reinterpreted in text)	Data from Hüls AG Marl cited in IUCLID (2000)	Use with care (carried out to OECD 202 and to GLP, but limited analytical confirmation of exposure concentrations)
	4-*tert*-octylphenol	Adults and young	Flow	ND	ND	ND	ND	21-day EC_{50} (based on adult survival) 21-day NOEC (based on adult mean length) 21-day NOEC (based on reproduction rate)	0.340 0.037 0.062	Unpublished report from Analytical Bio-chemistry laboratory 1988 (cited in SIDS 1994)	Valid (lack of full study; US EPA method carried out to GLP and measured concentrations data)
Gammarus pulex (scud or freshwater shrimp)	4-*tert*-octylphenol	First and second instar nymphs	Semi-static	ND	ND	ND	ND	96-hour EC_{50} (immobilisation) 96-hour LC_{50} (lethality)	0.0133 0.0196	Sims and Whitehouse (1998)	Valid (full study details and measured exposure concentration data)

Table 4.2 Continued (ND = no data)

Species	Chemical tested	Age/Size	Static/ Flow	Temp (°C)	Dissolved oxygen (mg/L)	Hardness (mg $CaCO_3$/L) or salinity (‰)	pH	Endpoint	Concentration (mg/L)	Reference	Validity
Saltwater											
Mysidopsis bahia (mysid shrimp)	4-*tert*-octylphenol	<24 hours	Static	25	5.9-7	20‰	7.8-8.3	96-hour EC_{50} (growth)	0.0534	Cripe *et al.* (1988)	Use with care (important study details lacking)
Acartia tonsa (copepod)	4-octylphenol	10-12-day-old adults	Static	20	ND	18	ND	48-hour LC_{10} (lethality) 48-hour LC_{50} (lethality)	0.23 0.42	Andersen *et al.* (2001)	Valid (study carried out to ISO 14669 and measured exposure concentration data)
Uca pugilator (fiddler crab)	4-octylphenol	ND	Semi-static	19-21	ND	34‰	ND	EC_{50} (enzymic changes)	10	Zou and Fingermann (1999a,b)	Use with care, (important study details lacking)

4.1.2.3 Summary of toxicity data for aquatic invertebrates

The lowest reliable toxicity data reported for freshwater invertebrates are:

- An acute 96-hour EC_{50} (immobilisation) of 13.3 µg/L and 96-hour LC_{50} (lethality) of 19.6 µg/L reported for the amphipod *G. pulex* (this species appears to be more sensitive than *D. magna*, which has 48-hour LC_{50}s in the range 90-270 µg/L);
- A 21-day juvenile reproduction NOEC of 62 µg/L for *D. magna*.

Only acute data are available for saltwater species. A 48-hour LC_{50} of 420 µg/L was obtained for the marine copepod *A. tonsa*. For the mysid shrimp *M. bahia* 96-hour LC_{50} values ranged from 53.4 to 113.1 µg/L in a 'use with care' study. The limited saltwater dataset restricts comparisons with the freshwater data, but the 48-hour LC_{50} values for the water flea *D. magna* and the copepod *A. tonsa* are of a similar order of magnitude.

4.1.3 Toxicity to aquatic algae and plants

All results are summarised in *Table 4.3*. The absence of measured exposure concentrations means these studies should be used with care. No information on freshwater aquatic macrophytes or saltwater species has been located.

Algal studies are considered to be multigenerational. The TGD recommends that 72-hour (or longer) EC_{50} values are considered as equivalent to a short-term result, and that a 72-hour (or longer) NOEC is considered as a long-term result. Based on effects on growth rate, 72-hour EC_{10}, EC_{50} and EC_{90} values of 300, 1100 and 4200 µg/L (nominal concentrations) have been reported for *Scenedesmus subspicatus* (IUCLID, 2000). A similar 96-hour EC_{50} of 1900 µg/L (nominal concentration) has been obtained for *Selenastrum capricornutum*[18] in a static test at 24-25°C and carried out according to GLP standards (IUCLID, 2000).

[18] *Selenastrum capricornutum* has recently been renamed *Pseudokirchneriella subcapitata*.

Table 4.3 Toxicity of 4-*tert*-octylphenol to freshwater aquatic algae and plants

Species	Chemical	Experimental conditions	Endpoint/Effect	Concentration (mg/L)	Reference	Validity
Scenedesmus subspicatus (green alga)	4-*tert*-octylphenol	Nominal concentration	72-hour EC_{10} (growth rate) 72-hour EC_{50} (growth rate) 72-hour EC_{90} (growth rate) 72-hour EC_{10} (biomass) 72-hour EC_{50} (biomass) 72-hour EC_{90} (biomass)	0.3 1.1 4.2 No data No data No data	Data from Hüls AG Marl cited in IUCLID (2000) and SIDS (1994)	Use with care (lack of full study; not carried out to GLP, nominal concentration data)
Selenastrum capricornutum (alga)	4-*tert*-octylphenol (high purity)	Static, 24-25°C	96-hour EC_{50} (growth rate) 96-hour NOEC (growth rate) 96-hour EC_{50} (biomass) 96-hour NOEC (biomass)	1.9 <1.0 No data No data	Unpublished report from Analytical Bio-chemistry laboratory 1984 (cited in SIDS 1994)	Use with care (nominal concentration data)

4.1.4 Quantitative structure–activity relationships

The absence of a fully valid data set for algae means that there is a potential gap in the basic hazard data set. Toxicity has therefore also been predicted using the quantitative structure-activity relationships (QSARs) given in the TGD. The OECD defines four modes of toxic action for chemicals in the aquatic environment, namely non-polar narcosis, polar narcosis, reactive and specific-acting. The mechanism of non-polar narcosis is primarily related to the hydrophobicity of the substance and is also referred to as minimum or baseline toxicity. The polar narcotic chemicals class consists of more polar chemicals such as esters, phenols and anilines. The mode of action of these is also not particularly specific, but they are significantly more toxic than is predicted by non-polar narcosis.

The structure of 4-*tert*-octylphenol suggests that it will fall into the class of polar narcotic chemicals. The TGD QSAR provides only two endpoints however, and therefore QSARs developed by SRC have also been used. The selected SRC QSAR was specifically derived for phenols, and is well established (EPISUITE, 2004). This QSAR provides a range of endpoints and between the two sets of predicted data it is possible to make some comparisons with the measured data presented in the previous sections. The QSARs have been run using the physico-chemical properties selected for use in this risk assessment in Section 1 (i.e., log K_{ow} of 4.12 and a water solubility of 19 mg/L).

Data derived from both of these QSAR sources are shown in *Table 4.4* alongside comparable measured data where possible.

Table 4.4 Comparison of experimental and estimated (QSAR) aquatic toxicity data

Trophic level	End point	Experimental concentration (mg/L)	TGD QSAR concentration (mg/L)	EPISUITE QSAR concentration (mg/L)
Freshwater fish	96-hour LC_{50}	0.25	1.4	1.5
	Chronic value	0.0061 (60-d)	-	0.03 (90-d)
Freshwater invertebrates	48-hour L/EC_{50}	0.27	1.65	1.5
	21-day Chronic value	0.062	-	0.16
Freshwater algae	EC_{50}	1.1 (72-h)	-	1.4 (96-h)
	Chronic value	0.3 (72 to 96-h NOEC (growth)	-	0.5 (96-h)

Where comparisons can be made, the QSAR data generated by both the TGD methods and the SRC software appear to underestimate toxicity, although they are generally within an order of magnitude of those generated in experimental studies for all three trophic levels. The estimates derived for algae for short-term and long-term toxicity (based on growth rate) are broadly similar to the experimental values. This is particularly relevant, because there is a discrepancy in algal sensitivity between nonylphenol and 4-*tert*-octylphenol (discussed further in Section 4.1.6). However, it has not been checked whether the measured algal data form part of the underlying data set for the QSAR model.

Given that for the most part valid laboratory toxicity data are available, it is considered more appropriate to use these experimentally derived data in the PNEC derivation.

4.1.5 Overall summary of standard endpoint toxicity data

The most relevant toxicity data for 4-*tert*-octylphenol are summarised in *Table 4.5*. The most sensitive trophic levels based on freshwater data would appear to be aquatic invertebrates and fish. Overall, the lowest valid long-term NOEC value was 6.1 µg/L for growth inhibition in rainbow trout (*On. mykiss*) fry after 60 days exposure, with a corresponding LOEC value of 11 µg/L. There is a possibility that effects on the endocrine system may have caused the observed toxicity. Note that the acute toxicity result for *G. pulex*, an EC_{50} of 13.3 µg/L, is only a little higher than the fish NOEC. This may indicate a higher sensitivity for some invertebrates.

4.1.5.1 Comparison with nonylphenol

Nonylphenol has a much larger toxicity dataset (ECB, 1999), and it is useful to compare this with the data for 4-*tert*-octylphenol, to test the potential for read-across. *Table 4.5* lists data for the same trophic levels and species (where available) for each substance.

Table 4.5 Comparison of the lowest reliable acute and chronic toxicity data for 4-*tert*-octylphenol and nonylphenol (data are valid unless identified as 'use with care', units are all µg/L)

Data type		4-*tert*-Octylphenol	Nonylphenol
Acute fish (freshwater)	96-hour LC_{50} Fathead minnow, *Pimephales promelas*	290	128
	6-day LC_{50} Rainbow trout *Oncorhynchus mykiss*	170	-
Chronic fish (freshwater)	60-day $NOEC_{growth}$ Rainbow trout *Oncorhynchus mykiss*	6.1	-
	33-day $NOEC_{survival}$ Fathead minnow, *Pimephales promelas*	-	7.4
Acute fish (saltwater)	96-hour LC_{50} Killifish *Fundulus heteroclitus*	280-340 (use with care)	-
	96-hour LC_{50} Sheepshead minnow *Cyprinodon variegatus*	720 (use with care, 72-h)	310
Chronic fish (saltwater)	-	-	-
Acute invertebrates (freshwater)	48-hour EC_{50} *Daphnia magna*	270	85
	96-hour EC_{50} *Gammarus pulex*	13.3	12.7
Chronic invertebrates (freshwater)	21-day $NOEC_{surviving\ offspring}$ *Daphnia magna*	62	24
Acute invertebrates (saltwater)	96-hour $EC_{50\ growth}$ *Mysidopsis bahia*	53.4 (use with care)	43
Chronic invertebrates (saltwater)	28-day $NOEC_{survival}$ *Mysidopsis bahia*	-	3.9
Algae (freshwater)	72-hour $EC_{10\ growth\ rate}$ *Scenedesmus subspicatus*	300 (use with care)	25.1 or 500
	72-hour $EC_{50\ growth\ rate}$ *Scenedesmus subspicatus*	1100 (use with care)	323 or 1300
Algae (saltwater)	96-hour EC_{50} cell growth *Skeletonema costatum*	-	27

For comparative purposes, the toxicity should be expressed on a molar basis, but since nonylphenol is not a pure substance, this is not considered essential in this case. In general the substances show similar toxicity for a particular taxonomic group (algae, invertebrates and fish) and test type (i.e., acute or chronic). In most cases the toxicity values are within a factor of three of one another. The major difference occurs for algal

toxicity. For nonylphenol, the lowest chronic algal value was a 72-hour EC_{10} of 3.3 µg/L for biomass inhibition in *S. subspicatus*. There are no fully valid algal data for 4-*tert*-octylphenol, and no data for biomass inhibition to compare against the nonylphenol results. However, the biomass end point is no longer preferred for PNEC derivation. In fact, invertebrates and fish are both more sensitive than algae for both alkylphenols when algal growth rate is used (i.e., the profile is the same). In addition, other results were available for the same algal species for nonylphenol, and these were very similar to those obtained for 4-*tert*-octylphenol. Comparisons with QSARs for phenols (see Section 4.1.4) show that both the short- and longer-term predicted algal toxicity values are broadly similar to those measured in the 'use with care' studies (see *Table 4.3*). The use of the nonylphenol algal biomass EC_{10} was, however, supported by a 28-day NOEC for survival in *M. bahia* of 3.9 µg/L. Since there are no comparative chronic *M. bahia* data available for 4-*tert*-octylphenol, it is possible that this could be an important data gap.

From the information available, it does not seem likely that 4-*tert*-octylphenol and nonylphenol exert their effects through different modes of action given the structural similarities between the substances. It may, therefore, be important to obtain further chronic invertebrate toxicity data for 4-*tert*-octylphenol in any refinement of the risk assessment. This is considered further in the risk characterisation (Section 5).

Valid saltwater species toxicity data for 4-*tert*-octylphenol were limited to acute data for invertebrates. Comparison of the 48-hour lethality data for freshwater and saltwater crustacean species suggests that sensitivity is similar with a 48-hour LC_{50} value of 270 µg/L for *D. magna* and a 48-hour LC_{50} value of 420 µg/L for *A. tonsa*. Values for acute fish toxicity tests were also comparable, although the data for the estuarine fish *F. heteroclitus* and for *C. variegatus* were from 'use with care' studies.

4.1.6 Endocrine disruption

4.1.6.1 Introduction

Alkylphenols are known to act as weak oestrogens in fish (SIDS, 1994; EA, 2004c), and so might cause effects on endocrine systems that are not identified by the usual 'base set' tests. This section compares the reported endocrine effects with those for more standard (and potentially related) endpoints. However, interpretation of such comparisons is not straightforward, and should be viewed with caution. The TGD does not provide guidance on how to use such data for risk assessment. Consequently, a short section containing some contextual background information is included before the main discussion of the available data. The validity of the available data was assessed in a similar manner to that for standardised toxicity endpoints. The key issues remain whether a valid experimental design was adopted in terms of the number and quality of the animals used, the number of exposure concentrations and the interval between them, whether exposure concentrations were analytically confirmed and the statistical treatment of the data.

4.1.6.2 Background

'Endocrine disrupting' substances can act directly through a specific receptor or indirectly in a system, and their effects on a range of physiological systems (including metabolism, immunity and behaviour) vary depending on the target tissue, the timing of exposure and

interactions with other endocrine disrupters. Since endocrine disrupters can mimic or modulate the activity of endogenous hormones, it can be difficult to distinguish altered responses from the range of basal hormone-regulated responses within organisms.

For this reason the Scientific Committee for Toxicology, Ecotoxicology and the Environment (CSTEE) in its *Opinion on Human and Wildlife Health Effects of Endocrine Disrupting Chemicals, with Emphasis on Wildlife and Ecotoxicology Test Methods* considered it important to realise and state 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" (CSTEE, 1999). Therefore, in line with the International Programme for Chemical Safety (IPCS) definition of an endocrine disrupter, substances should only be considered endocrine disrupters if they cause "adverse health effects in an intact organism, or its progeny, or (sub)populations, consequent to changes in endocrine function".

Various methods using a range of ecotoxicological endpoints are now claimed by different sources to be relevant to the assessment of endocrine disruption in 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. Furthermore, the assessment of adverse effects on wildlife is complicated by the limited information on what constitutes normal endocrine function for many species, particularly invertebrates. The data that are available are largely for mammals, birds, amphibians, reptiles and, particularly, fish. Information on the largest taxonomic group, the invertebrates, is limited because of the diversity of endocrine systems between taxa and the absence of available validated test methods (EU 2001). With respect to ecological risk assessment, the CSTEE has stated that "The strategy for ecotoxicity assessment must focus on relevant endpoints for the detection of population-community effects. The analysis of current protocols for ecological risk assessment indicates a concern on the capability of low tier levels to detect the ecological risk of endocrine disrupters because of problems related to the suitability of test species and the extrapolation from acute lethality to long-term effects" (CSTEE, 1999).

The endocrine-mediated responses of chemical substances on wildlife can be tested at two levels:

- Laboratory, semi-field or field based *in vivo* studies (involving testing of responses in intact organisms);
- Laboratory-based *in vitro* studies (involving the testing of responses at the sub-cellular, cellular or tissue level).

From the numerous recent reviews of potential test methods, such as the Detailed Review Paper published by the OECD (OECD, 2002), there is a clear consensus in terms of the hierarchy of the relevance of test methods to measure endocrine-mediated responses. 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 generally of greater relevance than *in vitro* assays. The greater relevance of chronic *in vivo* tests is also confirmed by the fact that these are the ecotoxicological methods being developed in the OECD Endocrine Disruption Testing and Assessment (EDTA) Programme.

The mechanistic limitations of *in vitro* methods were discussed in detail by OECD (2002) 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*. Furthermore, 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.

Hence, in this assessment the key data currently required to determine whether 4-*tert*-octylphenol can be considered to cause endocrine-mediated responses are longer-term *in vivo* assays (such as multigenerational tests) or those in which exposure is targeted towards critical windows of sensitivity in the life history of the organism. The endpoints of greatest significance are those associated with reproduction and/or development. For non-standard protocol endpoints, including *in vivo* screening studies, the assessment of endpoint relevance has been a subjective decision based on expert judgement. While consistent *in vitro* data are useful in making judgements about the presumption of hazard they are not currently linked directly to, nor predictive of, adverse and/or toxicological effects associated with endocrine disruption.

For similar reasons, assessment of mammalian toxicity data has not been included in this section as it is regarded that this information – while helpful in establishing mechanisms and possibly indicative of hazard in some vertebrates – is unlikely to be predictive of effects seen in the majority of non-mammalian wildlife. The endocrine system is reasonably conserved across many vertebrate species, but this is not the case for many other aquatic and terrestrial taxa. The issue of the limited current understanding of the endocrinology of most invertebrate groups also needs to be re-emphasised.

It needs to be recognised that there are a number of 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 (e.g., oestrogenic, anti-estrogenic, androgenic, anti-androgenic, thyroid and anti-thyroid) need to be considered.

4.1.6.3 Assessment of the endocrine-mediated effects of 4-*tert*-octylphenol

In this section the available *in vivo and in vitro* data on the endocrine-mediated responses of 4-*tert*-octylphenol are considered for aquatic and terrestrial species.

4.1.6.3.1 *In vivo* studies in aquatic organisms

Information on endocrine-mediated responses to 4-*tert*-octylphenol in aquatic organisms is summarised in *Table 4.7* at the end of this section. Key studies are discussed further in the text.

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 African clawed frog *Xenopus laevis* (see *Table 4.7*). Larvae at developmental stage 38/40 (2-3 days after hatching) were exposed to 2.1 and 21.0 µg/L (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-estradiol concentration of 27.2 µg/L resulted in the sex ratio being skewed to 5% males and 95% females, while a concentration of 2.7 µg/L resulted in a sex ratio of 30% males and 70% females. A change in the sex ratio of populations is clearly an important endpoint that could result from endocrine disrupting effects. However, given that 4-*tert*-octylphenol would be expected to be of lower potency than 17β-estradiol (see section on *in vitro* effects) it is unexpected that similar effects on sex ratios occurred at similar concentrations for these two substances. It needs to be recognised that this study was not carried out to a standardised regulatory test protocol and the data need to be considered in this context. In particular, the results of a similar study with bisphenol-A were not reproducible in a repeat test (ECB, 2003), which raises some questions about the validity of the findings and as such the study is classified as 'use with care'.

Tadpoles of the bullfrog *Rana catesbeiana* were exposed to 4-*tert*-octylphenol at 10^{-9}, 10^{-8} and 10^{-7} M, corresponding to 0.2, 2 and 20 µg/L (Mayer *et al*., 2003; see *Table 4.7*). The tadpoles were at development stages 32 to 36, which were identified as those just before, during and after sexual differentiation, and 12 tadpoles from each stage in this range were exposed to each of the concentrations. The concentrations were chosen to be below those reported to have toxic or lethal effects. Exposures were for 24 hours. After this time, the animals were killed, staged, weighed and one gonad from each was harvested for histological analysis. Sections of gonadal tissue were used to determine the sexual phenotype; the animals were identified as male, female or undifferentiated. Where the assignment was not clear, the animals were left out of the sex ratio and steroidogenic factor 1 (SF-1) protein expression determinations.

Exposure to 4-*tert*-octylphenol at all three levels increased the number of individuals who underwent sexual differentiation in stages 32-34 compared to the controls – differentiation was complete in exposed animals by stage 34, whereas it was not completed until stage 36 in the controls. Male differentiation occurred three stages earlier than in the controls; female differentiation occurred one stage earlier. The sex ratios of animals treated with 4-*tert*-octylphenol did not differ from those in the controls at any treatment level. (The authors comment that the exposures here were much shorter than those used by Kloas *et al*. (1999) where changes in the sex ratio were seen.)

In control animals, the expression of SF-1 increased in females at stage 34, and the level dropped in males. At stage 33, a significant increase in expression was seen in females exposed to 10^{-8} M relative to males. At stage 34, males exposed to 10^{-7} M had a significantly different (increased) level compared to other treatment groups and the

controls. At stages 35 and 36, there were no significant differences between the treatment groups. The results were interpreted as suggesting that SF-1 may not play a direct role in testicular differentiation.

Bevan *et al*. (2003) exposed embryos of *X. laevis* at the early gastrula stage to 4-*tert*-octylphenol (see *Table 4.7*). Twenty to thirty embryos were used at each of the exposure levels, which ranged from 10 nM to 10 µM (2 µg/L to 2 mg/L, individual concentrations between not specified). Control and vehicle exposed organisms came from the same mating as the test organisms. Exposures began at stage 10.5 and ended at stage 37 after around 48 hour's treatment; the end stage is at the end of the critical period for the detrimental actions of 17β-estradiol. Embryos were fixed, followed by measurement of body length, interocular distance and body shape, and examination for the numbers of melanocytes (reduced or absent). Significant reductions in length were noted at 500 nM (the next lowest concentration appears to have been 100 nM). (There are no comments on results for the other endpoints in relation to 4-*tert*-octylphenol exposure; it appears that 1 µM and possibly 500 nM increased the percentage with dorsal curvature, and possibly that 1 µM increased the interocular distance.) A difference in the number and pattern of melanocytes was noted for exposure to 4-*tert*-octylphenol, but was not described as significant.

Rohr *et al*. (2003) exposed eggs of the streamside salamander (*Ambystoma barbouri*) to three concentrations of 4-*tert*-octylphenol in water (5, 50 and 500 µg/L nominal) from 2 days after collection for 37 days (see *Table 4.7*). The solutions were changed every 2 days (50% of the volume changed at each time). The concentration in the removed solution showed that the exposure concentrations declined by no more than 4-8% over the period between renewals. There was a significant increase in the time to hatching at the highest concentration, no effect on embryo survival, a significant reduction in larval survival at the highest concentration and a significant reduction in snout-to-vent length (measured on day 50) at the highest concentration.

Van Wyk *et al.* (2003) dosed male *X. laevis* with 4-*tert*-octylphenol by injection on days 1, 7 and 14 of a 28-day experiment (see *Table 4.8*). The dose administered was 100 µg/g/week. Measurements were made on the epithelium height and gland areas for the breeding (nuptial) glands in male frogs. Both measurements differed significantly from control values after 28 days (both were reduced). The plasma testosterone and VTG levels did not differ significantly from those in the controls. Two frogs died during the exposure.

Fish

A range of different types of *in vivo* endpoints in fish have been considered including:

- Biochemical changes (e.g., the production of proteins);
- Histopathological changes in cells and tissues;
- Changes in reproductive activity and sexual development (e.g., 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 known as vitellogenin (VTG) (Chen, 1983). VTG 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, VTG 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, VTG 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 VTG 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 VTG 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 (i.e., male gonads showing 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 VTG. In whole organisms exposure of fish to 4-*tert*-octylphenol in the aqueous phase has been shown to result in the induction of VTG in juveniles and adult males of a range of species (see *Table 4.7*), including rainbow trout *On. mykiss* (Jobling *et al.,* 1996 – valid study; Routledge *et al.,* 1998 – valid study; Pedersen *et al.,* 1999 – 'use with care' study) and roach *Ru. rutilus* (Routledge *et al.,* 1998 – valid study).

The lowest aqueous exposure concentration of 4-*tert*-octylphenol which has been shown to result in a statistically significant induction of VTG in males (relative to controls) is 4.8-10 µg/L in rainbow trout (Jobling *et al.,* 1996, Routledge *et al.,* 1998), with a corresponding NOEC value of 1.6 µg/L. A higher exposure concentration of 100 µg/L was required to elicit VTG production in roach (Routledge *et al.,* 1998) and a similar concentration did not produce any response in zebrafish (Van den Belt *et al*., 2001), whereas a concentration of 11.4 µg/L produced an increase in VTG in medaka (Seki *et al*., 2003). The reason for these differences is unknown – there might be differing inter-species sensitivities, or the response may depend on the breeding condition of the fish and hence the time of year at which they were tested. Data for 17β-estradiol indicate that there is significant induction of VTG in male rainbow trout at concentrations of approximately 10 ng/L, with a corresponding NOEC value of 1-5 ng/L. These data indicate that 4-*tert*-octylphenol is approximately 1000 times less potent than 17β-estradiol for this endpoint (see also section on *in vitro* responses).

Flounder (*P. flesus*) were exposed to 4-*tert*-octylphenol through their diet, at dose levels of 10, 50 and 100 mg substance per kg body weight (Madsen *et al.*, 2002). Fish were force fed through a tube and syringe, and treatment occurred every 2 days for a period of

11 days. A significant increase in plasma VTG levels was noted at all dose levels, with the largest increase at the middle dose of 50 mg/kg. There was a significant accumulation of 4-*tert*-octylphenol in liver and muscle tissue, and the concentrations were correlated with the VTG levels.

Madsen *et al*. (2003) fed adult male flounder (*P. flesus*) a paste that contained 4-*tert*-octylphenol on every second day for a period of 10 days (six administrations) directly into the stomach. The first experiment had three treatment levels, equivalent to 10, 50 and 100 mg/kg bodyweight (bw; the doses were calculated for each fish individually). The concentrations in food were not measured, but similar preparations of spiked food for other work showed less than 30% deviation from nominal. Eight fish were used per exposure level, at 9-10°C. A group of fish were kept in the tanks but not fed to check for any influence of exposure via water. The second experiment had exposure levels of 1, 2.5, 5, 7.5, 10 and 25 mg/kg bw, and a temperature of 5-7°C. Water samples were taken at 0, 6 and 10 days.

Low mortality was seen in all fish. VTG levels were elevated in male fish in the first experiment at all three exposure levels when compared to the controls. The water control fish showed no response (the concentration in the control tank was 0.82 ± 0.09 µg/L, the concentration in water in the tank with the highest dose fish was 3.66 ± 1.8 µg/L).

In the second experiment, no significant difference in VTG levels was seen in fish dosed at the 1 and 2.5 mg/kg bw levels. A significant increase was seen at 10 mg/kg on day 6, and at 5 mg/kg on day 11. As only some of the fish responded at the lower concentrations, a dose–response relation could be constructed. The ED_{50} values were 8.2 and 5.6 mg/kg bw after 6 and 11 days, respectively. The water concentrations were 0.35 ± 0.09 µg/L in the controls, and 0.58 ± 0.22 µg/L in the highest dose. In both experiments there were no significant differences from the controls in the gonadosomatic index (GSI) at any exposure level. For the hepatosomatic index (HSI), a significant difference was only noted at the 50 mg/kg dose level.

The concentration of 4-*tert*-octylphenol in liver and muscle tissues was significantly higher in exposed fish at all levels compared to the controls in experiment 1 – 23 ng/g wwt in liver in the controls, 2195 ng/g wwt in the 10 mg/kg bw dosed fish. Levels in muscle were around 25% of those in liver. 4-*tert*-Octylphenol was measured in fish exposed at all levels in experiment 2; a significant increase was found at 2.5 mg/kg bw in liver, and at 5 mg/kg bw in testis and muscle. It was estimated that around 8% of the applied dose (50 mg/kg bw) was present in the fish at the end of the experiment.

Immature rainbow trout (*On. mykiss*) were dosed with 4-*tert*-octylphenol by administration of spiked food homogenate directly into the stomach after anaesthetising the fish (Pedersen *et al*., 2003). Fish were fed on days 0, 2, 4, 6, 8 and 10 of the experiment, and water samples were taken for analysis after 5 days and at the end of the exposures. The nominal concentrations in the food were 0.04, 0.2, 1 and 5 mg/mL in the first experiment, and 2, 3, 4 and 5 mg/mL in the second. These corresponded to nominal doses of 0.4, 2, 10 and 50 mg OP/kg fish in the first experiment and 20, 30, 40 and 50 mg/kg fish in the second. The measured concentrations in the food were 11-30% lower than nominal in the first experiment, and 10-22% higher in the second. The concentration of 4-*tert*-octylphenol in the water for the control fish was 0.2 ± 0.5 µg/L,

and 6.4 ± 1.8 µg/L in the tanks with the highest dose level. The concentration in water increased with the dose level. The faeces of fish given 10 mg/kg or more contained detectable levels of 4-*tert*-octylphenol.

Plasma VTG levels showed no change in fish dosed at 0.4, 2 and 10 mg/kg fish every second day in either males or females, and no individual fish showed a response. Dosing at 50 mg/kg fish produced a significant increase in males at 6 and 12 days, and a similar pattern was seen in females. In the second experiment, increases were seen in the dose range 30-50 mg/kg fish, in a dose- and time-related pattern, with significant increases in females at 40 mg/kg and in males at 50 mg/kg. No individual fish showed a response at 20 mg/kg, and only some individual responses were seen at 30 mg/kg and 40 mg/kg. Values calculated from the responses were ED_{50} of 34 and 36 mg/kg/2 days at 6 and 12 days, respectively; ED_{10} of 25 and 24 mg/kg/2 days at 6 and 12 days, respectively.

No 4-*tert*-octylphenol was found in the fish fed control food or those fed at 0.4 mg/kg fish. Twenty-four hours after the last feeding, the 2, 10 and 50 mg/kg fish exposures had detectable levels in all muscle samples and some liver samples. In the second study, 4-*tert*-octylphenol was detected in the muscles and livers of all fish in all exposures. It was estimated that at the highest dose, 4-*tert*-octylphenol in the muscle and liver at the end of the experiment accounted for 1-2‰ of the amount fed. The authors considered that most of the dose was absorbed from food and then eliminated, rather than being eliminated in the faeces directly without absorption.

Histopathological changes

A number of studies have investigated whether exposure to 4-*tert*-octylphenol can result in changes in the structure of reproductive tissues in both male and female fish. The endpoints are highly relevant if the modifications are endocrine mediated and impact on reproductive performance. Changes in GSI (ovosomatic or ovarian somatic index, OSI, in females and testis somatic index in males) are measured using the equation [100 x tissue weight/(body weight – tissue weight)].

Jobling *et al.* (1996) reported that there was significant inhibition of testicular growth (as measured by the GSI) in rainbow trout exposed to a single measured 4-*tert*-octylphenol concentration of 39 µg/L for 3 weeks during sexual development (see *Table 4.7*). Exposure of sexually maturing fish to 39 µg/L 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 (0.3-43.9 µg/L) no fish exposed to 4-*tert*-octylphenol displayed any significant differences in gonadal size. This latter study was carried out during the later stages of the reproductive cycle of the fish, whereas the single exposure used fish at an early stage of the cycle. The authors considered that this was likely to have lead to the different responses. No clear mechanism could be attributed to the potential inhibition of testicular growth.

Ashfield *et al.* (1998) investigated the effects of 4-*tert*-octylphenol on the ovosomatic index of female rainbow trout exposed from immediately post-hatch (see *Table 4.*7). In the (use with care) study fish were exposed to nominal 4-*tert*-octylphenol concentrations of 1, 10 and 30 µg/L for 35 days before being exposed to clean water for 431 days. The

ovosomatic index measured in the 4-*tert*-octylphenol exposed groups of fish after 466 days was not significantly different from that measured in the controls.

Guppies (*Poecilia reticulata*) were exposed to 4-*tert*-octylphenol at 100, 300 and 900 µg/L (20 males per group) in a flow-through system for 60 days (Kinnberg and Toft, 2003). The highest exposure was halted after 30 days because of 60% mortality. Measured concentrations were within 14% of the nominal levels, and were not corrected. The controls showed 15% mortality over 30 days (see *Table 4.*7).

The testes of fish exposed to the highest concentration of 4-*tert*-octylphenol had increased numbers of spermatozeugmata located in enlarged sperm ducts. Some ducts were ruptured, resulting in free spermatozoa. Only a few spermatogenetic cysts were seen around the periphery of the testes. Overall, the conversion of early stages to mature sperm took place without the early stages being replenished. The authors considered that the results indicated that an endocrine-mediated pathway was involved, as the changes were not as a result of the degradation of spermatogenetic elements, but rather as a change in the developmental stage.

The study does not derive a no-effect concentration. As it refers to effects at higher concentrations, it might be implied that such effects were not seen at the lowest (100 µg/L) concentration, but this is not confirmed. An LC_{50} of <900 µg/L could also be deduced from the results, as 60% mortality was seen at this concentration. As no excess deaths were noted at 300 µg/L, the NOEC for mortality appears to be 300 µg/L.

The effects of 4-*tert*-octylphenol on the gonads of guppies (*Po. reticulata*) exposed as adults via water or as embryos via the mother were investigated by Kinnberg *et al.* (2003; see *Table 4.*7). Exposures were carried out in a flow-through system. Initially, 20 tanks were populated with two male and two female fish each; when one female gave birth, the offspring and the other female were removed from the tank, and the remaining fish were exposed to 4-*tert*-octylphenol, 17β-estradiol or an acetone control. Males were exposed for 28 days, females were exposed until birth (26-36 days). The females were sampled after birth, and their offspring were raised in clean water for 70 days. The gonads of the parent fish were examined. Secondary sexual characteristics of the offspring were recorded, and gonadal examination was also carried out, including staging of the testes and ovas. The exposure concentration was monitored throughout, and was 26 ± 8 µg/L for 4-*tert*-octylphenol.

In the parent fish, there were no differences in survival or in the GSI for fish exposed to 4-*tert*-octylphenol (females exposed to 17β-estradiol had reduced GSI). Fish exposed to 4-*tert*-octylphenol (and to 17β-estradiol) tended to show the transformation of early to late stage in the testes, without corresponding replenishment of the earlier stages. The ovaries of control and octylphenol-exposed fish contained all stages of oocytes, although the amount of yolk oocytes appeared to be reduced in octylphenol-exposed fish.

In studying the offspring, only larger brood sizes were included in the analysis, as guppies tend to have naturally varying brood sizes. (There were no significant differences in brood sizes between control and treated fish.) There were no differences in weight, length or gonopodium index for octylphenol-exposed males with a developed gonadopodium. The distribution between sexes was not different between the exposed and control groups, and the numbers of fish not showing secondary sexual

characteristics were not different in all three exposure groups. The evaluation of sexual distribution was based on gonad histology, and the development of the gonads was not significantly altered by exposure to 4-*tert*-octylphenol or to 17β-estradiol. There was a tendency for the testis and ovary to be at more advanced stages in the offspring of treated mothers.

Young (up to 6 days old) guppies (*Po. reticulata*) were exposed to 4-*tert*-octylphenol for 90 days, with 50 fish in each exposure tank (Toft and Baatrup, 2003). The exposure levels used were 1, 10 and 100 µg/L in the first experiment and 100 and 200 µg/L in the second. The measured concentrations in the first experiment were 1.7, 11.7 and 149 µg/L (see *Table 4.7*).

Mortality in the groups exposed to 1 or 10 µg/L was similar to or less than that in the controls. At 100 µg/L, eight fish died in the first experiment, 20 in the second; 22 fish died at 200 µg/L. Exposure to 4-*tert*-octylphenol had no effect on the sex ratio, which was 50:50 as in the controls. The length of males was significantly increased at 200 µg/L. The gonopodium (copulatory organ) length was increased at all exposure concentrations, but only significantly at 100 µg/L. Male colouration (orange spots which are attractive to females) was markedly reduced on exposure to 200 µg/L, but not at lower concentrations. The intensity of the orange colouration was also reduced at 200 µg/L.

The ovary weights of females were markedly reduced after exposure to all 4-*tert*-octylphenol concentrations. This was generally reflected in the number of mature oocytes or embryos in the ovaries. Expressed as a percentage of body weight (i.e., the GSI) a significant reduction was seen at 100 µg/L. Testes weights were not affected by exposure to 4-*tert*-octylphenol. Sperm count tended to increase with increasing concentration, but was significantly different to controls only at 100 µg/L.

The sexual behaviour of exposed males was examined after the end of the exposures, by pairing individual males with mature non-exposed females. A significant increase in the time males spent in posturing behaviour was seen for those exposed to 100 µg/L. The number of displays was also increased, but not significantly as there was considerable variation in the controls.

Sand goby (*Pomatoschistus minutus*) were exposed to 4-*tert*-octylphenol for 28 days in a dose-ranging study and for 6 months in a temporal response study (Robinson *et al*., 2004; see *Table 4.7*). The sand goby is an estuary or inshore marine fish, which can spend all of its life-cycle in an estuary, and was selected as a sentinel species for the UK Endocrine Disruption in the Marine Environment (EDMAR) programme. The fish in this case came from the Ythan estuary in north-east Scotland. Exposures took place in a flow-through system using seawater with nominal concentrations of 10, 30, 100 and 300 µg/L for the 28 day study. The concentrations were measured in samples taken from the outlets of the tanks each week, and the mean measured levels were 3, 20, 31 and 101 µg/L, respectively. No treatment related effects were seen on the GSI or HSI, nor on male sperm duct gland somatic index (SDGSI), urogenital pupilla length index (UGPLI) or nuptilla colouration. Males exposed to 31 and 101 µg/L had elevated VTG mRNA expression.

The longer-term exposures began in winter, and the temperature varied between 5.9°C in February and 12.5°C in May. Three exposure levels were used, with measured levels of 7, 28 and 119 µg/L (these are median concentrations as a small number of extreme concentrations were observed). Mortality in the 119 µg/L exposure was 100% after 7 weeks, while the mortality in the 28 µg/L exposure was significantly greater than in the controls. (The 7 µg/L exposure had to be terminated after 8 weeks when the water supply failed.) No treatment-related effects on weight, length, HSI or UGPLI were noted for males or females. The GSI showed the same seasonal variation in controls and the exposed fish. The SDGSI increased in the control fish as they matured; this was not seen in the 28 µg/L exposure, and the SDGSI was significantly lower in the exposed fish after 46 and 80 days (the SDGSI was also reduced in the 7 µg/L exposures, although not significantly). Nuptilla colouring showed a similar pattern, the colouration score increasing in the controls, but being inhibited in exposed fish. Exposure to 7 µg/L did not increase VTG mRNA expression compared to the controls; at 28 µg/L mRNA expression was not elevated at 27 days, but was significantly elevated compared to the controls at 46 days.

Changes in reproductive success and development

True reproductive success can only really be measured at the level of the population where fertilisation occurs and viable young are produced, which can themselves develop and reproduce, thus leading to a stable population. There are many endpoints in this cycle that can be measured, such as fertilisation rate and/or percentage, hatching of eggs, survival of hatchlings and development of eggs, embryo and/or fry. However, using any one of these in isolation still leaves considerable uncertainty in the overall ecological significance of the results.

Gray *et al.* (1999a) investigated the effects of 4-*tert*-octylphenol (and 17β-estradiol) on the sexual differentiation and development of Japanese medaka (*O. latipes*) in a series of (use with care) static renewal studies (see *Table 4.*7):

Study 1: Medaka were exposed to a nominal concentration of 100 µg/L beginning at 1, 3, 5, 7 21 and 35 days post-hatch and continuing to 100 days post-hatch.

Study 2: Medaka were exposed to a nominal concentration of 100 µg/L beginning at 1 day post-hatch 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 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 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 17β-estradiol control (100 µg/L) was used in all the studies.

For exposures beginning at 1, 3, 7, 21 and 35 days post-hatch (study 1), the incidence of testis-ova (an intersex condition) at 100 days post-hatch was highest (and statistically significant) in the 3 day post-hatch treatment (29%) and declined when exposures were

initiated with older fry. In study 2, exposure to 100 µg/L 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 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 treatment. Overall, these data indicate that only prolonged exposure of male medaka to 4-*tert*-octylphenol, 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 in the same species (medaka). In this (use with care) study all of the male fish were exposed, but the females were divided into exposed and unexposed groups. The nominal exposure concentrations were 10, 25, 50 and 100 µg/L, 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*-octylphenol. Eggs produced by unexposed females from reproduction trials 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. Within the trials eggs produced by unexposed females mated with males from the 10, 50 and 100 µg/L treatments (but not the 25 µg/L treatment) were significantly 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 treatments (but not the 50 µg/L 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:

1. Unexposed females mating with exposed males in the 10 and 25 µg/L treatments (but not the 50 and 100 µg/L treatments);
2. Exposed females mating with exposed males in the 10 µg/L treatments (but not the 25, 50 and 100 µg/L treatments).

These developmental problems included circulatory system difficulties, incomplete eye development (anisophthalmia) and failure to inflate the swim bladder upon hatch.

Gray *et al.* (1999b) concluded that “Exposure to 4-*tert*-octylphenol 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 4-tert_octylphenol.” 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. This makes it difficult to identify suitable effect levels for use in a risk assessment. Some developmental abnormalities were recorded in the eggs and fry of both exposed and unexposed females fertilised by exposed males, although only at 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 that one male fish with the intersex condition was able to fertilise eggs.

Gronen *et al.* (1999) investigated the effects of 4-*tert*-octylphenol on reproductive impairment in adult male Japanese medaka (*O. latipes*) and the link to induction of VTG (see *Table 4.*7). Organisms were exposed to nominal concentrations of 20, 50, 100 and 300 µg/L for 21 days, with chemical analysis showing actual exposure concentrations of 20, 41, 74 and 230 µg/L. At the end of the exposure period measurements were made of serum VTG 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 of exposure increased with increasing 4-*tert*-octylphenol concentrations, but decreased after 4-*tert*-octylphenol 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 appear to be closely linked phenomena. Histological examination indicated that spermatogenesis in 4-*tert*-octylphenol-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, which suggests that 4-*tert*-octylphenol may be teratogenic as well as oestrogenic.

Japanese medaka (*O. latipes*) were exposed to 4-*tert*-octylphenol from fertilised eggs to 60 days post-hatch under flow-through conditions (Seki *et al*., 2003; see *Table 4.*7). Sexual differentiation and hepatic VTG induction were examined, as well as embryological development, hatching success, post-hatch survival and growth. The study also investigated whether changes seen were reversible after transfer to clean water. The nominal exposure concentrations were 6.25, 12.5, 25, 50 and 100 µg/L. Exposures begin at less than 12 hours after fertilisation, with 60 embryos per treatment. Concentrations in the exposures were measured at least once per week. Measured concentrations ranged from 91.9% to 111% of nominal. The results are based on the mean measured concentrations, which were 6.94, 11.4, 23.7, 48.1 and 94.0 µg/L.

There were no significant effects on hatchability or on the time to hatch in any of the exposure groups. No abnormal behaviour or appearance was noted in any of the exposure groups. Post-hatch mortality was elevated in the 23.7 µg/L exposure group, but not in any of the others. There were no significant differences in total length or body

weight in fish exposed to 4-*tert*-octylphenol, with the exception of the 23.7 µg/L group, in which both of these measurements were increased over those in the controls.

Based on an examination of the external sex characteristics, the sex ratio in the exposed fish was affected at concentrations of 48.1 and 94.0 µg/L. The ratio was skewed towards females, with no males at the highest concentration. The ratio was also skewed at 23.7 µg/L, but not significantly so. From histological examination post-exposure, testis-ova were found in fish exposed to 11.4 µg/L and higher concentrations, and the sex ratio based on the gonadal histology was significantly different from that in the controls at 48.1 µg/L and above. Spermatogenesis was observed in all specimens with testis-ova, and spermatocytes and spermatids could be differentiated. The testes of medaka treated with 6.94 µg/L 4-*tert*-octylphenol were histologically identical to those in the controls. No histological abnormalities were noted in the ovaries of fish exposed at concentrations up to and including 48.1 µg/L; fish at higher exposure levels showed regressed condition of the ovaries. Hepatic VTG was induced in a dose-dependent manner in exposed fish. Levels of VTG in male fish exposed to 11.4 µg/L and above were significantly higher than the levels in the controls; for female fish, levels were significantly higher than in the controls at exposure levels of 48.1 µg/L and above. The study concluded that the LOEC for abnormal sexual differentiation and VTG induction was 11.4 µg/L.

Fish from the highest exposure level were transferred to clean water. All fish in this exposure showed only female external sex characteristics on transfer. After 30 days in clean water, two of the 16 fish showed secondary male characteristics; the number increased to six fish after 60 days. The results suggest that 4-*tert*-octylphenol may have acted to depress the natural androgen levels in genetically male fish, resulting in the feminisation of the secondary sexual characteristics. Transfer to clean water allows the androgen levels to recover.

Toft and Baatrup (2001) assessed the effects on the sexual characteristics of adult male guppies (*Po. reticulata*) of short-term exposure to 4-*tert*-octylphenol (and 17β-estradiol). In the study groups of fish were initially exposed to nominal 4-*tert*-octylphenol concentrations of 100, 300 and 900 µg/L 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 treatments were then divided into two groups:

- One group was exposed to 100 and 300 µg/L 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 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 treatments (but not the 900 µg/L treatment) caused significant increases in the sperm counts after 30 days, while only the 300 µg/L treatment (but not the 100 µg/L 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 treatments (but not in the 100 µg/L treatment) after 30 days, but only in the 300 µg/L treatment (but not in the 100 µg/L treatment) after 60 days. Significant increases in sperm count and decreases in coloration index were measured after 30 days exposure to a nominal 17β-estradiol concentration of 1 µg/L.

Preliminary results on male reproduction capability indicated that males exposed to the highest exposure concentration of 900 µg/L 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 produced larger numbers of offspring (8.2 per untreated female). Exposure of males to a nominal 17β-estradiol concentration of 0.1 µg/L 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) 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 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. The study consisted of three periods and was initiated with 100 fertilised eggs of unexposed zebra fish in each test set (*Table 4.6*). The first 42 days of the full life-cycle test corresponded to the fish early life stage toxicity test (with the 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 (the 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 as in *Table 4.6*.

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 were 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 (in controls mean length = 2.0 ± 0.2 cm compared to 1.9 ± 0.16 cm at 35 µg/L).
- Time to first spawning was significantly delayed by 3-4 weeks at 35 µg/L (in controls time to first spawning = 104-116 days compared to 132-138 days at 35 µg/L).
- The total number of eggs per test female and day was significantly reduced at 35 µg/L (in controls mean total number of eggs per female and day = 56.6 compared to 11.6 at 35 µg/L).
- The fertilisation capacity (%) and cumulative number of fertilised eggs was significantly reduced at 35 µg/L (in controls fertilisation capacity = 86.7% compared to 30.3% at 35 µg/L).

No effects on the sex ratios of resulting adult fish were evident at any exposure concentration.

Table 4.6 Surveillance timing for zebrafish test (Wenzel *et al.*, 2001)

Period	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 period 3, even at the highest concentration of 35 µg/L, 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 (*Da. rerio*) using spawning and fertilisation success, GSI and plasma VTG levels as endpoints (see *Table 4.*7). Adult male and female zebrafish were exposed under semi-static (daily renewal) conditions to 12.5, 25, 50 and 100 µg/L for 3 weeks and analytical confirmation of exposure levels was conducted. The experiments were performed twice with, respectively, five and seven successful breeding 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. 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 percentage of spawning females and the males with a post-exposure fertilisation above 70% were counted. For each treatment group the mean of the percentage of successful spawning females and the mean of the percentage of fertile males were calculated. After 5 days of evaluation plasma was collected for VTG analysis and GSIs (ovosomatic or OSI in females and testis somatic index in males) were measured. To allow improved interpretation of the GSI 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 OSI in females exposed to 25, 50 and 100 µg/L (but not 12.5 µg/L) 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.

Segner *et al*. (2003a) presented a qualitative summary of the findings of the IDEA project (identification of endocrine disrupting effects in aquatic organisms). The aim of the project was to investigate what parameters and endpoints allow the identification of endocrine-mediated effects on development and reproduction in zebrafish (*Da. rerio*). 4-*tert*-Octylphenol was one of the substances tested. In full life-cycle studies on zebrafish, 4-*tert*-octylphenol showed induction of VTG, alterations to gonad differentiation, delay of first spawning and reduced fertilisation success. The pattern of effects is similar to that produced by EE2, but at higher concentrations with 4-*tert*-octylphenol. The effects were partly irreversible on transfer to clean water. Partial life-cycle exposures produced lasting effects only when the exposure was during the period of juvenile bisexual gonad differentiation.

A full life-cycle exposure test has been conducted with zebrafish, *Da. rerio* (Segner *et al*., 2003b). Four concentrations were used, at nominal levels of 1.2, 3.7, 11.9 and 38 µg/L, with two replicates of each. Exposures were carried out under flow-through conditions; concentrations were measured and were close to nominal, so the results are based on the nominal concentrations. One hundred fertilised eggs were used per exposure vessel and exposure was continued until the fish reached sexual maturity. After 42 days, the numbers of fish in each tank were adjusted to 50, and were reduced again to 30 at days 75-78. The parameters monitored or measured were mortality, behavioural anomalies, growth, time to first spawning, egg production and fertilisation success. The authors comment that lifetime exposure of zebrafish to oestrogenic compounds alters a number of reproductive parameters, but fertilisation is affected most consistently and reproducibly. Therefore the endpoint chosen from this study, and for comparison with a number of *in vitro* studies reported in the same article, was fertilisation success. The result was an EC_{50} of 136 nM (28 µg/L).

Knörr and Braunbeck (2002) exposed Japanese medaka (*O. latipes*) to 0, 2, 20 and 50 µg/L of octylphenol in a flow-through system. Concentrations were monitored, and differed by less than 10% from the nominal concentrations indicated above. Exposure was from 2 to 4 hours post-fertilisation until maturity, which occurred at 12-13 weeks post-hatch in all experimental groups (a further group exposed to 100 µg/L octylphenol all died within 72 hours post-fertilisation). Different numbers of eggs were used in each exposure.

All three exposure levels resulted in 30-40% mortality (combined before and after hatch; see *Table 4.*7). At 2 µg/L 28.6% mortality was seen before hatching, with 3.6% after hatching. Control mortality was 10% (before and after). Mortality pre-hatch was not

related to the concentration of octylphenol; post-hatch mortality increased in a dose-dependent manner.

The length and weight of fish (both male and female) were reduced significantly at the highest concentration tested, but not at lower concentrations. The report notes that control males and all fish exposed to 2 µg/L octylphenol were significantly smaller than the control female fish. At the higher concentrations no differences between the sexes could be detected.

Sex ratios in the controls were biased towards males, with 41:59 female:male in the water control and 42:58 in the solvent control (0.01% dimethyl sulphoxide was used in preparing the exposure solutions). In the exposed animals the ratios were reversed, with 55:45 female:male at 2 µg/L after 14 weeks; there was a slight reduction in the preponderance of females at the higher exposure levels. Testis-ova was noted in 3% of the animals across the three exposure levels (four fish in total, two in the lowest exposure level and one in each of the other two levels).

After reaching maturity, fish were used in tests to investigate the effects on fertility and reproductive success. Exposed fish were mated and eggs collected 2-4 hours post-fertilisation. In addition, exposed males were mated with unexposed females and vice versa. For mating of exposed males and females, the fertilisation rate dropped from 81% in the controls to 73% at 2 µg/L, 70% at 20 µg/L and 69% at 50 µg/L. There was a slight increase in the mean number of eggs produced per fish per day in the exposed fish, but this was not statistically significant. Mortality in the F_2 generation did not show a clear trend, although the overall mortality (pre- and post-hatch) was higher for offspring of exposed fish than for those of control fish. Mortality of the progeny of exposed females and control males showed a dose-related increase, with significantly increased mortality at 20 and 50 µg/L. The reverse combination (exposed males with control females) showed a significant increase in mortality only at 50 µg/L. Fertilisation rates for exposed females with control males were higher than in the controls, whereas for the reverse combination the rates were reduced.

Karels *et al*. (2003) exposed sheepshead minnows (*C. variegatus*) to 4-*tert*-octylphenol in filtered natural seawater adjusted to around 14 g/L salinity, at pH 8.54 and 27°C. The exposure concentrations were measured as 11.5, 33.6 and 66.1 µg/L. VTG levels increased significantly over those in the controls at all exposure concentrations. The number of testes with abnormalities was significantly increased at 33.6 and 61.1 µg/L. Exposed males were mated with unexposed females; the number of viable eggs was significantly reduced at 33.6 and 61.1 µg/L (see *Table 4.*7). The authors also noted a decrease in the lobules containing the later stages of sperm development in the gonads, in particular spermatozoa. Most lobules contained the earlier stages of cell development. No significant differences were observed in the number of abnormal fry, nor in the percentage of embryo and fry survival in the F_1 generation to 3 days post-hatch, so the sperm capable of fertilising eggs were functionally undamaged.

Pregnant female eelpout (*Zoarus viviparus*) were obtained from the wild and acclimated for a week before being exposed to 4-*tert*-octylphenol in a continuous flow-through system at nominal concentrations of 25 and 100 µg/L (Rasmussen *et al.*, 2002). Samples of the exposure medium were taken every three days for analysis. Exposures lasted for up to 35 days, beginning at the time when the embryos of the pregnant eelpout

were in the late yolk-sac phase. After exposure, the fish were weighed, their total length measured, and blood and ovarian fluids collected. The liver and ovaries were removed and weighed. Embryos were dissected from the ovaries, anaesthetised, counted and scored for survival.

The concentration in water declined after the addition of the fish, but remained reasonably stable at 8-17 µg/L (nominal 25 µg/L, OP25) and 57-79 µg/L (nominal 100 µg/L, OP100). The mean concentrations were 14 µg/L and 65 µg/L. The concentrations of 4-*tert*-octylphenol measured in blood plasma were 5026 µg/L and 54904 µg/L in the OP25 and OP100 exposure groups respectively. The mean concentrations in ovarian fluids were 4.8 µg/L and 1421 µg/L at the lower and higher exposure levels respectively.

The GSI increased in the controls with time. The GSI in exposed fish tended to be lower than that in the controls, but only significantly so in the OP25 exposure group. The HSI of the controls decreased significantly during the experiment; the higher dose fish had significantly higher HSI than the controls after 35 days. The mortality in the embryos was significantly higher in the OP100 exposure group than in the controls. The mass and length of the embryos were significantly reduced in both exposures compared to the controls after 35 days. This may be attributed to disturbances in the maternal and foetal trophic relationship (i.e., effects on the mother).

At the start of the experiment 48% of the embryos in the control group had female gonads (early differentiated ovary) and 52% had presumptive male gonads. After 35 days, 52% of the controls had female gonads (ovary with oocytes) and 48% had presumptive male gonads. The embryos in the lower exposure group had gonads that were similar in appearance to those in the control group, with 58% having ovaries with oocytes after 35 days and 42% having presumptive male gonads. At the higher exposure level 46% had normal ovaries with primary oocytes after 35 days, but only 22% had normal presumptive male gonads resembling those in the controls. The remaining 32% of embryos had abnormal gonads, which exhibited both male and female features.

The authors concluded that the study showed that 4-*tert*-octylphenol can be transferred from water via the mother fish to the ovarian fluid and can subsequently affect the embryos.

Changes in behaviour

Bayley *et al.* (1999) investigated the effects of 4-*tert*-octylphenol (and 17*β*-estradiol) on the sexual behaviour of adult male guppies (*Po. 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 µg/L (a predicted exposure concentration of 42 µg/L). A nominal 17β-estradiol concentration of 10 µg/L also caused a marked reduction in the rate and intensity of sexual display.

In vivo systems involving fish exposed via intra-peritoneal injections

Table 4.8 summarises the *in vivo* data from a series of studies in which different fish species were given intra-peritoneal injections of 4-*tert*-octylphenol. The environmental relevance of these studies are low because of the route of administration, 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 VTG in certain studies (Pedersen *et al.*, 1999; Andreassen and Koorsgaard, 2000). Pedersen *et al.* (1999) in a study on the *in vivo* oestrogenic activity of linear and branched alkylphenols found that exposure to a given level of 4-*tert*-octylphenol by aqueous exposure or intra-peritoneal injection resulted in similar ultimate levels of VTG induction, though the timing of production differed.

Octylphenol was administered by intra-peritoneal injection to rainbow trout (*On. mykiss*) on days 1, 4 and 7 of a study examining the effect on the expression of cytochrome P450 isoforms in rainbow trout liver (Katchamart *et al.*, 2002). Fish were sacrificed on day 9. Octylphenol (and other chemicals) caused an increase in the plasma VTG levels, decreased hepatic lauric acid hydroxylase activity and repressed the expression of cytochrome P450 isoforms in rainbow trout liver. The first two endpoints were significantly different from the controls; the last one was not.

Table 4.7 Summary of *in vivo* studies on aquatic vertebrates involving 4-*tert*-octylphenol 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	Study Validity
Amphibians					
African clawed frog *Xenopus laevis*	2-3 days post-hatch larvae	Semi-static: 2.1 and 21 µg/L (nominal)	Significant increase (relative to controls) in proportion of females at metamorphosis after 84 days exposure to 21 µg/L with a NOEC of 2.1 µg/L	Kloas *et al.* (1999)	Use with care – nominal concentrations, and question over reproducibility
	Embryos, stage 10 to 11	Static; 2 µg/L to 2 mg/L (nominal, intervals not specified)	48-hour exposure to stage 37. Significant reduction in length at 103 µg/L, NOEC 26 µg/L	Bevan *et al*. (2003)	Use with care – nominal concentrations, levels not specified
Bullfrog *Rana catesbeiana*	Tadpoles, stages 32 to 36	Static; 10^{-7}, 10^{-8} and 10^{-9} M (0.2, 2 and 20 µg/L)	24-hour exposure. Earlier completion of sexual differentiation at all concentrations; no change to sex ratios	Mayer *et al*. (2003)	Use with care – nominal concentrations
Streamside salamander *Ambystoma barbouri*	Eggs	Static renewal. 5, 50 and 500 µg/L (measured)	35 days exposure. Significant effects on time to hatch, larval survival and snout-vent length at 500 µg/L	Rohr *et al*. (2003)	Valid (but wide separation of levels)
Leopard frog *Rana pipiens*	Newly hatched tadpoles	Static; 1 nM and 1 µM (0.2 and 200 µg/L)	10 day exposure. Altered gene expression in hypothalamic tissue at 200 µg/L, NOEC 0.2 µg/L. No effects on body weight or development at 200 µg/L	Crump *et al*. (2002)	Use with care – nominal concentrations widely separated
Snapping turtle *Chelydra serpentina*	Hatchling and 1-year-old males	Static	17 day and 35 day. Altered gene expression in hypothalamic tissue at 10 µg/L (LOEC). No effect on growth or feeding rate at 10 µg/L	Trudeau *et al*. (2002)	Use with care – nominal concentrations
Fish (freshwater)					
Rainbow trout *Oncorhynchus mykiss*	Adult males (2-year-old)	Flow through: 38.5 µg/L only (measured)	Significant increase (relative to controls) in plasma VTG levels after 21 days exposure at 38.5 µg/L Significant decrease (relative to controls) in testicular growth after 21 days exposure at 38.5 µg/L Significant inhibition of spermatogenesis (relative to controls) after 21 days exposure at 38.5 µg/L	Jobling *et al.* (1996)	Use with care – single exposure level
		Flow through: 0.3, 0.6, 1.6, 4.8 14.6 and 43.9 µg/L (measured)	Significant increase (relative to controls) in plasma VTG levels after 21 days exposure at 4.8 µg/L with a NOEC of 1.6 µg/L No significant effect (relative to controls) on gonadal size after 21 days exposure at any test concentration		Valid
	Post-hatch females	Flow through: 1.0, 10 and 30 µg/L (nominal)	No significant effect (relative to controls) in ovosomatic index in females after 35 days exposure to any test concentration	Ashfield *et al.* (1998)	Use with care – nominal concentrations
	Adult males	Flow through: 1.0, 10 and 100 µg/L (measured)	Significant increase (relative to controls) in plasma VTG levels after 21 days exposure at 100 µg/L	Routledge *et al.* (1998)	Valid
	Juveniles (103-168 g)	Flow-through: 41µg/L only (measured)	Significant increase (relative to controls) in plasma VTG levels after 9 days exposure at 41µg/L	Pedersen *et al.* (1999)	Use with care – single exposure level
	Juvenile	Static, 30 µg/L	Significant induction of VTG	Van den Belt *et al.* (2003)	Use with care – single exposure level

Table 4.7 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	Study Validity
Roach *Rutilus rutilus*	Adult females	Flow through: 1.0, 10 and 100 µg/L (measured)	No significant effect (relative to controls) in plasma VTG levels after 21 days exposure at 100 µg/L	Routledge *et al.* (1998)	Valid
	Adult males	Flow through: 1.0, 10 and 100 µg/L (measured)	Significant increase (relative to controls) in plasma VTG levels after 21 days exposure at 100 µg/L		
Guppy *Poecilia reticulata*	Adult males	Flow through: 150 µg/L only (nominal)	Significant decrease (relative to controls) in the rate and intensity of sexual display after 28 days exposure followed by 10 days in clean water at 150 µg/L	Bayley *et al.* (1999)	Use with care – single exposure level
	Adult males	Flow through: 100 and 300 µg/L only (measured)	Significant increase (relative to controls) in sperm count after 30 days exposure at 100 µg/L Significant increase (relative to controls) in sperm count after 60 days exposure at 300 µg/L	Toft and Baatrup (2001)	Valid
		Flow through: 100, 300 and 900 µg/L (measured)	Significant decrease (relative to controls) in colouration index after 30 and 60 days exposure at 300 µg/L Significant decrease (relative to controls) in GSI after 60 days exposure at 900 µg/L		
	Adult males	Flow through: 100, 300 and 900 µg/L (measured)	60% mortality after 30 days exposure to 900 µg/L. No excess mortality over controls at 100 or 300 µg/L to 60 days Conversion of early sperm stages to mature sperm without replenishment of early stage seen at higher concentrations	Kinnberg and Toft (2003)	Valid
	Adults and offspring to 70 days post-hatch	Flow through, 26 ± 8 µg/L (measured)	Parent: no effects on survival or GSI Offspring: no difference in weight, length or gonadopodium index in males compared to controls. No difference in numbers of fish showing no secondary sexual characteristics compared to controls. No effects on gonadal development compared to controls	Kinnberg *et al.* (2003)	Use with care – single exposure level
	Up to 6 days old	Flow through; 1.7, 11.7, 149 and 200 µg/L (measured)	90 days. Significant increase in mortality and gonadopodium length, changes in colouration, GSI in females and sexual behaviour (all at 149 µg/L)	Toft and Baatrup (2003)	Valid (but wide separation of levels)

Table 4.7 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	Study Validity
Japanese medaka *O. latipes*	Embryos (1-35 days post-hatch)	Static renewal: 100 µg/L only (nominal)	Significant increase (relative to controls) in 3 days post-hatch males developing testis ova at 100 days post-hatch at 100 µg/L. No significant effects in 1, 7 21 and 35 days post-hatch organisms at 100 µg/L No significant effect (relative to controls) on sex ratio at any exposure scenario	Gray *et al.* (1999a)	Use with care – nominal concentrations
	Embryos (1 day post-hatch)	Static renewal: 100 µg/L only (nominal)	No significant effect (relative to controls) in 1 day post-hatch males developing testis ova after 3 months exposure at 100 µg/L No significant effect (relative to controls) on sex ratio at any exposure scenario		
	Adult males	Static renewal: 200 and 300 µg/L (nominal)	Significant degeneration (relative to controls) of testicular tissue after 18 and 36 days exposure at 100 µg/L No significant effect ratio (relative to controls) on sex at any exposure scenario		
	Embryos (1 day post-hatch)	Static renewal: 10, 25 and 50 µg/L (nominal)	Significant decrease (relative to controls) in number of approaches of males after 6 months exposure at 50 µg/L with a NOEC of 25 µg/L Significant decrease (relative to controls) in number of circles of males after 6 months exposure at 25 µg/L with a NOEC of 10 µg/L Significant decrease (relative to controls) in number of copulations of males after 6 months exposure at 50 µg/L with a NOEC of 25 µg/L 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 with a NOEC of 10 µg/L	Gray *et al.* (1999b)	Use with care – nominal concentrations

Table 4.7 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	Study Validity
Japanese medaka *O. latipes*	Adult males	Flow through: 20, 41, 74 and 230 µg/L (measured)	Significant decrease (relative to controls) in egg production (eggs/day) in males exposed for 120 days at 20 µg/L followed by 9 days in clean water and mated with unexposed females No significant effect in fertilisation rate of eggs or percent survival of embryos produced by unexposed females mated with males exposed for 12 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 12 days at 20 µg/L followed by 9 days in clean water	Gronen *et al.* (1999)	Valid
	Adult males	Flow through: 20, 41, 74 and 230 µg/L (measured)	Significant decrease (relative to controls) in egg production (eggs/day) in males exposed for 120 days at 20 µg/L followed by 9 days in clean water and mated with unexposed females No significant effect in fertilisation rate of eggs or percent survival of embryos produced by unexposed females mated with males exposed for 12 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 12 days at 20 µg/L followed by 9 days in clean water		
	2-4 hours post-fertilisation to 12-13 weeks post-hatch	Flow through: 0, 2, 20 and 50 µg/L (measured)	Increased mortality pre- and post-hatch combined at all levels compared to controls Length and weight of fish significantly reduced compared to controls at 50 µg/L Sex ratio altered at all levels compared to controls Reduction in fertilisation rate in eggs from exposed fish relative to controls, reduced by >10% at 20 µg/L Significant increase in mortality relative to controls for offspring of exposed fish mated with control fish, for exposed males at 20 µg/L, for exposed females at 50 µg/L	Knorr and Braunbeck (2002)	Valid
	Fertilised eggs	Flow through: 6.94, 11.4, 23.7, 48.1 and 94 µg/L (measured)	Exposure to 60 days post-hatch. No effects on hatchability, time to hatch, post-hatch mortality. Sex ratio affected at 48 and 94 µg/L; testis-ova found at 11.4 µg/L and above; VTG increased significantly at 11.4 µg/L.	Seki *et al*. (2003)	Valid

Table 4.7 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	Study Validity
Zebrafish *Danio rerio*	Fertilised eggs (F_0 generation)	Flow through: 1.2, 3.2, 12 and 35 µg/L (measured)	No significant effect (relative to controls) on survival or growth of fish at any test concentration	Wenzel *et al.* (2001)	Valid
	38-day-old animals	Flow through: 1.2, 3.2, 12 and 35 µg/L (measured)	Significant reduction (relative to controls) in growth of fish at 35 µg/L , with a NOEC of 12 µg/L Significant increase (relative to controls) in the time to first spawning of fish at 35 µg/L , with a NOEC of 12 µg/L Significant reduction (relative to controls) in total number of eggs per female and day, fertilisation capacity and cumulative number of fertilised eggs of fish at 35 µg/L , with a NOEC of 12 µg/L No significant effect (relative to controls) on sex ratios at any test concentration		
	Fertilised eggs (F_1 generation)	Flow through: 1.2, 3.2, 12 and 35 µg/L (measured)	No significant effect (relative to controls) on survival or growth of fish at any test concentration		
	Adult males	Semi-static: 12.5, 25, 50 and 100 µg/L (measured)	No significant effect (relative to controls) on spawning success or plasma VTG concentration at any exposure concentration Significant decrease (relative to controls) in ovarian somatic index at 25 µg/L with a NOEC of 12.5 µg/L	Van den Belt *et al.* (2001)	Valid
	Adult females		No effect (relative to controls) on male fertilisation success, testis somatic index or plasma VTG concentration at any exposure concentration		
	Fertilised eggs	Flow through. 1.2, 3.7, 11.9 and 38 µg/L (measured)	Lifetime exposure. Endpoint selected for reporting was fertilisation success, EC_{50} 28 µg/L	Segner *et al*. (2003b)	Valid
	Juvenile	Static: 12.5-100 µg/L (nominal)	No significant induction of VTG at any level	Van den Belt *et al.* (2003)	Use with care – nominal concentrations
Fish (estuarine or marine; tested in seawater)					
Sand goby *Pomatoschistus minutus*	Taken from wild	Flow through: 3, 20, 31 and 101 µg/L (measured)	28 day. Elevated VTG at 31 µg/L	Robinson *et al*. (2004)	Valid
Sheepshead minnow *Cyprinodon variegatus*	8-9 months	Intermittent flow through. 11.5, 33.6 and 68.1 µg/L (measured)	24 days exposure. Significant elevation of VTG at all levels; testicular abnormalities at 33.6 µg/L	Karels *et al*. (2003)	Valid

Table 4.8 Summary of *in vivo* studies on aquatic vertebrates involving 4-*tert*-octylphenol 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
African clawed frog *Xenopus laevis*	Adult males	Intra-peritoneal, on days 1, 7 and 14 of 28. Dose equivalent to 100 mg/kg/week	Reduction in epithelium height and gland area for breeding (nuptial) glands. No change to plasma testosterone or VTG	Van Wyk *et al.* (2003)	Use with care – single exposure level
Rainbow trout *Oncorhynchus mykiss*	Juveniles (103-168 g)	Intra-peritoneal injection – 50 mg/kg	Significant increase (relative to controls) in plasma VTG levels after 12 days	Pedersen *et al.* (1999)	Use with care – single exposure level
	Embryos – eyed (21 days post-fertilisation)	Injection – 0.01, 0.1 and 1.0 mg/kg	No significant effect (relative to controls) on sexual development after 6 months	Carlson *et al.* (2000)	Valid
Eelpout *Zoarces viviparous*	Adult males	Intra-peritoneal injection – 10 mg/kg on days 2 and 14	Significant increase (relative to controls) in plasma vitellogenesis at 2 and 14 days	Andreassen and Korsgaard (2000)	Use with care – single exposure level
Summer flounder *Paralichthys dentatus*	Sexually immature juveniles	Intra-sinus injection – 2, 20 and 200 mg/kg	Significant decrease (relative to controls) in GSI in males 4, 6 and 8 weeks after injection at 200 mg/kg No significant effect on plasma VTG levels in males 4, 6 and 8 weeks after the first injection at any exposure concentration Significant increase (relative to controls) in plasma 17β-estradiol in males 4 weeks after first injection at 2 mg/kg. Levels not changed significantly after 6 and 8 weeks. Significant decrease (relative to controls) in plasma testosterone in males 4 weeks after first injection at 200 mg/kg. Levels not changed significantly after 6 and 8 weeks	Mills *et al.* (2001)	Valid
			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/kg No significant effect on testis development 8 weeks after first injection at any test dose	Zarogian *et al.* (2001)	Valid
Killifish *Fundulus heteroclitus*	Adult males from wild	Intra-peritoneal injection – 0, 10, 50, 100 and 150 mg/kg.	VTG significantly increased compared to controls at 100 and 150 mg/kg No significant effects on GSI, HSI and condition index	Pait and Nelson (2003)	Valid

In vitro systems using aquatic vertebrate cells and tissues

Table 4.9 summarises the *in vitro* data for 4-*tert*-octylphenol available for aquatic organisms. Jobling and Sumpter (1993) reported that because alkylphenols are bioaccumulative they are likely to be more potent at lower concentrations *in vivo* than *in vitro*.

Lutz and Kloas (1999) assessed the *in vitro* oestrogenic potency of 4-*tert*-octylphenol as part of a programme to study the effects of endocrine disruption on the African clawed frog *X. laevis*. Competitive displacement effects for oestrogen receptor binding of 4-*tert*-octylphenol in a liver cytosol fraction were investigated. The IC_{50} value was 78 µM (16,068 µg/L) compared with an IC_{50} value for 17β-estradiol of 42 nM (11.4 µg/L), which indicates that 4-*tert*-octylphenol has a relative potency (compared to 17β-estradiol) of 0.00054.

Jobling and Sumpter (1993) measured the production of VTG in superfused rainbow trout hepatocytes after exposure to a range of alkylphenolic compounds, including 4-*tert*-octylphenol. VTG production in the hepatocytes showed a U-shaped response curve with levels increasing at concentrations from 1 µM/L (206 µg/L) to 50 µM/L (10,300 µg/L) and decreasing markedly at 100 µM/L (20,600 µg/L) through cytotoxic effects. The ED_{50} for octylphenol was 2.11 µM (434 µg/L), which compared with the mean ED_{50} value for 17β-estradiol of 0.00181 µM (0.492 µg/L), indicating that 4-*tert*-octylphenol has a relative potency *in vitro* (compared to 17β-estradiol) of 0.0037.

Navas and Segner (2000) also found that VTG production in rainbow trout liver cells exposed to 4-*tert*-octylphenol was significantly higher than in unexposed control cells at concentrations of 1 µM/L (206 µg/L). The data showed a traditional S-shaped concentration–response curve with VTG production increasing as the 4-*tert*-octylphenol concentration increased.

Monteverdi and Giulio (1999) and Toomey *et al.* (1999) also investigated the production of VTG in hepatocytes of channel catfish (*Ictalurus punctatus*) and brown bullhead catfish (*Americurus nebulosus*), respectively. Significant increases in VTG production (relative to controls) were evident at 0.01 µM/L (2.06 µg/L) for channel catfish hepatocytes and 10 µM/L (2060 µg/L) for brown bullhead catfish hepatocytes. In the study with channel catfish hepatocytes a traditional S-shaped concentration–response curve was observed, with VTG production increasing as the 4-*tert*-octylphenol concentration increased. The 4-*tert*-octylphenol induced VTG concentration (303 ± 67 ng VTG/mL) was approximately 12-fold greater than that for nonylphenol (25 ± 8 ng VTG/mL). In contrast, in the study with brown bullhead catfish hepatocytes the concentration–response curve was an inverted U-shape with the VTG production increasing in the range 10-50 µM/L (2060-10,300 µg/L) and decreasing at 100 µM/L (20,600 µg/L), probably through cytotoxic effects.

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 VTG. These findings indicated that the effect of 4-*tert*-octylphenol was mediated through the oestrogen receptor (White *et al.*, 1994; Monteverdi and Giulio, 1999; Toomey *et al.,* 1999) and that it is an agonist.

Table 4.9 Summary of *in vitro* studies on aquatic vertebrates with 4-*tert*-octylphenol

Cell type	Exposure series	Endocrine disruption measurement parameter(s)		Potency (relative to 17β estradiol = 100)	Reference
		Description of effect	Concentration–response type		
African clawed frog (*Xenopus laevis*) liver cytosol fraction	10^{-3} to 10^{3} µM (0.21-206,000 µg/L)	Significant decrease in competitive displacement of [^{3}H]estradiol binding at 10 µM (2060 µg/L)	Decreasing S-shaped	0.00054	Lutz and Kloas (1999)
Rainbow trout (*Oncorhynchus mykiss*) hepatocytes	10^{-1} to 10^{2} µM (20.6-20,600 µg/L)	Significant increase (relative to controls) in VTG after 96hours exposure at 10 µM (2060 µg/L)	U-shaped	0.0037	Jobling and Sumpter (1993)
	10^{-1} to 10^{2} µM (20.6-20,600 µg/L)	Significant increase (relative to controls) in VTG at 0.1µM (20.6 µg/L)	Increasing S-shaped	No data	White *et al.* (1994)
	Not given	4-day exposure. VTG production: half maximum induction concentration (EC_{50}) 41.4 µM (8528 µg/L)	Increasing S-shaped*	0.00063	Segner *et al.* (2003b)
	10^{-3} to 10 µM (0.21-2060 µg/L)	Significant increase (relative to controls) in VTG synthesis after 72 hours exposure at 1 µM (206 µg/L)	Increasing S-shaped	No data	Navas and Segner (2000)
		No significant effect on basal EROD activity after 72 hours exposure at any exposure concentration	None	No data	
Channel catfish (*Ictalurus punctatus*) primary hepatocytes	10^{-2} to 10 µM (2.1-2060 µg/L)	Significant increase (relative to controls) in VTG synthesis after exposure at 0.01 µM (2.06 µg/L)	No data	No data	Monteverdi and Giulio (1999)
Brown bullhead catfish (*Americurus nebulosus)* Hepatocytes	10, 25, 50 and 100 µM (2060, 5650, 10,300 and 20,600 µg/L)	Significant increase (relative to controls) in VTG synthesis after 24 hours exposure at 10 µM (2060 µg/L)	U-shaped	No data	Toomey *et al.* (1999)
		Significant increase (relative to controls) in the disruption of the membranes of hepatocytes after 24 hours exposure at 100 µM (20,600 µg/L)	No data	No data	
		Significant increase (relative to controls) in apoptotic cell death after 24 hours exposure at 100 µM (20,600 µg/L)	No data	No data	
Eelpout (*Zoarces viviparous*) hepatic cytosolic extracts	10^{-5} to 10^{2} µM (0.0021-2060 µg/L)	Significant decrease in binding affinity to oestrogen-binding sites as concentration of competitor inhibiting specific binding of [^{3}H] estradiol at 5.9 µM (1215 µg/L)	Decreasing S-shaped	0.0011	Andreassen and Korsgaard (2000)
Carp (*Cyprinus carpio*) hepatocytes	Not given	4-day exposure. VTG production: half maximum induction concentration (EC_{50}) 38.2 µM (7870 µg/L)	Increasing S-shaped*	0.0024	Segner *et al.* (2003b)
Carp (*Cyprinus carpio*) liver cytosol fraction	Not given	Competitive displacement of ^{3}H estradiol binding; EC_{50} 32 µM (6603 µg/L)	Increasing S-shaped*	0.001	

* Response shape inferred from use of sigmoidal curve fitting to calculate EC_{50}.

Andreassen and Korsgaard (2000) investigated the effects of 4-*tert*-octylphenol on the oestrogen-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 inhibited binding at high ligand concentrations.

Recently, the Chemicals Evaluation and Research Institute in Japan (CERI, 2001) developed a competitive binding assay for the Japanese medaka (*O. latipes*) oestrogen receptor α. This assay has been used to measure the relative binding affinity (RBA) of 4-octylphenol (and other alkylphenols). Linear 4-octylphenol had an RBA of 0.077 compared to 100 for 17β-estradiol. 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.

Invertebrates

Limited information is available on the endocrine-disrupting effects of 4-*tert*-octylphenol on aquatic invertebrates. These data are summarised in *Table 4.10*, and details are highlighted below.

In a 'use with care' study, Zou and Fingermann (1997) investigated the effects of 4-*tert*-octylphenol on the moulting of the freshwater water flea *D. magna*. *D. 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 <12-hour-old neonates and the organisms were exposed to nominal 4-*tert*-octylphenol concentrations of 10, 20 and 40 µg/L until the fourth instar (day 4-7 of the experiment). 4-*tert*-Octylphenol did not inhibit the moulting and development at the highest concentration tested (40 µg/L).

In the fiddler crab (*Uca pugilator*), Zou and Fingermann, (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. At concentrations up to 10000 µg/L no significant effects on epidermal chitobiase activity were recorded, while 7 days of exposure to 10000 µg/L significantly inhibited hepatopancreatic chitobiase activity. Since 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 oestrogenic agents could account for at least some slowing of moulting that occurs when crustaceans are exposed to these agents. However, the concentrations that produce these effects were significantly higher (at least an order of magnitude) than those that caused effects on more traditional endpoints in other aquatic invertebrates.

Andersen *et al.* (2001) investigated the effects of 4-*tert*-octylphenol on the larval development of the marine copepod *A. 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 and 13 µg/L, respectively. The corresponding EC_{10} and EC_{50} values for 17β-estradiol were 370 and 720 µg/L, respectively, which indicates that the effect on naupliar development is probably not oestrogenically mediated.

Table 4.10 Summary of *in vivo* studies on aquatic invertebrates with 4-*tert*-octylphenol

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	Study Validity
Freshwater – water exposures					
Water flea *Daphnia magna*	12-hour-old neonates	Static renewal: 10, 20 and 40 µg/L (nominal)	No significant effect (relative to controls) at any test concentration after 4-7 days (on reaching fourth instar)	Zou and Fingermann (1997)	Use with care – nominal concentrations
Apple snail *Marisa cornuarietis*	Adults	Semi-static renewal: 1, 5, 25 and 100 µg/L (nominal)	Significant increase (relative to controls) in mortalities of animals after 5 months exposure at 1 µg/L	Oehlmann *et al.* (2000)	Use with care – nominal concentrations
	Egg masses	Semi-static renewal: 1 and 100 µg/L (nominal)	No significant effect (relative to controls) on vas deferens sequence index after 6 and 12 months exposure at either test concentration. Increases in numbers of eggs produced and size of spawning masses at 1 µg/L		
Mud snail *Potamopyrgus antipodarum*	Adults	Static renewal (every 4 days): 1, 5, 25 and 100 µg/L (nominal)	Significant increases in numbers of new embryos at 5 µg/L (NOEC 1 µg/L)	Jobling *et al*. (2003)	Use with care – nominal concentrations, wide separation
Freshwater – sediment exposures					
Mud snail *Potamopyrgus antipodarum*	Adults	Artificial sediment: 1, 10, 30, 100 and 300 µg/kg dw (nominal)	At 2 weeks all concentrations but 10 µg/kg had significant increase in new embryos; at 4 weeks all concentrations showed significant increase in new embryos, concentrations above 10 µg/kg had increased total embryos. EC_{10} for new embryo increase calculated as 4 ng/kg, for total embryos as 2.1 µg/kg	Duft *et al*. (2003)	Use with care – nominal concentrations, some results extrapolated well below lowest concentration used.
Saltwater					
Dog whelk *Nucella lapillus*	Adults	Semi-static renewal: 1, 25 and 100 µg/L (Nominal)	Significant increase (relative to controls) in relative numbers of females with oocytes after 2-3 months exposure at 1 µg/L Significant increase (relative to controls) in length of capsule gland and weight of female pallial glands after 3 months exposure at 1 µg/L Significant increase (relative to controls) in length of penis and the prostate gland after 3 months exposure at 1 µg/L.	Oehlmann *et al.* (2000)	Use with care – Nominal concentrations
Copepod *Acartia tonsa*	Eggs	Semi-static: concentration series not given (measured values)	Threshold concentration for effects on naupliar development of 5.2 µg/L after 5 days exposure	Andersen *et al.* (2001)	Valid
Harpacticoid copepod *Tigriopus japonicus*	<24-hour-old nauplii	Static renewal: 0.01, 0.1, 1 and 10 µg/L (nominal)	Delay in completion of naupliar stages at 0.1 and 1 µg/L in parent generation, and at all but 0.1 µg/L in F_1 generation. Conclusion was that exposure would have little effect on demographic profile	Marcial *et al*. (2003)	Use with care – nominal concentrations

Oehlmann *et al.* (2000) investigated the effects of suspected endocrine-disrupting chemicals, including octylphenol, on a freshwater prosobranch snail (Apple snail *Marisa cornuarietis*) and a marine prosobranch snail (Dog Whelk *Nucella lapillus*). In a series of three studies (all 'use with care', since they were based on nominal concentrations only), animals were exposed to octylphenol at nominal concentration ranges between 1 and 100 µg/L. The studies were:

1. *Ma. cornuarietis* parental generation test in which adult animals of comparable age were exposed to nominal concentrations of 1, 5, 25 and 100 µg octylphenol per litre 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. *Ma. 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 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 1-year-old. They reached sexual maturity in their eighth 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.
3. *N. lapillus* test in which groups of adults were exposed to nominal concentrations of 1, 25 and 100 µg/L (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 *Ma. cornuarietis*, octylphenol induced a complex syndrome of alterations in females (referred to as 'superfemales') at the lowest concentration of 1 µg/L. 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 4-*tert*-octylphenol resulted in inverted U-type concentration–response relationships for egg and spawning mass production. Adult *Nucella* from the field were tested for 3 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 because of 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. Since statistically significant effects were observed at the lowest nominal test concentrations (1 µg/L), the authors concluded that even lower concentrations might 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 *Ma. cornuarietis* is an especially promising candidate for a future organismic invertebrate model to identify endocrine-mimetic test compounds. However, it needs to be recognised that these were basic research studies, and the data need to be considered in this context. A similar effect has been noted for the substance bisphenol-A, and currently work is underway to develop a standardised regulatory test protocol for this species.

As part of studies to investigate the relative sensitivities of fish and molluscs, Jobling *et al*. (2003) exposed the freshwater mud snail *Potamopyrgus antipodarum* (a temperate species of prosobranch snail, common in Europe) to 4-*tert*-octylphenol in water. The exposures lasted up to 90 days in a semi-static system with 50% of the dosed water being replaced every 4 days. The exposure levels were nominally 1, 5, 25 and 100 µg/L. No analysis of the exposure solutions was performed. The endpoints monitored were growth and embryo production. Growth was measured by the length of the shell and the width of the shell opening. For embryo production, the number of embryos in the brood pouch was counted, distinguishing between shelled and unshelled embryos. The former are at a much later stage of development, and so this gives a measure of new embryo production.

No effects were seen on survival at any concentration of 4-*tert*-octylphenol (or for bisphenol-A at the same concentrations, or for EE2 at a concentration three orders of magnitude below). Shell height and operculum width were also little affected for the most part, but at 9 weeks the shell height in the 25 µg/L exposure were significantly increased over the 6-week value. There were no significant effects at higher doses. Embryo production was significantly increased over that in the controls after 3 weeks at 5 µg/L. At 42 days the number of embryos was increased, but not significantly so. At 63 days, the 5 and 25 µg/L exposures had significantly more embryos than the controls, whereas the 1 and 100 µg/L exposures had higher numbers, but not significantly so. This indicates an inverted U-shaped response, similar to that seem with EE2 (at ng/L levels). From the studies reported here and from other studies, the authors concluded that fish appear more sensitive to disruption in reproductive output caused by EE2, but that snails may be more responsive to low concentrations of some xenoestrogens than are fish.

The freshwater mud snail *Ps. antipodarum* was exposed to 4-*tert*-octylphenol in artificial sediments for up to 8 weeks (Duft *et al*., 2003). The sediment was composed of 95% quartz sand and 5% ground beech leaves. 4-*tert*-Octylphenol was added to the sediment dissolved in ethanol and the sediment left for 1 day for the solvent to evaporate. Water was added and the sediment allowed to equilibrate for 5 days with aeration. Exposure concentrations were 1, 10, 30, 100 and 300 µg/kg dw. Experiments were carried out at 15 ± 1°C.

Eighty snails were added to the flasks containing sediment at the start of the exposures, and 20 were removed after 0, 2, 4 and 8 weeks. Embryos were removed from the brood pouch and the number of ‘grown up’ embryos (with shells) and ‘new’ embryos (without shells) were counted. The occurrence of egg cells in the oviduct and the maturity of the ovary were noted, as was any mortality in the treatments.

All exposure concentrations, except for 10 µg/L, resulted in significant increased production of unshelled embryos after 2 weeks. There were no significant trends in the total number of embryos, or in the number of shelled embryos. At 4 weeks the total number of embryos was significantly above that in the controls at concentrations above 10 µg/kg. The numbers of unshelled embryos were elevated at all concentrations. At 8 weeks, the higher concentrations showed only slight increases in numbers of unshelled embryos over the controls, while concentrations of 30 µg/kg or lower continued to show significant increases. (Non-linear regression was used to fit the results and to derive concentrations that give 10% and 50% stimulation. For embryo production at 4 weeks the values were 4 and 70 ng/kg, and for total embryo numbers the values were 2.1 and

108 µg/kg, respectively. Values could not be calculated at 8 weeks. The extrapolated values for embryo production are well below the lowest exposure concentration of 1 µg/L.)

No analysis of the sediment was undertaken because of the reported short half-life of 4-*tert*-octylphenol in sediment. Attempts were made to analyse the soft tissues of the snails to obtain a measure of the levels in the organisms, but insufficient tissue was recovered to allow the analysis to be performed.

The effects of 4-*tert*-octylphenol on the harpacticoid copepod *Tigriopus japonicus* have been studied (Marcial *et al*., 2003). This is an intertidal organism, which thrives at a wide range of temperatures and salinities. It has six naupliar and six copepodid stages, of which the last is the adult. Tests were conducted at 2.5% salinity. Stock solutions of 4-*tert*-octylphenol were changed every week.

Acute toxicity was determined in 48 hour exposures, the result being a LC_{50} of 0.62 mg/L (95% confidence limits 0.56-0.67 mg/L). Longer-term exposure was carried out at four concentrations, 0.01, 0.1, 1.0 and 10 µg/L, with three replicates at each concentration. Nominal concentrations are reported, the test solutions were renewed daily by replacing around 50% of the working volume each time. Twenty nauplii, less than 24 hours old, were used at each exposure level. The survival and developmental stage of the organisms was assessed at the renewal of the solutions, at which time they were also fed. For the first 8 days the exposures were in 24-well plates; after this time the surviving copepodids were transferred to the chambers of 6-well plates, with food and fresh solution, to initiate copulation. After 2-3 days, six mature females (bearing ovisacs) were randomly selected from the population and transferred individually to new plates. The number of nauplii produced up to the third brood was monitored for each organism. After 21 days, the sex ratio of the copepodids and the percentage survival were determined. The first brood of nauplii were cultured in the same conditions and the same parameters were monitored for 21 days.

Survival rates were not affected by 4-*tert*-octylphenol exposure in either the parent or the F_1 generation. A significant delay in completion of the naupliar stages (compared to the controls) was seen at concentrations of 0.1 µg/L and 1.0 µg/L in the parent generation, and at all concentrations except 0.1 µg/L for the F_1 generation. The time to sexual maturity was not different from that in the controls in the parent generation, but was increased at 10 µg/L for the F_1 generation. The sex ratios of copepodids were not significantly different from the controls at any concentration, for either of the generations. There was an increase in fecundity (as measured by the average number of nauplii per female) in the 1 µg/L exposure. The authors concluded that 4-*tert*-octylphenol (and the other chemicals tested, nonylphenol, bisphenol-A and 17-β-estradiol) had no extensive effect on reproductive parameters, and would have little impact on the demographic profile of the copepod. However, the effects on development could be a potential indicator of exposure to oestrogens for crustacean species.

In vivo studies in terrestrial organisms

The only information that has been identified for the potential endocrine-mediated responses to 4-*tert*-octylphenol of terrestrial species is a study by Millam *et al.* (2001) on the adult performance of zebra finches. In the study the short-term effects of oral post-

hatch exposure of 4-*tert*-octylphenol (and estradiol benzoate) on the reproductive performance of zebra finches were investigated. 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 of 100 mM 4-*tert*-octylphenol (99% purity) dissolved in canola oil, which resulted in a dose of 100 nmol/g body mass/day. A 1 µL/g body mass dose of canola oil alone was used as a control. The 100 nmol/g 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 doses of estradiol benzoate.

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 treatment 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 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 trial and production of the first egg;
- Number of eggs laid which operationally are 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 that 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 estradiol benzoate resulted in marked effects on a number of endpoints and profound disruption of reproduction.

4.1.6.4 Summary of endocrine-mediated responses

When examining the various endpoints from studies that investigated endocrine-mediated responses in wildlife species it is important to try to consider their ecological significance. It can be argued that any irreversible physiological or histological change following exposure to a chemical (which is outside normal background limits) is inherently adverse and should be avoided, especially if it can be linked mechanistically with anatomical or physiological effects. However, it may be more appropriate to try to protect against effects related to reproductive activity and sexual development. In terms of protecting the 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 males and females of species to breed and produce viable offspring that can develop and then successfully reproduce.

A consideration when reviewing endocrine studies is the possibility of dose–responses (such as an inverted U-shape) that do not fit the usual pattern for 'standard' toxicity. Such responses may be expected in cases where a substance can produce a stimulatory effect at low concentrations and a toxic effect at higher ones. Responses of this type have been observed with 4-*tert*-octylphenol. They are difficult to interpret in the conventional risk assessment sense, but are nevertheless included in the discussions on the PNEC derivation and risk characterisation.

In the aquatic environment the lowest NOEC from a valid study assessing the endocrine disrupting effects in fish was 1.6 µg/L for VTG induction in adult male rainbow trout (*On. mykiss*) after 21 days exposure to 4-*tert*-octylphenol (Jobling *et al.* 1996). Routledge *et al.* (1998) reported a NOEC of 10 µg/L for VTG induction in the same species and with the same exposure duration. The reason for this difference is not clear, but may result from the different times of year at which the studies were conducted. In other species higher concentrations of 4-*tert*-octylphenol have been required to elicit VTG induction. While induction of VTG 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 established clearly.

Wenzel *et al.* (2001) in a life-cycle study with zebrafish (*Da. rerio*) reported a NOEC of 12 µg/L (based on measured concentrations) for a range of endpoints affecting the reproductive success of fish that 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. All these parameters were significantly affected at an exposure concentration of 35 µg/L. Segner *et al*. (2003b) reported a lifetime exposure study on the same species, from which an EC_{50} of 28 µg/L was determined. They considered that this was the reproduction parameter affected most consistently and reproducibly. A valid study by Van den Belt *et al.* (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. These data, while initially appearing contradictory, indicate that there are probably differences in species sensitivity to 4-*tert*-octylphenol in terms of endocrine-mediated responses and that the critical window of exposure for the effects on reproduction may be during the early life stage of the fish.

In a valid study of reproductive impairment in adult male Japanese medaka (*O. latipes*), Gronen *et al.* (1999) found that breeding groups composed of exposed males (at 20 µg/L 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) were also evident. These data once again indicate that, as might be expected, there are probably differences in species sensitivity to 4-*tert*-octylphenol in terms of endocrine-mediated responses.

Knorr and Braunbeck (2002) reported similar results in Japanese medaka. When exposed males were mated with control females, increased mortality was seen at 20 µg/L. When exposed females were mated with control males, the increase in

mortality was not seen until 50 µg/L. Mortality was not increased at 2 µg/L, which could be taken as a NOEC for mortality (although there is a factor of 10 between the concentrations). Other endpoints in the same study indicated significant changes at 20 µg/L, with smaller effects at 2 µg/L.

Seki *et al*. (2003) observed testis-ova in medaka exposed to concentrations of 4-*tert*-octylphenol of 11.4 µg/L and above, with increases in VTG at the same concentrations. The NOEC from this study was 6.94 µg/L.

In a study on the guppy (*Po. reticulata*), a number of parameters, including mortality, male colouration, GSI in females and sexual behaviour, were all affected at 149 µg/L but not at 11.7 µg/L (Toft and Baatrup, 2003). The separation between these concentrations is large.

Mortality of embryos in the viviparous fish eelpout (*Z. viviparus*) was increased following exposure of pregnant female fish to 64 µg/L, and the development (weight and length) of the embryos was reduced at 14 µg/L (Rasmussen *et al*., 2002).

In the estuarine fish sheepshead minnow (*C. variegatus*) abnormalities in the testes were observed at 33.6 µg/L, but not at 11.5 µg/L (Karels *et al*., 2003). VTG levels were elevated at both concentrations.

For amphibians, a study by Kloas *et al.* (1999) reported a NOEC of 2.1 µg/L for effects on sex ratios in *X. 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 need to be considered in this context. The findings of a similar study on bisphenol-A could not be reproduced in a repeat study by a different laboratory, and the study is therefore classed as ‘use with care’.

Changes in the development of the bullfrog (*R. catesbeiana*) were observed by Mayer *et al*. (2003). The stage at which sexual differentiation was completed in tadpoles was advanced by exposure to 0.2 µg/L and above. The significance of this change is not clear, and there were no changes to the sex ratios.

For invertebrates, a study by Oehlmann *et al.* (2000) (with a ‘use with care’ status) showed significant effects on the reproductive anatomy of freshwater (*Ma. cornuarietis*) and marine (*N. lapillus*) prosobranch snails. In experiments with *Ma. Cornuarietis*, octylphenol induced a complex syndrome of alterations in females (referred to as ‘superfemales’) at the lowest concentration of 1 µg/L. Since statistically significant effects were observed at the lowest nominal test concentrations (1 µg/L), the authors concluded that even lower concentrations may have a negative impact on the snails. As with the amphibian study, this test was not carried out to a standard regulatory test protocol and the data need to be considered in this context.

Studies on another snail species *Ps. antipodarum*, showed an increase in embryo production, significant at 5 µg/L but not at 1 µg/L (Jobling *et al*., 2003). In the estuarine copepod *T. japonicus*, delays in the completion of the naupliar stage in the F_1 generation were observed at concentrations down to 0.01 µg/L (Marcial *et al*., 2003). However, the authors concluded that exposures at up to 10 µg/L would have little impact on the

demographic profile of the species. An EC_{10} of 5.2 µg/L was determined by Andersen *et al*. (2001) for the marine copepod *A. tonsa*, also for delay in naupliar development.

Overall, on the basis of the fully *valid* studies, the lowest NOEC for endocrine modulation of reproductive performance in aquatic organisms is 12 µg/L, based on the data from the Wenzel *et al.* (2001) study with fish. However, effects on VTG production in rainbow trout (*On. mykiss*) have been observed at concentrations above 1.6 µg/L. Furthermore, data from studies (of use with care status) in molluscs and amphibians indicate that a lower NOEC, potentially less than 1 µg/L, may be appropriate. These data are difficult to use in the risk assessment at present, and would benefit from further, more reliable, evidence to support them (i.e., there may be a need for further testing). This is considered in the discussion of PNEC derivation and risk characterisation (Sections 4.1.8 and 5).

4.1.7 Wastewater treatment plant micro-organisms

A 6-hour EC_{50} of >1700 mg/L was reported for *Pseudomonas putida* based on inhibition of oxygen consumption. The concentrations were nominal and the test was carried out according to GLP (SIDS, 1994; IUCLID, 2000).

A 3-hour EC_{50} of >10 mg/L was observed for activated sewage sludge organisms when tested by the OECD Guideline 209 method 'activated sludge, respiration inhibition test' (SIDS, 1994; IUCLID, 2000).

4.1.8 Predicted no-effect concentrations for the aquatic compartment

4.1.8.1 Calculation of a PNEC for surface water

In principle, the PNEC is calculated by dividing the lowest short-term median lethal (effect) concentration, $L(E)C_{50,}$ or long-term NOEC value by an appropriate assessment factor. The assessment factors reflect the degree of uncertainty in extrapolation from laboratory toxicity test data for a limited number of species to the real environment. Lower assessment factors can be used with larger and more relevant data sets, for example if data are available on the toxicity to organisms at a number of trophic levels, belonging to taxonomic groups and with lifestyles representing various feeding strategies. This is discussed in detail in the TGD.

A summary of the toxicity data for 4-*tert*-octylphenol is provided in Section 4.1.5. For this substance, valid short- and long-term data are available for freshwater invertebrates and fish, but not for green algae (the available algal studies all have a 'use with care' status). The lowest and most reliable freshwater aquatic toxicity data are summarised in *Table 4.11*. Unless otherwise stated these are considered valid data for use in the calculation of the PNEC.

Based on these data for 'traditional' toxicity endpoints, the NOEC (growth) of 6.1 µg/L from the 60-day post-hatch early life stage toxicity study with rainbow trout (*On. mykiss*) is the most relevant value for use in the PNEC derivation. Nevertheless, note that the invertebrate *G. pulex* is more sensitive in acute tests than other organisms, and a chronic NOEC is not available for this species. There is also evidence from nonylphenol that

invertebrates (and possibly algae) are more sensitive than fish in chronic tests (see Section 4.1.6). An assessment factor of 50 is therefore appropriate, and this gives a PNEC of 0.122 µg/L.

Table 4.11 Summary of valid traditional endpoint toxicity data used for aquatic PNEC

Trophic level	Species	End point	Concentration (µg/L)
Freshwater fish	Fathead minnow (*Pimephales promelas*)	96-hour LC_{50}	290
	Rainbow trout (*Oncorhynchus mykiss*)	6-day LC_{50}	170
	Rainbow trout (*Oncorhynchus mykiss*)	60-day post-hatch early life stage NOEC	6.1
Freshwater invertebrates	Freshwater shrimp (*Gammarus pulex*)	96-hour EC_{50} (immobilisation)	13.3
	Water flea (*Daphnia magna*)	48-hour LC_{50}	270
		21-day NOEC (juvenile production by surviving adults)	62
Freshwater algae	*Scenedesmus subspicatus*	72-hour EC_{50} (growth rate)	1100 (use with care)
		72-hour EC_{10} (growth rate)	300 (use with care)

A number of the exposure scenarios involve intermittent releases, for which a PNEC based on acute toxicity is more appropriate. The lowest acute toxicity value is the 96-hour EC_{50} for *G. pulex* of 13.3 µg/L; the intermittent exposure PNEC is therefore 0.13 µg/L using an assessment factor of 100. This is very similar to the PNEC value derived from long-term data, and so the latter is preferred.

4.1.8.1.1 Consideration of 'endocrine disruption' data

On the basis of valid studies, the lowest most relevant NOEC for endocrine-mediated responses in aquatic organisms is 12 µg/L from a life-cycle study with zebrafish (*Da. rerio*) (Wenzel *et al.*, 2001). This is higher than the lowest NOEC (growth) for general ecotoxicological effects of 6.1 µg/L from the 60-day post-hatch early life stage toxicity study with rainbow trout (*On. mykiss*).

However, effects on VTG production in rainbow trout (*On. mykiss*) have been observed at concentrations above 1.6 µg/L, which is the lowest NOEC value for fish. There are also NOEC values of 11.7 µg/L for the guppy, 6.94 µg/L for the medaka and 11.5 µg/L for the sheepshead minnow. In addition, data from studies (of 'use with care' status) in molluscs and amphibians suggest that a lower NOEC – potentially less than 1 µg/L – may be appropriate for these organisms. A possible NOEC of 1 µg/L is indicated for *Ps. antipodarum*. Changes in the development of bullfrog tadpoles were observed at 0.2 µg/L, but the significance of these is not clear. Delays in the completion of the naupliar stage in marine copepods were observed at 0.01 µg/L, but the authors considered these would have little impact on the demographic profile of the organism at concentrations up to 10 µg/L. The PNEC from the 'standard' toxicity test results is lower than all of the above values, with the exception of that for copepods. Neglecting this exception in light of the authors' comments, there is a margin of around eight-fold between the PNEC and the level at which effects seen in snails, and less than double for bullfrogs (but with no indication of the significance or otherwise of this effect). Although a second PNEC could be derived from the data, the choice of concentration to use (as some are effects at the lowest tested concentration) and assessment factor would be somewhat arbitrary.

Instead, the risk characterisation considers which endpoints would give a margin of safety of 10 based on the data above.

4.1.8.1.2 Comparison with nonylphenol

The aquatic toxicity databases for nonylphenol and 4-*tert*-octylphenol are compared in *Table 4.5*. For nonylphenol, a PNEC of 0.33 µg/L (0.0015 µmoles/L) was derived by applying an assessment factor of 10 to the 72-hour EC_{10} (biomass endpoint) of 3.3 µg/L for the green alga *S. subspicatus* – this value was supported by a 'use with care' 28-day NOEC (for survival) of 3.9 µg/L for the mysid *M. bahia*. The PNEC for 4-*tert*-octylphenol is 0.122 µg/L (0.00059 µmol/L).

The toxicity data for 4-*tert*-octylphenol for different taxonomic groups and different endpoints are generally similar to those for nonylphenol. In most cases the toxicity values are within a factor of three of one another. However, there is a difference in the algal toxicity data, and there is no information about chronic toxicity to mysids. If 4-*tert*-octylphenol were to have similar toxicity, a lower NOEC from 'traditional' endpoint studies of around 3 µg/L might be expected if further testing was performed. With an assessment factor of 10, this implies that the PNEC would be comparable to that for nonylphenol (i.e., around 0.3 µg/L). However, since the endocrine-mediated effects may occur at lower concentrations, any further refinement of the 4-*tert*-octylphenol PNEC should be focussed on those endpoints. This is considered further in the risk characterisation (Section 5). Note that comparable data for nonylphenol on endocrine-mediated effects in molluscs did not exist at the time the assessment for that substance was completed (1999).

In conclusion, the PNEC of 0.122 µg/L is used in the risk characterisation, but the consequences of a lower PNEC are also considered (see Section 5).

4.1.8.2 Calculation of PNEC for sediment

The only test result found for exposure of organisms via sediment is the study on *Ps. antipodarum* described in the invertebrate section of Section 4.1.6.3.1. In this study there were increases in the number of unshelled embryos relative to controls at 4 weeks at all concentrations used (the lowest was 1 µg/kg dw), and in the total numbers of embryos at concentrations of 30 µg/kg dw and above. As effects were seen at all concentrations, no NOEC value can be derived from the study. The data at 4 weeks allowed for a non-linear regression analysis that gave estimates of the EC_{10} value for stimulation of embryo production, 4 ng/kg for unshelled embryos and 2.1 µg/kg for total embryos. The data at 2 and 8 weeks did not allow a similar analysis. The value for unshelled embryos is extrapolated over two orders of magnitude below the lowest (nominal) exposure level and as such has a high degree of uncertainty. The production of additional embryos is considered to be a potentially harmful effect, as it involves the use of energy resources in the organism out of the normal embryo production period, and also the release of young into the environment at possibly less than optimum times. However, it is not clear whether the numbers of new unshelled embryos or the total embryos should be used as a parameter for this effect, and it is not considered possible at present to determine a reliable no-effect level from this study.

In the absence of a reliable test result, the equilibrium partitioning method may be used to estimate the $PNEC_{sediment}$. In using this method it is assumed that sediment-dwelling organisms and water column organisms are equally sensitive to 4-*tert*-octylphenol and that the concentration in sediment, interstitial water and benthic organisms is at thermodynamic equilibrium.

The following formula is used to derive the $PNEC_{sediment}$ from the $PNEC_{aquatic}$

$$PNEC_{sediment} = \frac{K_{susp-water} \times PNEC_{aquatic} \times 1000}{RHO_{susp}}$$

where $PNEC_{aquatic}$ is 0.122 µg/L, $K_{susp\text{-}water}$ is the suspended matter–water partition coefficient (69.4 m^3/m^3 EUSES) and RHO_{susp} is the bulk density of suspended matter (1150 kg/m^3).

The provisional $PNEC_{sediment}$ calculated using the value for surface water and equilibrium partitioning is 0.0074 mg/kg wwt, which is used in the risk characterisation.

The equilibrium partition result (as a PNEC) is of the same order as the estimated EC_{10} value for total embryo production in *Potamopyrgus*, but as the derivation of the aquatic PNEC from which it is calculated includes an assessment factor of 50, the effective no-effect level is higher. This provides additional evidence that snails might be especially sensitive to the effects of 4-*tert*-octylphenol. However, it is difficult to derive a suitable no-effect level for use in risk assessment from the currently available test results on snails for both sediment and water. The EC_{10} value for unshelled embryo production is extrapolated too far below the lowest concentration used to be reliable. The value for total embryo production is better defined, but it is not clear if this is the appropriate value to use. In addition to the 'conventional' PNEC derived by equilibrium partitioning above, the lowest concentration tested with snails (at which effects were seen) is therefore also considered in the risk characterisation. This is 1 µg/kg dw; converting to a wwt value and to the standard TGD suspended sediment (for which the PEC values are calculated) gives a value of 0.92 µg/kg wwt.

4.1.8.2.1 Comparison with nonylphenol

The $PNEC_{sediment}$ for nonylphenol is 0.039 mg/kg wwt (based on equilibrium partitioning – no sediment organism toxicity data were available). The lower PNEC for 4-*tert*-octylphenol reflects the lower PNEC for surface water and the lower $K_{susp\text{-}water}$ value (69.4 m^3/m^3 for 4-*tert*-octylphenol compared to 135 m^3/m^3 for nonylphenol).

A recent study has investigated the toxicity of nonylphenol to the sediment dwelling dipteran larvae of *Chironomus riparius* (Bettinetti *et al.*, 2002). First instar larvae obtained from populations at three different sites were used. To spike the sediments, an equilibrium procedure between water and sediment was adopted to avoid the use of solvents. Ten-day LC_{50} values obtained for two populations of *Ch. riparius* from clean environments (315-465 and 315-350 mg/g dw, respectively) were lower than those of a strain from a population collected in a polluted river (600-680 mg/g dw). Larval growth always decreased with increasing 4-nonylphenol concentration, but without any defined trend.

On a hypothetical basis an assessment factor of 1000 could be applied to the LC_{50} values for *Ch. riparius* from control locations. The resulting PNEC value would be in the range of 0.158-0.233 and 0.158-175 mg/kg wwt (assuming that the dry weight of the sediment was 50% of the wwt). This results in estimated values that are generally within a factor of five of the value derived on the basis of the equilibrium partitioning approach.

4.1.8.3 Calculation of PNEC for WWTP micro-organisms

The lowest and most reliable data considered valid for use in the calculation of a PNEC is the 3-hour EC_{50} of >10 mg/L observed for activated sewage sludge organisms. A PNEC of >100 µg/L is derived using an assessment factor of 100, in accordance with the TGD.

4.1.8.3.1 Comparison with nonylphenol

A limited micro-organism data set is available for nonylphenol. An EC_{50} of 950 mg/L was obtained from an OECD 209 inhibition of activated sludge respiration study. A PNEC of 9.5 mg/L was derived using an assessment factor of 100. It is therefore highly likely that the PNEC for 4-*tert*-octylphenol is too conservative. This is considered further in the risk characterisation (Section 5).

4.2 TERRESTRIAL COMPARTMENT

4.2.1 Terrestrial toxicity data

No experimental data on the toxicity of 4-*tert*-octylphenol to terrestrial organisms (particularly plants and invertebrates) are available.

4.2.2 Calculation of PNEC for the soil compartment

In the absence of data for terrestrial effects, a provisional $PNEC_{soil}$ can be calculated using the $PNEC_{aquatic}$ and an equilibrium partitioning approach. The following formula is used:

$$PNEC_{soil} = \frac{K_{soil-water} \times PNEC_{aquatic} \times 1000}{RHO_{soil}}$$

where $PNEC_{aquatic}$ is 0.122 µg/L, $K_{soil\text{-}water}$ is the soil–water partition coefficient (82.4) and RHO_{soil} is the bulk density of wet soil (1700 kg/m^3).

The provisional $PNEC_{soil}$ calculated using the PNEC value for surface water and equilibrium partitioning is 0.0059 mg/kg wwt, which is used in the risk characterisation.

4.2.2.1.1 Comparison with nonylphenol

The $PNEC_{soil}$ of 0.3 mg/kg wwt for nonylphenol is based on experimental data[19]. An assessment factor of 10 was applied to a 21-day EC_{10} (reproduction) of 3.44 mg/kg wwt for the earthworm (*Apporec-todea calignosa*).

Based on the similarity of the PNECs for surface water for the two substances, the low terrestrial PNEC for 4-*tert*-octylphenol needs careful consideration. If any refinement of the 4-*tert*-octylphenol PNEC were necessary it would be prudent to consider a toxicity test on earthworms in the first instance. This is considered in the risk characterisation (Section 5).

4.3 ATMOSPHERIC COMPARTMENT

There are no data on the effects of 4-*tert*-octylphenol through aerial exposure of non-mammalian organisms. Direct emissions to the atmosphere are likely to be very low. Biotic or abiotic effects are unlikely to occur because of the limited release, low volatility and rapid atmospheric degradation of 4-*tert*-octylphenol. A PNEC has therefore not been derived.

4.4 NON-COMPARTMENT-SPECIFIC EFFECTS RELEVANT TO THE FOOD CHAIN (SECONDARY POISONING)

4-*tert*-Octylphenol is not readily biodegradable and may have a low to moderate potential to bioconcentrate in aquatic organisms as suggested by a BCF of 634 calculated using EUSES. It is therefore possible that the substance can accumulate in food chains.

4.4.1 Mammalian toxicity data

This section contains summaries of those studies considered to be of most relevance for the risk assessment. It is not intended to be a comprehensive summary of the mammalian toxicity data for 4-*tert*-octylphenol. The Norwegian government reviewed the mammalian toxicity data in 2004 for a human health classification proposal document. Some of these data are included here; other studies in the proposal would not affect the conclusions in this assessment and so were not reviewed, but further references from the proposal are listed in this assessment at the end of the main reference section for information.

[19] The source of the terrestrial effect data was a Danish Environment Protection Agency report. No raw data or test reports were available, and the Danish report did not explicitly mention whether the values were based on wet or dry weight. However, there is one value quoted for earthworm accumulation that is marked as 'dry weight'. Since this was specifically identified as such, the assumption has been that all the other values were in terms of wet weight. There is no mention of normalisation to organic carbon. The nonylphenol soil PNEC is therefore based on wet weight and not normalised to standard (EU) soil.

4.4.1.1 Summary of acute toxicity

No human data are available, but in laboratory mammals 4-*tert*-octylphenol has a low order of acute toxicity by the oral route, with LD_{50} values greater than 2000 mg/kg bodyweight for mice and rats (SIDS, 1994).

4.4.1.2 Summary of repeat dose toxicity

4.4.1.2.1 Injection exposure

A two-month repeat-dose study was carried out in Fischer 344 rats in which 4-*tert*-octylphenol was dosed by subcutaneous injection three times per week (Blake and Boockfor, 1997; Boockfor and Blake, 1997). Dosing was calculated as being equivalent to 30 or 160 mg/kg bodyweight/day. The results of this study appear to indicate some changes in the sex organs (at both macroscopic and microscopic level) and also in hormone levels at both dose levels. The effects were more severe after 2 months' exposure. These studies cannot readily be used to derive a PNEC for oral exposure, so are not considered further.

4.4.1.2.2 Oral exposure

Four repeat-dose toxicity studies have been performed in which animals were exposed to 4-*tert*-octylphenol orally. Two of these were sub-chronic 90-day dietary studies, while the other two were sub-acute oral gavage studies of 28 and 29 days duration.

A pre-GLP 90-day study with albino rats, carried out by Rohm and Haas (1961), and cited in IUCLID (2000), involved 15 young male and female animals treated with a 35% aqueous solution of 4-*tert*-octylphenol in the diet in an amount calculated to achieve a 5% concentration of the active ingredient. During the study urine was collected and tested, haematological tests were carried out and, at sacrifice, histopathological analysis was undertaken. Organ-to-bodyweight ratios were determined for liver, kidney, spleen and heart. Tissues taken for histopathological study were heart, lung, liver, spleen, gasteroenteric, bladder, bone marrow, muscle, skin, brain, thyroid, adrenal and pancreas. No effects of treatment were apparent in this study and there were no deviations in food consumption between treated and control animals.

A more recent 90-day non-GLP study with rats (BOR/WIWS, SPF Cpb), carried out by Bayer (1982) and cited in SIDS (1994) and IUCLID (2000), used 20 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 issuing 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 4-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. Throughout the study, no clinical signs of toxicity were observed. While both sexes displayed some organ weight changes, males exposed to 300 ppm of the test substance experienced the only statistically significant dose-related organ weight change – this was in relative brain

weight. Haematological parameters in all treated male rats were unaffected by exposure to the test substance. 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. The NOEL for this study was reported as 30 ppm in the diet, but the lack of dose response in the only effect reported and the lack of any other treatment-related effects does not support the use of this value as a reliable NOEL for the risk characterisation.

An oral gavage study carried out to GLP in rats (Sprague Dawley), reported by the Chemical Investigation Promoting Committee in 1994 and cited in SIDS (1994) and IUCLID (2000), used 12 animals (six males and six females) for each of the control and three treated groups (15, 70 and 300 mg/kg/day). The test was conducted to the Japanese Guidelines for the 28-day dose toxicity test of chemicals using 4-*tert*-octylphenol (98.2%). Excessive salivation was observed on test-substance administration in the medium (70 mg/kg/day) and high (300 mg/kg/day) 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 no observed adverse effect level (NOAEL) of 15 mg/kg/day was reported, although if this is based on the salivation it could be an artificial dosing effect rather than systemic toxicity. If this effect is neglected, the NOAEL is 70 mg/kg/day for reduction in body weight gain.

Another 29-day oral gavage study in rats (Sprague Dawley), carried out to GLP and cited in SIDS (1994) and IUCLID (2000) used 10 animals (five males and five females) for each of the control and three treated groups (15, 150 and 250 mg/kg/day). The test was carried out to OECD TG 407 using octylphenol (purity 98.7%). A dose of 150 mg/kg/day led to the following symptoms: slightly higher food consumption in females, higher water consumption of females, lower cholesterol levels in female rats and basophilic epithelium occasionally with mitotic figures in proximal convulated tubules in male rats. A dose of 250 mg/kg/day 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 convoluted tubules in male and female rats. For this study a NOAEL of 15 mg/kg/day and a lowest observed adverse effect level (LOAEL) of 150 mg/kg/day were reported. The NOAEL is conservative, as the effects reported at the next dose are not major.

There are some similarities between the two studies in terms of effects on water consumption, but there are also differences in terms of effects on food consumption. There are no obvious reasons for the differences in the summaries reported in SIDS (1994) and IUCLID (2000). The values that are derived from the studies are not very different.

4.4.1.2.3 Reproductive and developmental toxicity

A 6-week reproduction/developmental screening test (in accordance with OECD 421, draft dated 12 January 1993)) in the rat with gavage administration reported a NOAEL for reproductive performance and development of the offspring of 250 mg/kg bw/day (cited in SIDS, 1994).

A two-generation study on CD rats with oral exposure via the diet has been conducted (Tyl *et al*., 1999). Thirty rats of each sex were used at each of four exposure levels (0.2, 20, 200 and 2000 ppm, corresponding to 0.015, 1.5, 15 and 150 mg/kg bw/day) and in the controls. The study was conducted to GLP and followed acceptable procedures. Effects were only seen at the highest dose level of 150 mg/kg/day. The effects noted included significantly decreased body weight in adult males in both the F_0 and F_1 generations, and in offspring during the later part of the lactation period. A statistically significant reduction in absolute and relative uterine weights was observed in F_0 females at the highest dose. Significant delays in vaginal opening and in preputial separation were also noted at this dose in the F_1 and F_2 generations. There were no observed effects at any dose on a number of other reproductive parameters, including testes, prostate and ovary weights or morphology, sperm counts, production, motility and morphology, and oestrus cycling. The NOAEL for systemic toxicity and postnatal toxicity from this study was 15 mg/kg/day, and for reproductive effects it was 150 mg/kg/day or above.

Sharpe *et al*. (1995) reported exposing male Wistar rats to 4-*tert*-octylphenol through drinking water during gestation and/or as neonates. The testis weights and spermatogenesis were measured at adulthood. In study 1, rats were treated from days 1-22 after birth; in study 2 the dams were treated from a 2-week period before mating throughout gestation up to 22 days after giving birth; in study 3 the treatment was from 2 weeks before mating, through two mating and gestation periods and on to 22 days after the second generation was born. The top-dose group were exposed to 1000 µg/L of octylphenol, which reportedly equates to 367 µg/kg per day at maximal intake levels (postnatal day 22). The top doses (100 and 1000 µg/L) resulted in a small but significant ($p < 0.01$ or $p < 0.0001$) reduction in mean testicular weight in studies 2 and 3, an effect that was still evident when testicular weight was expressed relative to body weight or kidney weight. Exposure during gestation also appeared to reduce the adult testis weight in the highest dose group measured at 90-95 days after birth. Comparable but more minor effects of treatment on testicular size were observed in study 1. There was a slight decrease in sperm production, but no signs of abnormalities. A NOAEL of 10 µg/L of octylphenol could be put forward based on the changes in adult testicular weight at the next dose level, and this reportedly equates to 3.67 µg/kg per day. The real biological significance of such an effect is uncertain, since no subsequent testing of fertility or reproductive performance was carried out. A subsequent article by the same authors (Sharpe *et al*., 1998) reported that the findings could not be repeated, and suggested that this was because of unknown biological factors. They noted that the testis weights of control animals had changed significantly over the period between the test and the repeat. As a result this study is not considered for risk assessment.

Blake *et al*. (2004) exposed rats to 4-*tert*-octylphenol in drinking water for 4 months. The concentrations in the water were 10^{-5}, 10^{-7} and 10^{-9} M and the rats could drink as much as they required. Water and food consumption were monitored for three 3-day periods during the exposures. Consumption did not vary between the exposed groups. The body weight increased during the exposures without a similar increase in drinking water consumption. The uptake at the start of the experiments was approximately 35 ng/kg, 3.5 µg/kg and 350 µg/kg (all bw/day), and 20 ng/kg, 2.0 µg/kg and 200 µg/kg (all bw/day) during the later part of the experiment for the 10^{-9}, 10^{-7} and 10^{-5} M concentrations, respectively. No significant effects were seen on: body weight gain, haematocrit,

reproductive organ weights, mean serum luteinising hormone, follicle-stimulating hormone or testosterone concentrations, germ cell yield or relative numbers of different classes of testicular cells, or testicular sperm number. All doses caused an increase in epididymal sperm with tail abnormalities, which would affect sperm motility, and the highest dose decreased epididymal sperm numbers. The results were compared with those for administration by injection, in which significant effects were found on general health and reproductive system parameters. The significance of the effects seen in terms of impact on environmental populations is not clear.

A three-day subcutaneous injection test with young female rats appeared to show a doubling of uterine weight with octylphenol (Bicknell *et al.*, 1995). There are accepted uterine weight study protocols (the rat uterotrophic assay) that should be followed if such an effect is to be investigated and properly interpreted in the context of other known promoters of uterine growth. It is not clear that this was the case here and the study should be viewed as 'use with care'. The route of administration means the study is not useful for environmental risk assessment.

Bicknell *et al.* (1995) also reported that administration of 4-*tert*-octylphenol to pregnant rats and also postnatally had no effect on the development in the offspring of one area of the brain known to be dependent on levels of perinatal oestrogen. The authors suggested that the lack of results could reflect the inability of the substance to cross the blood–brain barrier, or to the low dose used.

4.4.2 Derivation of $PNEC_{oral}$

The most appropriate data for use in estimating the likely risk to predators are those from chronic dietary studies. There is one full chronic study available, a two-generation study on rats with exposure through food (Tyl *et al*., 1999), which gives a NOAEL of 15 mg/kg/day. This is supported by the results of two shorter (28- or 29-day) oral gavage studies, both of which have NOAEL values of 15 mg/kg/day for relatively minor effects (the next tested levels were 70 mg/kg and 150 mg/kg). The Sharpe *et al*. (1995) study is not considered valid in view of the later comments from the authors. Effects on sperm (tail abnormalities) have been seen at lower doses (down to 20-35 ng/kg/day) in a drinking water study (Blake *et al*., 2004), with reductions in the numbers of epididymal sperm at doses of 0.2-0.5 mg/kg/day. The significance of these observations for the environment is not clear. No effects on sperm numbers or morphology were seen in the two-generation rat study, where the dose levels overlap those at the higher end in the drinking water study, and there were no effects on reproduction overall. The two-generation study is therefore preferred in this assessment.

The NOAEL used for the PNEC derivation is 15 mg/kg/day. The conversion factor to concentration in food is 20 for rats that are more than 6 weeks old, giving a NOEC of 300 mg/kg. As the result is from a chronic study, the appropriate assessment factor is 30, giving a PNEC of 10 mg/kg in food.

4.4.2.1.1 Comparison with nonylphenol

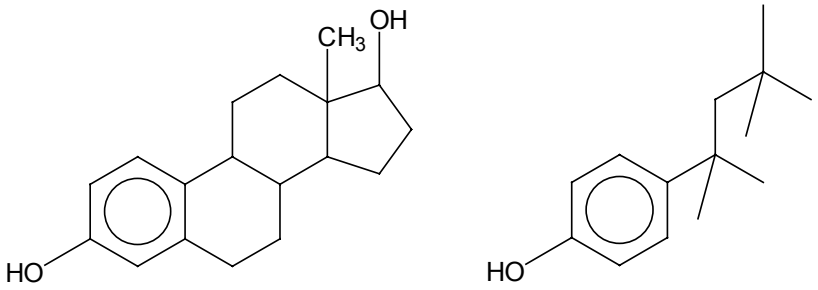
The $PNEC_{oral}$ for nonylphenol is also 10 mg/kg food; both PNECs are based on a NOAEL of 15 mg/kg/day in a chronic study and use the same assessment factor.

4.5 MODE OF ACTION

4-*tert*-Octylphenol may exert its effects on organisms by more than one mode of action. As outlined in Section 4.1.4 on QSAR-generated data, the substance falls into the category of polar narcotics as defined by OECD toxicity classes. However, this classification does not provide an indication of the actual mode of toxic action at a cellular level, and a number of mechanisms could be operating to disrupt cellular function and produce toxicity. The standard toxicity data do not provide any indication of exactly which systems are being affected.

Endocrine-mediated responses, on the other hand, are most likely to be mediated by a specific mechanism, and the majority of the data for this substance point towards interference and/or competition with the binding of natural oestrogens (such as 17β-estradiol) to receptor sites and mimicry of their effects (i.e., an oestrogen agonist). There are some structural similarities between 4-*tert*-octylphenol and certain hormones (see *Figure 4.1*), and 4-*tert*-octylphenol has been demonstrated to bind to the oestrogen receptor in almost exactly the same way as estradiol.

Figure 4.1 Structures of the hormone estradiol and 4-*tert*-octylphenol



Estradiol 4-*tert*-Octylphenol

There are also indications that 4-*tert*-octylphenol may act as an anti-androgen, by displacing androgen from the androgen receptor (Paris *et al.,* 2002). There are, however, other possible non receptor-mediated modes of action for the endocrine disruption effects reported, some of which may also be important in the more general toxicity seen. These mechanisms include calcium-dependent apoptosis, and also inhibition of the testicular calcium ATPase enzyme (leading to disruption of testicular development and a decrease in fertility). One cellular mechanism reported in a number of recent articles involves the disruption of cytochrome P450 enzymes, which has consequential effects on steroidogenesis – this could potentially produce effects on the endocrine system.

There are insufficient data to adequately describe a definitive mechanism of action for either general toxicity or endocrine effects for 4-*tert*-octylphenol, but it is possible that more than one may be acting, and that the two types of effects may be linked.

4.6 CLASSIFICATION FOR ENVIRONMENTAL HAZARD

Agreement on the environmental classification of 4-*tert*-octylphenol according to Directive 67/548/EEC was reached at an EU expert meeting in September 2004. The substance is classified as 'dangerous to the environment' with the following risk phrase:

R50/53: Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

This is based on the following data:

Aquatic toxicity: 48-hour/96-hour L(E)C_{50} <1 mg/L.
log K_{ow} >3 and low to moderate measured BCFs.
Not readily biodegradable

This classification is the same as the provisional classification included on manufacturer's Safety Data Sheets (Aldrich Chemicals, 2002; SASOL, 2001). For comparison, nonylphenol has the same classification.

The human health classification is not considered in this assessment.

5 RISK CHARACTERISATION

The following sections characterise risks for the aquatic, terrestrial and atmospheric compartments and the risk of secondary poisoning of predators in the food chain. The risk characterisation is performed by comparing the PECs with the PNECs to derive a risk characterisation ratio (RCR). An RCR of less than one implies that any risk resulting from that level of exposure is acceptable. An RCR above one implies a potential risk, and all such values are highlighted in bold in the following tables.

This assessment concerns the current use pattern of 4-*tert*-octylphenol. Appendix 4 presents a separate hypothetical risk assessment that assumes total replacement of nonylphenol by 4-*tert*-octylphenol in all of its applications. A risk characterisation for non-deliberate release as a consequence of nonylphenol production and use has also been performed as an illustration in Appendix 5.

5.1 AQUATIC COMPARTMENT

5.1.1 Surface water and sediment

5.1.1.1 Risk characterisation ratios

4-*tert*-Octylphenol enters the aquatic compartment directly due to industrial releases of the substance itself, and indirectly due to degradation of some its derivatives in use (e.g. ethoxylates). The RCRs for the aquatic compartment are shown in *Table 5.1*. **Note**: Since both the PECs and PNEC for sediment are derived assuming equilibrium partitioning, the sediment RCRs are similar to those for water.

Table 5.1 PECs and RCRs for the aquatic compartment

Life-cycle stage		PECs for surface water (µg/L)	RCR for surface water	PECs for WWTP organisms (mg/L)	RCR for WWTP
Production of 4-*tert*-octylphenol		5.4	**44.5**	0.215	**<2.15**
Phenol–formaldehyde resins	Resin manufacture	5.6	**46.2**	0.22	**<2.23**
	Resins in rubber formulation	0.49	**4.05**	0.004	<0.04
	Resins in varnishes	0.22	**1.82**	0.001	<0.014
	Resins in printing inks	7.85	**64.4**	0.078	<0.78
	Ethoxylated resin production	9.8	**80.3**	0.098	<0.98
	Marine paint formulation	0.14	**1.17**	6×10^{-4}	<0.006
	Marine paint application	8.47	**66.2**	0.08	<0.80
	Marine paint disposal	14.3	**117**	0.14	**<1.4**
Octylphenol ethoxylates	Production	3.1	**25.6**	0.39	**<1.22**
	Formulation (textile, pesticide)	1.33	**10.9**	-	-
	Emulsion polymerisation	0.125	**1.03**	-	-
	Textiles	3.0	**24.6**	-	-
	Paint formulation	0.86	**7.06**	-	-
	Paint application	0.096	0.79	-	-
	Pesticide application	0.34	**2.56**	-	-
	Veterinary medicine	0.18	**1.35**	-	-
	Ether sulphate production	5.9	**48.3**	-	-
Regional		0.084	0.69	-	-

Note: $PNEC_{aquatic}$ = 0.122 µg/L; PNEC WWTP = >0.1 mg/L.

Potential risks for surface water and sediment are identified for all of the scenarios, with the exception of the application of paints formulated with OPEs. The RCR for the regional concentration in water is 0.69, which indicates no risk from the background concentration. However, the background concentration does make a significant contribution to the PEC for a small number of scenarios – marine paint formulation, emulsion polymerisation and veterinary medicine applications. These three scenarios would not show a risk based on their local concentrations alone.

The PNEC selected for this assessment is similar to the draft maximum acceptable concentration Quality Standard for surface waters (0.13 µg/L) proposed under the Water Framework Directive (European Commission, 2004). The draft annual average Quality Standard (0.06 µg/L) is lower by a factor of two (this is based on an assessment factor of 100 applied to the lowest 'traditional' fish NOEC of 6.1 µg/L). Risks calculated using these Quality Standards would therefore either be similar to or twice as severe as those identified in *Table 5.1*.

5.1.1.1.1 Measured environmental concentrations

In addition to the modelled PECs given above, some values calculated from monitoring data are available and these can be compared to the predicted concentrations. Production site A has a PEC of <0.089 µg/L, which does not indicate a risk. The PEC estimated for site B is <0.11 µg/L, which gives a ratio just below one. The calculated PEC for a UK site releasing OPE to sewer from emulsion polymerisation was 2.2 µg/L, giving a ratio of 18. Site-specific factors (e.g. dilution) were not taken into account.

Otherwise, typical concentrations in surface water appear to be around 0.4 µg/L or below, although higher levels have been detected on occasion (e.g., 13 µg/L). Some of these are above the PNEC, and therefore a risk is suggested for the surface water compartment. However, the source of these high levels is unknown, and could be related to accidental discharges or to the presence of 4-*tert*-octylphenol as an impurity in nonylphenol.

Concentrations of 4-*tert*-octylphenol have been measured in industrial wastewaters and in the effluents of WWTP. The most recent measurements in The Netherlands found that the highest level in municipal effluent was 1.3 µg/L. If dilution by a factor of 10 is assumed (the TGD default), this would correspond to a surface water concentration slightly above the PNEC, which implies that the majority of measurements would lead to concentrations below the PNEC. (Note that measured levels in industrial wastewaters in Canada would, if dilution by 10 were assumed, give rise to potential risks in surface waters for some industries.)

5.1.1.1.2 Other ethoxylate breakdown products

The calculations for uses of OPEs are based on the breakdown of OPEs to 4-*tert*-octylphenol during wastewater treatment, with the production of 4-*tert*-octylphenol being 2.5% of the OPE released to the WWTP. Other products from the breakdown of the ethoxylates have been identified, as described in Appendix 1. Some of these, in particular the short-chain ethoxylates with one or two ethoxy units, and similar carboxylic acid derivatives, may also have toxic effects, although their relative hazards are unclear because of a lack of information (EA, 2002). White *et al* (1994) investigated the effects

of various alkylphenols in *in vitro* tests, using two different human breast cancer cell line cultures. Their work concentrated on octylphenol and *p*-nonylphenols and the ethoxylated and carboxylated derivatives of *p*-nonylphenol. In general the order of oestrogenicity of the compounds tested in each of the bioassays were:

octylphenol	>	p-nonylphenoxy-carboxylic acid	>	p-nonylphenol	=	p-nonylphenol diethoxylate

OPEs with greater than two ethoxylate groups were found to possess very little oestrogenic activity. Jobling *et al*. (1996) reported that nonylphenol monocarboxylate was as equipotent an oestrogen as nonylphenol itself.

Based on the limited evidence currently available, the OPEs and carboxylate derivatives appear to be less toxic than the parent alkylphenol. Although these degradation products are not strictly part of an assessment of 4-*tert*-octylphenol, being neither made from the substance nor arising from its breakdown, they are related to the use of derivatives of the substance. In Appendix 1, the production of OP1EO, OP2EO and short-chain carboxylates is estimated to be around 25% of the ethoxylate input. If this is added to the 2.5% of 4-*tert*-octylphenol produced, and the products are assumed to have a similar level of toxicity as a worst case, *all* scenarios involving ethoxylate use would give rise to a risk using the current assumptions on emissions and the current PNEC value.

However, it is understood that two articles are in press that contradict the earlier findings on relative potencies (APERC and CEPAD, 2005). One *in vivo* study in juvenile rainbow trout (*On. mykiss*) is reported to conclude that the relative estrogenic potency of NP1EO and NP1EC to NP was 0.22 and 0.03 respectively. The other study with medaka (*O. latipes*) is reported to find that:

- NP1EC at concentrations up to 2010 µg/L is essentially not toxic, with no effect on sex ratio or VTG induction noted.
- NP1EO had only a weak estrogenic response at 105 µg/L (an order of magnitude higher than the LOEC for nonylphenol).
- No estrogenic responses were seen with NP1EC (2010 µg/L), NP4EO (380 µg/L) or NP9EO (540 µg/L).

The evidence about the relative toxicity of these substances should be re-evaluated once the new data cited in APERC and CEPAD (2005) are published.

5.1.1.2 Uncertainties and possible refinements

There are significant uncertainties in the risk characterisation for the aquatic environment (including sediment). These are discussed below.

5.1.1.2.1 Emissions and exposure

The predicted environmental concentrations have been calculated using default release estimations from the TGD and ESDs, and data on production and use tonnage supplied by CEPAD. Measured environmental levels tend to suggest that emissions might not be as high (or the substance is not as persistent) as predicted in this assessment. The limited site-specific data for 4-*tert*-octylphenol producers indicate lower concentrations

(see Section 3.1.2.1). These levels need to be confirmed for other sites and preferably by measurements with a lower detection limit. The absence of site-specific measured data from downstream users means the calculated PECs (based on many default assumptions) are used to indicate the levels that arise from the different industrial uses. There are no monitoring data linked to actual processes to confirm these levels.

This reliance on default values implies that it should be possible to refine the PECs further. To do this, further site-specific information is required on releases of 4-*tert*-octylphenol by those EU companies that manufacture and distribute the end products, such as resins for tyres and ethoxylates. More specific information on the residual content of 4-*tert*-octylphenol in resins for different uses would be useful; at present, the assessment assumes that there is 3% residual 4-*tert*-octylphenol in all resins.

It is assumed that 100% of the activities involving a substance take place in the region, unless there is good reason to consider a more dispersed distribution. For many of the scenarios considered, there is not sufficient information to assume a different distribution, although in a small number of cases the assumption of 10% use in one region has been applied. One area for potential improvement of the PEC values is therefore to obtain realistic estimates of the amounts of 4-*tert*-octylphenol used on sites per day for the various areas of application.

As noted in Section 3.3.1.2.8 the calculated regional concentration is of the same order as the maximum measured values in surface water, and may therefore be an over-estimation. More extensive monitoring of levels in surface waters at suitable locations (away from the direct influence of point sources) would allow a measured regional background concentration to be used, which may have an impact on the conclusions for those scenarios which currently have ratios just above one.

5.1.1.2.2 Persistence

There are some indications in studies using natural waters, including seawater, that degradation may occur more rapidly than is estimated based on the classification of biodegradation. The determination of realistic half-lives in surface water and sediment could have an impact on the regional concentrations, which may be especially significant for sediment. Further investigation of the degradation in WWTP may allow a conclusion of 'inherently biodegradable meeting the criteria' to be reached. This would permit a reassessment of the fate of 4-*tert*-octylphenol in WWTP (this is briefly described in Section 3.2.5). Calculations using this revised distribution would reduce the risk ratios to around 60% of the current values for higher ratios, though less so for the smaller ratios. Only the scenario for marine paint formulation would have a ratio reduced to below one. This change would therefore have little impact on the assessment. (The scenarios involving ethoxylates are not affected.)

5.1.1.2.3 Effects

The PNEC of 0.122 µg/L is derived using an assessment factor of 50, and so could be revised. By comparison with the data available for nonylphenol, further testing might be expected to lead to a NOEC of the order of 3 µg/L, which would give a PNEC of 0.3 µg/L with an assessment factor of 10. This value would remove from concern the use of resins in varnishes, marine paint formulation and the use of ethoxylates in emulsion

polymerisation and in veterinary medicines. Taking the current data set, the highest PNEC achievable with an assessment factor of 10 would be 0.61 µg/L. This value would also remove the use of resins in rubber and pesticide applications (with OPEs) from concern. This would, however, still leave over half of the scenarios as showing a risk. To confirm the PNEC, long-term testing on *Gammarus* may be necessary, since this is the most sensitive species in short-term tests, and it is notably more sensitive than other invertebrates tested. Alternatively, long-term toxicity data on mysid shrimps could be obtained, since these are sensitive to nonylphenol. It is considered that testing with invertebrate species would be more beneficial than a repeat test on algae to confirm the somewhat uncertain data for that trophic level, although a fully valid algal result would provide reassurance about relative sensitivities.

The above comments relate to a PNEC derived from' traditional' toxicity endpoints. There are tests showing effects at lower concentrations (especially in snails) from which it is currently difficult to draw values to use as NOECs (see Sections 4.1.6 and 4.1.8). If the concentration of 1 µg/L is taken as an indication of the level at which effects in snails are seen (noting that this was the lowest concentration tested), only the application of paints containing OPEs gives a margin of safety greater than 10, and several scenarios have margins of safety less than one. Further research into these effects at low concentrations would therefore be beneficial. For example, a range of snail species could be tested, although no standard test methods are currently available. A further possible avenue of investigation could be the field monitoring of prosobranch molluscs. This would provide evidence of whether the apparent sensitivity of molluscs to substances such as 4-*tert*-octylphenol in laboratory tests is also demonstrated in the field. There would be difficulties in relating effects seen to specific substances, and so such work would need to be combined with targeted monitoring.

A more detailed investigation of the effects of some of the intermediate breakdown products of the ethoxylates could also be considered. It is understood that some relevant data might be published in the scientific literature in the near future.

5.1.1.2.4 Summary

From the above discussion, the most promising area for refining the assessment is in the estimation of emissions, including the amounts used at sites. Targeted monitoring to support this may also be useful. Further testing on conventional toxicity endpoints should await any refinement of the exposure assessment. In view of the difficulty in deriving suitable NOECs from the data on endocrine effects, further testing on aquatic snails could also be considered, noting that this may result in a lower value for the PNEC.

These comments relate to both water and sediment exposures. The limited results of tests on snails exposed via sediment strengthen the arguments for further testing on snails. Using the tentative alternative sediment PNEC of 0.92 µg/kg derived in Section 4.1.8.2 (based on the highest concentration tested in a study on snails with sediment exposure), all scenarios would give rise to a risk.

5.1.2 Wastewater treatment plant micro-organisms

5.1.2.1 Risk characterisation ratios

The PECs and RCRs for WWTP organisms are shown in *Table 5.1*. A potential risk for micro-organisms in WWTP is indicated for the production of 4-*tert*-octylphenol and the production of octylphenol–formaldehyde resins and ethoxylates.

No risks to micro-organisms are identified for the use of resins, with the exception of the scenario for removal and disposal of marine paints. An assessment for the use of OPEs is not appropriate, because 4-*tert*-octylphenol is not produced during the aerobic phase of the WWTP that is the subject of this assessment.

5.1.2.2 Uncertainties and possible refinements

The assessment could be refined with better information on releases to WWTPs (or direct measurements) at production and resin production sites. The same information is required for the aquatic assessment. For example, site-specific information from two production sites shows much lower concentrations in the WWTP effluents, and these do not indicate a risk. These sites also use 4-*tert*-octylphenol to produce resins and/or OPEs, and so in at least some cases there would appear to be no risk for production or resin production. The data are limited, however, and it would be useful to confirm them at these and other sites. Further investigation of the possible releases from the removal and disposal of marine paints containing resins would be useful, as would information about realistic amounts of paint handled at such sites.

As noted in Section 4.1.8.1.2, the PNEC is also likely to be too conservative, and may be nearer the PNEC for nonylphenol (9.5 mg/L). A PNEC of this order would indicate no risks at the concentrations currently estimated using defaults, and so further testing for effects might refine the assessment (e.g., OECD 209 test at higher concentrations than the existing limit test). However, the emission information would still be required for the aquatic compartment, and so the need for toxicity data could be reviewed following any refinement of the exposure assessment.

5.1.3 Marine waters and sediment

5.1.3.1 Risk characterisation ratios

A marine risk assessment has been carried out (see Appendix 2). The RCRs appear to indicate a significant risk to marine waters and sediments from all scenarios, with the exception of the use of ethoxylates in emulsion polymerisation and the application of paints containing OPEs.

5.1.3.1.1 PBT assessment

4-*tert*-Octylphenol meets two of the PBT criteria; it can be considered to be potentially persistent or very persistent and toxic, but it does not exceed the bioaccumulation criterion given in the TGD. The measured fish BCFs are substantially lower than the threshold value of 2000 (only just over 10%). No further studies are therefore required to

test whether this criterion is met and 4-*tert*-octylphenol is not considered to be a PBT substance.

5.1.3.2 Uncertainties and possible refinements

The PEC assessment relies on the same estimates of emissions as for freshwater. The PNECs are derived from a data set of freshwater toxicity values with limitations as discussed in Section 4.1.8.1, together with values for a small number of saltwater species. As for the freshwater compartment, further site-specific information on releases to water would enable a refined assessment to be performed. The need for long-term toxicity testing with (preferably marine) aquatic and/or sediment organisms could be reviewed following any refinement of the exposure assessment.

5.2 TERRESTRIAL COMPARTMENT

5.2.1 Risk characterisation ratios

Direct releases of 4-*tert*-octylphenol to the terrestrial compartment are unlikely to occur given its production method and use pattern. However, high concentrations are predicted in all soil types because of the application of sewage sludge from some processes that use the substance (or its derivatives) and discharge aqueous effluent to water. The RCRs for the terrestrial compartment are shown in *Table 5.2*.

Table 5.2 PECs and RCRs for the terrestrial compartment

Life-cycle stage		PECs for soil (mg/kg wwt)	RCR for soil
Production of 4-*tert*-octylphenol		0.45	**76.2**
Phenol–formaldehyde resins	Resin manufacture	0.47	**79.1**
	Resins in rubber formulation	0.009	**1.49**
	Resins in varnishes	0.004	0.65
	Resins in printing inks	0.16	**27.7**
	Ethoxylated resin production	0.20	**34.6**
	Marine paint formulation	0.001	0.21
	Marine paint application	0.17	**28.5**
	Marine paint disposal	0.30	**50.7**
Octylphenol ethoxylates	Production	0.49	**82.9**
	Formulation (textile, pesticide)	0.61	**103**
	Emulsion polymerisation	0.02	**3.43**
	Textiles	1.43	**241**
	Paint formulation	0.38	**64.4**
	Paint application	0.006	**1.03**
	Pesticide application	0.029	**4.9**
	Veterinary medicine	0.003	0.51
	Ether sulphate production	2.84	**481**
Regional		2.5×10^{-4}	0.042

Note: $PNEC_{soil}$ = 0.00591 mg/kg wwt (provisional).

The RCR for the regional agricultural soil concentration (for both direct and indirect releases) is 0.042. All local scenarios indicate a possible risk to the soil compartment, with the exception of the use of resins in varnishes, formulation of marine paints and use of ethoxylates in veterinary medicine.

Concentrations of around 0.1 µg/L have been measured in water infiltrating a noise barrier made from shredded tyres in Norway (Aabøe *et al*., 2004). Detailed results are not included in the article, but the concentration is described as being just over the PNEC value of 0.122 µg/L for aquatic organisms in some cases (and hence the equivalent soil concentration would be just over the soil PNEC). After mixing in a storm water basin, the concentrations were all below 0.1 µg/L.

5.2.2 Uncertainties and possible refinements

There are significant uncertainties in the risk characterisation for the terrestrial environment. Both the predicted environmental concentrations and PNEC are based on those for the aquatic compartment, which are themselves uncertain (see Section 5.1.1.2). There are no monitored data to confirm the predicted levels. Any refinement to the aquatic PECs will therefore have an effect on the terrestrial PECs.

More specific information on the fate of sludges that contain 4-*tert*-octylphenol would also be useful for the revision of the assessment (e.g., information on sewage sludge disposal practice in the different use areas). Measurements on the concentrations of 4-*tert*-octylphenol in sludges would be useful to confirm the estimates regarding the fate of the substance in WWTP, and especially the calculations of the production of 4-*tert*-octylphenol from ethoxylates. One study (Rhind *et al.*, 2002) showed no accumulation in soils following bi-annual applications, with the level of 4-*tert*-octylphenol being below the limit of detection (although the detection limit in this case was above the PNEC value). This suggests that studies on biodegradation in soils might also be valuable.

Any refinement to the PNEC for surface water will also have a direct effect on the PNEC for soil organisms derived by the equilibrium partitioning method. Based on data for nonylphenol, the PNEC may be closer to 0.3 mg/kg wwt. The value for nonylphenol is based on test results with terrestrial organisms rather than on aquatic organisms through the equilibrium partitioning approach. A PNEC of this order would still indicate concerns for production, manufacture of resins and OPEs, and for some uses of OPEs. Since the substance is expected to partition to soil, direct testing with soil organisms would be useful even if further aquatic testing is performed, especially as some of the RCRs for soil are large and the read-across of effects data from nonylphenol is uncertain. A 28-day earthworm reproduction study is suggested, since this species and trophic level was the most sensitive for nonylphenol. In view of the endocrine-related effects in aquatic organisms, consideration should be given to using tests that have the ability to show similar effects in terrestrial organisms (though at the moment there are no methods available).

In the first instance, it is recommended that further information on releases be collected. The need for biodegradation and toxicity testing could be reviewed if the exposure assessment is refined, although it could usefully be performed concurrently.

5.3 ATMOSPHERIC COMPARTMENT

No effects data are available for non-mammalian species that can be used to derive a PNEC. Although the lack of toxicity data for suitable species cannot be taken as implying no concern, there is unlikely to be a risk for this compartment because air concentrations

are predicted to be very low (because of low releases to air, low volatility and a short atmospheric half-life). Abiotic effects are similarly unlikely.

5.4 NON-COMPARTMENT-SPECIFIC EFFECTS RELEVANT TO THE FOOD CHAIN (SECONDARY POISONING)

5.4.1 Risk characterisation ratios

Predators may be exposed to 4-*tert*-octylphenol via both the aquatic and terrestrial food chains. The RCRs are shown in *Table 5.3*.

No risks are identified for any of the scenarios. There is therefore no need to refine this aspect of the assessment. Improvements to the estimates of emissions are likely to lead to a further reduction in the ratios. An additional calculation of a level in food was made for direct application of pesticides containing OPE to plants, giving a concentration on plants of 4.5 mg/kg. This also does not indicate a risk, even if this was taken as the only food source in the diet.

Table 5.3 PECs and RCRs for secondary poisoning

Life-cycle stage		PECs for fish (mg/kg wwt)	RCRs for fish eating predators	PECs for worms (mg/kg wwt)	RCRs for worm eating predators
Production of 4-*tert*-octylphenol		1.45	0.15	0.58	0.06
Phenol–formaldehyde resins	Resin manufacture	1.5	0.15	0.60	0.06
	Resins in rubber formulation	0.16	0.02	0.012	<0.01
	Resins in varnishes	0.068	<0.01	0.006	<0.01
	Resins in printing inks	2.08	0.21	0.21	0.02
	Ethoxylated resin production	0.73	0.07	0.26	0.03
	Marine paint formulation	0.069	<0.01	0.002	<0.01
	Marine paint application	2.13	0.21	0.22	0.02
	Marine paint disposal	3.76	0.38	0.39	0.40
Octylphenol ethoxylates	Production	0.19	0.02	0.33	0.03
	Formulation (textile, pesticide)	0.086	0.01	0.78	0.08
	Emulsion polymerisation	0.064	<0.01	0.027	<0.01
	Textiles	0.21	0.02	1.84	0.18
	Paint formulation	0.19	0.02	0.49	0.05
	Paint application	0.056	<0.01	0.008	<0.01
	Pesticide application	0.054	<0.01	0.078	<0.01
	Veterinary medicine	0.054	<0.01	0.007	<0.01
	Ether sulphate production	0.81	0.08	3.66	0.67

Note: $PNEC_{oral}$ worm/fish = 10 mg/kg food (assessment factor on mammalian toxicity data = 30).

5.5 SUMMARY OF CONCLUSIONS

4-*tert*-Octylphenol enters the environment directly through industrial releases of the substance itself, and indirectly through degradation of some its derivatives in use (e.g.,

ethoxylates). The assessment identifies potential risks for the freshwater and marine aquatic (including sediment) compartments, WWTP and the terrestrial compartment. This applies to production and most uses, although it is based on exposure estimates that have many uncertainties.

The substance does not pose a risk to the atmosphere or for secondary poisoning through the food chain at current levels of use. Note that there is no concern for WWTP organisms for several uses.

More detailed information on emissions, biodegradation and measured concentrations in the environment could allow more realistic estimates to be made. There is also scope to refine the effects assessment for most compartments. Data that could usefully be provided are:

- Further site-specific information on releases of 4-*tert*-octylphenol by those EU companies that manufacture and distribute the end products, including information on amounts used on realistic worst-case sites for local PEC calculations.
- Confirmation of the true reasonable worst case percentage of the substance present within phenolic resins[20].
- Targeted monitoring to confirm the PEC values (especially in water and WWTP sludges).
- Further testing of environmental half-life, particularly in soils.
- Further aquatic toxicity testing (e.g., chronic tests on aquatic snails and other invertebrates, such as mysids or *Gammarus*).
- WWTP organism toxicity testing (e.g., an OECD 209 test).
- A 28-day earthworm toxicity test. Consideration should also be given to methods that have the ability to test for endocrine effects in terrestrial organisms (though at the moment there are no suitable methods available).

The largest initial impact on the conclusions would be provided by substantiated representative information on environmental releases. The need for toxicity and/or degradation testing could be reviewed if the exposure assessment were refined, although some studies could usefully be performed concurrently. The most useful would be studies on snails (although there are no agreed test methods currently available). A more detailed investigation of the effects of some of the intermediate breakdown products of the ethoxylates could also be considered.

It is now known that branched octylphenol isomers similar to 4-*tert*-octylphenol can be a significant impurity in commercial nonylphenol. Potential risks from this source have been predicted for several uses of nonylphenol (see Appendix 5). Risk-reduction measures are being put in place for nonylphenol, so this source should decline in importance. Nevertheless, the consequences of co-release of nonylphenol and 4-*tert*-octylphenol should be considered where they are used in the same processes, for example in site-specific assessments.

[20] It is understood that Industry is planning to conduct analytical studies to determine the concentration of free octylphenol in resin as well as the water extractable amount of octylphenol in resin and tyres (APERC and CEPAD, 2005).

Finally, the hypothetical complete substitution of nonylphenol by 4-tert-*octylphenol could lead to similar risks as predicted for nonylphenol* (see Appendix 4 for further details).

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Property	**Value**
Water solubility	5 mg/L at 25°C
Vapour pressure	64 mPa 4.7 Pa at 74°C
HLC	0.699 $Pa.m^3/mol$ at 20°C
K_{oc}	log K_{oc} 4.3
BCF	6000

Since this reference was not available for review, these data have not been considered further in this report. However, in general they are not very different from the values chosen for this assessment.

7 GLOSSARY OF TERMS

Term	Description
Biochemical oxygen demand (BOD)	A measure of degradation potential
Bioconcentration factor (BCF)	A measure of chemical uptake, being the ratio between the concentration in an organism and the concentration in an environmental compartment (usually water)
CAS number (no.)	An identifying code number assigned to chemicals by the Chemical Abstract Services. The CAS number is a generally recognised identification reference for a chemical; it is possible that a substance can have more than one such number
Lowest observed effect concentration	The lowest concentration in a toxicity test that gives rise to adverse effects (relative to a control)
Median effective concentration (EC_{50})	The concentration in a toxicity test at which a particular effect is observed in half of the organisms exposed for a specified time
Median lethal concentration/dose (LC/D_{50})	The concentration in a toxicity test that can be expected to cause death in half of the organisms exposed for a specified time
No observed effect concentration (NOEC)	The highest concentration in a toxicity test that does not give rise to adverse effects (relative to a control)
Octanol-water partition coefficient (K_{ow})	This parameter gives an indication of the partitioning behaviour of a substance between water and lipid-containing materials such as cell membranes or organic matter in soils and sediments
Readily biodegradable	Rapid environmental degradation to carbon dioxide and water, etc., as measured by laboratory screening tests involving micro-organisms

8 ABBREVIATIONS

Acronym	Description
AF	Assessment factor
APE	Alkylphenol ethoxylates
APERC	Alkylphenols and Ethoxylates Research Council
APnEC where n is an integer	AP carboxylate with n ethoxy units
APnEO where n is an integer	APE with n ethoxy units
ASTM	American Society for Testing and Materials
BACS	British Association for Chemical Specialities
BCF	Bioconcentration factor
BOD	Biochemical oxygen demand
bw	Body weight/*Bw*, *b.w.*
CAS	Chemical Abstract Services
CCP	Carbonless copy paper
CEFIC	European Chemical Industry Council
CEPAD	Conseil Européen des Phénols Alkylés et Derivés (the European Council for Alkylphenols and Derivatives): a trade association representing the major European producers of alkylphenols, and some of the users (http://www.cefic.be/cepad/)
CMR	Carcinogenic, mutagenic and toxic to reproduction
COD	Chemical oxygen demand
CTPA	Cosmetic, Toiletry and Perfumery Association
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
Defra	Department of the Environment, Food and Rural Affairs
DETR	Department of the Environment, Transport and the Regions
DIN	Deutsche Industrie Norm (German norm)
dw	Dry weight
EAA	European Adjuvants Association
EC	European Communities
EC_{50}	Median effect concentration
EC_x	As EC_{50}, but for x% effect; x usually being 0, 10, or 100
ECB	European Chemicals Bureau
EDMAR	Endocrine disruption in the marine environment
EDTA	Endocrine disruption testing and assessment
EEC	European Economic Communities
EINECS	European Inventory of Existing Commercial Chemical Substances – this lists all chemical substances that were supplied to the market prior to 18th September 1981
EN	European Norm
EOSCA	European Oilfield Speciality Chemicals Association
EPA	Environmental Protection Agency (USA)
EPDLA	European Polymer Dispersion and Latex Association
EPRA	European Phenolic Resins Association
EQS	Environmental quality standard

Acronym	Description
ESD	Emission Scenario Document
ESR	The Existing Substances Regulation – Council Regulation (EEC) 793/93 on the evaluation and control of the risks of 'existing' substances
EU	European Union
EU-15	The 15 European Union Member States prior to May 2004
EUSES	European Union System for the Evaluation of Substances (software tool in support of the TGD on risk assessment)
GLP	Good laboratory practice
GSI	Gonadosomatic index
HERTG	Health, Environmental and Regulatory Task Group (part of the American Chemistry Council's Petroleum Additives Panel)
HSI	Hepatosomatic index
HLC	Henry's Law constant
HPLC	High pressure liquid chromatography
HPV	High Production Volume (supply > 1000 tonnes/year)
HPVC	High production volume chemical (supply > 1000 tonnes/year)
IC	Industrial category
IC50	median immobilisation concentration or median inhibitory concentration
IPC	Integrated pollution control
IPCS	International Programme on Chemical Safety
IPPC	Integrated Pollution Prevention and Control (EC Directive 96/61/EEC)
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database: contains data collected under the Existing Substances Regulation (ESR)
IUPAC	International Union for Pure and Applied Chemistry – the IUPAC name is the formal chemical name
K_{hw}	n-Hexanol–water partition coefficient
K_{oc}	Organic carbon normalised distribution coefficient
K_{ow}	Octanol–water partition coefficient
Kp	Solids–water partition coefficient
$L(E)C_{50}$	Median lethal (effect) concentration
LD_{50}	Median lethal dose
LOAEL	Lowest observed adverse effect level
LOEC	Lowest observed effect concentration
LOEL	Lowest observed effect level
log K_{ow}	Log of the octanol-water partition coefficient (K_{ow})
LPV	Low production volume (supply 10-1000 tonnes/year)
LPVC	Low production volume chemical (supply 10-1000 tonnes/year)
LQ	Quantification limit
MITI	Ministry of International Trade and Industry, Japan
N	Dangerous for the environment (symbol and indication of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
NO(A)EL	No observed (adverse) effect level
NOEC	No observed effect concentration
NP	Nonylphenol

Acronym	Description
NPE(s)	Nonylphenol ethoxylate(s)
NPnEC where n is an integer	NP carboxylate with n ethoxy units
NPnEO where n is an integer	NPE with n ethoxy units
n.t.p.	Normal temperature and pressure
OECD	Organisation for Economic Cooperation and Development
OP	Octylphenol
OPE(s)	Octylphenol ethoxylate(s)
OPnEC where n is an integer	OP carboxylate with n ethoxy units
OPnEO where n is an integer	OPE with n ethoxy units
OPE-S	Octylphenol ether sulphates
OSI	Ovarian somatic index
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic, http://www.ospar.org
P	Persistent
PBT	Persistent, bioaccumulative and toxic
PEC	Predicted environmental concentration
PFPA	Pentafluoropropionic acid anhydride
pH	Logarithm (to the base 10) of the hydrogen ion concentration [H^+]
pKa	Logarithm (to the base 10) of the acid dissociation constant
PNEC	Predicted no effect concentration
ppb	Parts per billion
ppm	Parts per million
(Q)SAR	(Quantitative) Structure-Activity Relationship
RBA	Relative binding affinity
RCR	Risk characterisation ratio
SDGSI	Sperm duct gland somatic index
SEPA	Scottish Environmental Protection Agency
SETAC	Society of Environmental Toxicology And Chemistry
SIAR	SIDS Initial Assessment Report, OECD
SIDS	Screening Information Data Set, OECD
SMILES	Simplified Molecular Input Line Entry System – the SMILES code is a chemical notation system used to represent a molecular structure by a linear string of symbols; it is a simple way of entering chemical structural information into a computer programme
SRC	Syracuse Research Corporation
STW	Sewage treatment works
STP	Sewage treatment plant
TG	Test guideline
TGD	Technical Guidance Document
UBA	Umweltbundesamt (Federal Environment Protection Agency in Austria and Germany)
UGPLI	Urogenital pupilla length index
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet region of the electromagnetic spectrum
vB	Very bioaccumulative

Acronym	Description
vP	Very persistent
vPvB	Very persistent and very bioaccumulative
VTG	Vitellogenin
w/v	weight per volume ratio
w/w	Weight per weight ratio
wt	Weight
wwt	Wet weight
WWTP	Wastewater treatment plant

APPENDIX 1 FORMATION OF OCTYLPHENOL FROM THE DEGRADATION OF ETHOXYLATES AND OTHER DERIVATIVES IN THE ENVIRONMENT

One of the major indirect inputs of alkylphenols to the environment is via the biodegradation of alkylphenol ethoxylates (APE) (for nomenclature used in this section see below). Numerous authors (e.g., Rudling and Solyom, 1974; Brüschweiler *et al.*, 1982; Giger *et al.*, 1984, 1987; Reinhard *et al.*, 1982; Ahel *et al.*, 1996; Holt *et al.*, 1992) have determined the biodegradation sequence of APE in laboratory tests and during biological treatment in sewage works (see *Figure A1.1*). The first step in the breakdown of APEs is the rapid removal of ethylene oxide (EO) groups, eventually giving rise to 'lower' APEs with less than four ethoxy units, and usually one or two ethoxy units (AP1EO and AP2EO). Under aerobic conditions, these may be further oxidised to the corresponding carboxylic acids (predominantly AP1EC and AP2EC; Frazee *et al.*, 1964; Ball *et al.*, 1989; Ahel *et al.*, 1996; Field and Reed, 1996; Lee and Peart, 1998) or they may be converted into the alkylphenols, especially where anaerobic conditions prevail (Bennie, 1999).

Ball *et al.* (1989) carried out an extensive study of the biodegradation of 'lower' octylphenol ethoxylates (OPnEOs) and the corresponding carboxylic acids (OPnECs) under a variety of aerobic and anaerobic conditions. The OPnEO material used was a mixture of 13% OP1EO, 40% OP2E0, 29% OP3E0, 14% OP4E0 and 4% OP5E0. In addition, the corresponding OPnEC (same relative composition of oligomers) was used in some tests. The test substances were incubated with either (a) BOD dilution water seeded with solids from an activated sludge plant (500 µg/L of OPnEO with 180 mg/L of solids at 20°C), (b) settled effluent from the same plant (10 mg/L of OPnEO or OPnEC at 20°C) or (c) anaerobic bacteria maintained under anaerobic conditions (25 µmole per litre of OPnEO or OPnEC at 35°C).

The experiments using activated sludge inoculation (a) clearly show that the OPnEOs degrade to OPnEC (mainly OP2EC) with little or no mineralisation. Results from the primary sludge inoculated tests (b) show that the longer chain OPnEO (>3EO units) degraded rapidly (within 2 days) with a concurrent increase in OP2EO. Degradation of OP1EO and OP2E0 appeared to require an adaptation period of approximately 5 and 17 days respectively before degrading to unidentified products. Some oxidation of the OPnEO to OPnEC did occur, but this was only a very minor route of degradation since little or no OPnEC with >3 EO units were seen in the test. OP12EC were degraded to some extent under the conditions used, with the possible formation of small amounts of octylphenol. The test media were analysed for degradation products at selected periods throughout the experiments and the results are tabulated below (*Tables A1.1-A1.5*).

Table A1.1 Results of incubation of OPnEO with activated sludge

Components	Concentration (μmole/L)					
	0 hour	2 hour	6 hour	12 hour	18 hour	24 hour
Starting compounds						
OP1E0	0.18	0.05	0.03	0.02	0.01	tr
OP2EO	0.54	0.20	0.14	0.03	0.01	tr
OP3EO	0.40	0.06	0.02	nd	nd	nd
OP4EO	0.18	nd	nd	nd	nd	nd
OP5EO	0.10	nd	nd	nd	nd	nd
Total starting compounds	1.4	0.31	0.19	0.05	0.02	tr
Products						
Octylphenol		tr	tr	tr	tr	tr
OP1EC		0.26	0.17	0.19	0.23	0.14
OP2EC		0.61	0.39	0.50	0.85	0.72
OP3EC		0.19	0.12	0.08	0.20	0.15
Total components	1.4	1.4	0.87	0.82	1.3	1.0

nd, not detected; tr, trace amount (<0.005 μmole/L).

Table A1.2 Results of incubation of OPnEO with primary sewage

Components	Concentration (μmole/L)						
	day 0	day 2	day 5	day 17	day 36	day 64	day 127
Starting compounds							
OP1E0	3.6	2.3	2.9	0.13	nd	nd	nd
OP2EO	11	19	21	26	5.1	0.04	nd
OP3EO	8.0	0.58	0.33	0.19	0.03	nd	nd
OP4EO	3.7	tr	tr	nd	nd	nd	nd
OP5EO	0.88	nd	nd	nd	nd	nd	nd
Total starting compounds	27	22	24	26	5.1	0.04	nd
Products							
Octylphenol		nd	0.01	0.01	nd	nd	nd
OP1EC		nd	0.07	nd	nd	nd	nd
OP2EC		0.03	0.13	0.03	nd	nd	nd
OP3EC		tr	nd	0.03	0.03	0.02	0.03
Total components	27	22	24	26	5.2	0.06	0.03

nd, not detected; tr, trace amount (<0.005 μmole/L).

Table A1.3 Results of incubation of OPnEC with primary sewage

Components	Concentration (µmole/L)						
	day 0	day 2	day 5	day 17	day 36	day 64	day 127
Starting compounds							
OP1EC	0.32	0.35	0.32	tr	nd	nd	nd
OP2EC	9.2	6.6	7.6	0.05	nd	0.01	nd
OP3EC	7.7	5.5	6.5	6.3	5.5	2.8	0.14
OP4EC	4.3	2.6	3.4	3.4	3.4	1.3	nd
OP5EC	2.0	1.0	1.3	1.2	1.3	0.01	nd
OP6EC	0.69	0.99	0.92	1.1	0.38	nd	nd
Total starting compounds	24	17	20	12	11	4.1	0.14
Products							
Octylphenol		0.02	0.01	0.01	0.01	0.01	nd
OP1EO		0.03	0.03	0.20	0.02	nd	nd
OP2EO		0.01	0.01	0.19	0.40	0.01	nd
OP3EO		Nd	nd	tr	0.03	nd	nd
Total components	24	17	20	12	11	4.1	0.14

nd, not detected; tr, trace amount (<0.005 µmole/L).

Table A1.4 Results of incubation of OPnEO under anaerobic conditions

Components	Concentration (µmole/L)						
	day 0	day 10	day 23	day 46	day 66	day 116	day 190
Starting compounds							
OP1E0	3.8	26	18	2.8	0.51	0.15	tr
OP2EO	11	0.23	0.03	nd	nd	nd	nd
OP3EO	8.3	0.26	nd	nd	nd	nd	nd
OP4EO	3.8	tr	nd	nd	nd	nd	nd
OP5EO	0.91	0.37	nd	nd	nd	nd	nd
Total starting compounds	28	27	18	2.8	0.51	0.15	tr
Products							
Octylphenol		0.19	1.1	2.2	5.1	2.7	2.2
OP1EC		tr	0.39	nd	nd	nd	nd
OP2EC		1.0	0.85	0.91	0.89	1.1	0.82
OP3EC		0.72	0.44	0.56	0.48	0.33	0.11
OP4EC		nd	nd	0.13	0.17	0.17	0.06
Total components	27	22	24	26	5.2	0.06	0.03

nd, not detected; tr, trace amount (<0.005 µmole/L).

Table A1.5 Results of incubation of OPnEC under anaerobic conditions

Components	Concentration (µmole/L)						
	day 0	day 10	day 23	day 46	day 66	day 116	day 190
Starting compounds							
OP1EC	0.34	nd	nd	nd	nd	nd	nd
OP2EC	9.7	9.8	8.7	8.6	7.9	9.4	12
OP3EC	8.1	7.8	6.6	6.2	5.1	7.0	7.7
OP4EC	4.6	2.8	1.9	2.2	1.5	2.2	2.6
OP5EC	2.1	-	-	-	-	-	-
OP6EC	0.73	nd	nd	nd	nd	nd	nd
Total starting compounds	26	20	17	17	15	19	22
Products							
Octylphenol		0.27	0.19	0.19	0.19	0.17	0.17
OP1EO		0.24	0.13	0.11	0.07	0.05	0.04
Total components	26	21	18	17	15	19	23

nd, not detected; tr, trace amount (<0.005 µmole/L); - = not determined (because of interferences).

Under anaerobic conditions OPnEO was degraded predominantly to OP1EO within 10 days, and this was subsequently converted into octylphenol, which appeared to be stable under the conditions of the test. The oxidative pathway (to form the carboxylates) did not occur under these conditions. The OP24ECs were not degraded under anaerobic conditions, although OP1EC was rapidly degraded with octylphenol again being produced.

Most of the quantitative biodegradation studies have been carried out on NPEs (ECB, 1999), but these should also be indicative for OPEs in respect of the percentage conversion of OPE to octylphenol. For example, a major study for NPEs in WWTPs in Switzerland (Ahel *et al.*, 1994a) showed that the overall removal of NPnEO (n > 2) is 92%. Of the total entering the plant, 19% was released via the effluent as NPnEC, 11% released via the effluent as NP1EO + NP2EO, 25% released as nonylphenol (22.5% is adsorbed onto digested sludge and <2.5% is released as nonylphenol in effluent) and 8% released untransformed.

Little transformation of the ethoxylates into the alkylphenols occurs in rivers, but transformation can occur once the ethoxylate is sorbed to sediments. As a consequence of this and deposition of solid material that contains sorbed nonylphenol, measured concentrations of NP, NP1EO and NP2EO in river sediments tend to be higher than those determined in the overlying water (Ahel *et al.*, 1994b; Bennie, 1999). In marked contrast to the water column, nonylphenol was the most abundant alkylphenolic compound associated with the sediment with concentrations of 364-5100 times higher than in the water. Recent research (Johnson *et al.*, 1998) suggests that bed and suspended sediments play a key role in the fate of octylphenol in rivers, although their relative importance in sequestering octylphenol appears to differ between river catchments. In addition, studies of aerobic biodegradation of NPEOs in river water, using LC-ESP-MSMS (liquid chromatography coupled to tandem mass spectrometry with electrospray ionisation) to identify products have shown that degradation of the nonyl chain also occurs concomitantly with degradation of the ethoxylate chain (Jonkers *et al.*, 2001). This results in the formation of metabolites having both carboxylated ethoxylate and alkyl chains of varying lengths. Degradation was shown to be initiated by ω-carboxylation of the individual ethoxylate chains, and metabolites with long carboxylated

ethoxylate chains were identified. Further degradation proceeded gradually into short-chain carboxylated EO with the most abundant species being NPE2C.

From the available data for APEs (predominantly for NPEs), reasonable worst-case assumptions for the fate of OPEs during anaerobic wastewater treatment would be (based on % weight):

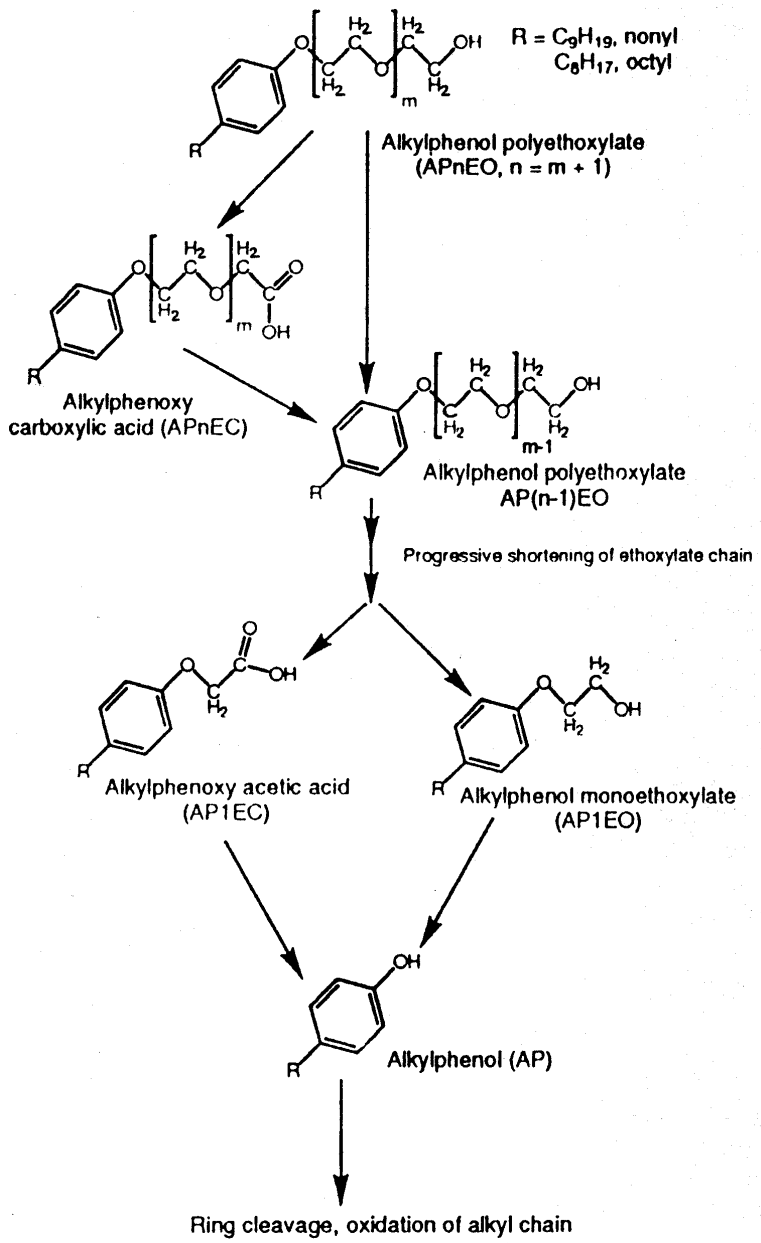
Mineralised/highly degraded	45%
Released as OP1EO/OP2EO/OPnEC in effluent	25%
Released as OPnEO (n > 3)	8%
Released as octylphenol in effluent	2.5%
Octylphenol in anaerobically digested sludge	19.5%

The OPEs released to the environment (OP1EO, OP2EO, OPnEO, OPnEC) will undergo some further degradation. The available information indicates that the alkylphenol is at most only a minor product from the aerobic degradation of such compounds in river water and soil, but may be produced in larger quantities if alkylphenols adsorb to anoxic sediments. As a worst case it could be assumed that a further 2.5% of the OPnEO released to the environment would eventually end up as octylphenol. The overall conversion is likely to have a fairly long half-life, probably of the order of 100 days in water and 30 days in soil.

Nomenclature:

APnEO	Alkylphenol ethoxylate with n ethylene oxide units. In commercial products, it is normal for a range of EO chain lengths to be represented and the value of n indicates the average number of EO units on the molecule. The most common length of the EO chain is around nine and, in the case of nonylphenol, this is denoted as NP9EO.
AP1EO	alkylphenol monoethoxylate
AP2EO	alkylphenol diethoxylate
AP1EC	the carboxylic acid of AP1EO formed by oxidation of the terminal OH group
AP2EC	the carboxylic acid of AP2EO formed by oxidation of the terminal OH group

Figure A1.1 Biodegradation scheme for alkylphenol ethoxylates



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APPENDIX 2 MARINE RISK ASSESSMENT

A2.1 Derivation of marine PECs

The calculation of marine PEC values has been performed according to the TGD, using the EUSES 2 program, unlike those calculated for the most recent OSPAR background document (OSPAR, 2001 – see the main report for the references cited in this Appendix). The input data are the same as those used for the freshwater calculations; a major difference between the freshwater and marine assessments is that the effluent from industrial activity is assumed to enter the marine environment directly, rather than after treatment in a WWTP. This direct release has been assumed for the calculations for production use as an intermediate and for all of the resin scenarios.

Releases from the use of OPEs have to be considered in a different way. The risk assessment considers that the degradation of the ethoxylates in WWTP leads to a release of 4-*tert*-octylphenol. Direct release of ethoxylates would not contain significant levels of 4-*tert*-octylphenol. To take into account the possible releases of 4-*tert*-octylphenol from these uses, it has been assumed that the effluents from WWTP that serve these industries discharge into the marine environment (this is done within the EUSES program). The resulting PEC values are given in *Table A2.1* and cover marine waters, sediments, predators and top predators.

Two scenarios from the main assessment – pesticide and veterinary medicine applications – are not relevant here and so are excluded.

There are at least three possible additional direct pathways to the marine environment for this substance, as follows:

- It has been used in the offshore oil industry, and discharges from production or exploration platforms could lead to direct entry into marine waters. However, no current data indicate this route to be significant. The use of 4-*tert*-octylphenol by the offshore oil industry was phased out by the UK in 1999 (OSPAR, 2001). As noted in Section 2.2.5, EOSCA is conducting a study on the possible presence of octylphenol in alkylphenol-based resins used offshore, and this work is due to be reported in 2005 (OSPAR, 2004). This is not considered in the current assessment.

- Resins derived from 4-*tert*-octylphenol have been used in special paints for marine applications because of the high resistance to saline waters that they provide. The tonnage of resin used for this application is assumed to be 800 tonnes/year. Scenarios for the formulation, application and removal of this type of paint are included in the main risk assessment (Section 3.1.3.1.5), and calculations for releases to marine waters from these are included in *Table A2.1*. Estimates of release over the service life of the paint are also included in Section 3.1.3.1.5; these have to be included in the releases to freshwater as there is no direct route for emissions to the marine environment on the larger scale, but their contribution is included.

- Finally, there is a very small reported use of OPE in lubricants, some of which might be used in marine engines. Further details are provided in the

confidential annex. Due to the very low tonnages involved, this source is unlikely to be significant.

Table A2.1 Estimated PECs for 4-*tert*-octylphenol for the local marine risk assessment

Life-cycle stage		PEC local seawater (µg/L)	PEC local sediment (µg/kg)	PEC oral, predator (mg/kg)	PEC oral, top predator (mg/kg)
Production of 4-*tert*-octylphenol		2.89	174	0.755	0.155
Phenol–formaldehyde resins	Resin manufacture	3.0	181	0.783	0.161
	Resins in rubber formulation	0.063	3.79	0.019	0.0078
	Resins in varnishes	0.026	1.59	0.0068	0.0053
	Resins in printing inks	1.05	63.5	0.277	0.059
	Ethoxylated resin production	1.31	79.3	0.096	0.023
	Marine paint formulation	0.016	0.95	0.007	0.0053
	Marine paint application	1.08	65.3	0.285	0.061
	Marine paint disposal	1.92	116	0.504	0.105
Octylphenol ethoxylates	Production	1.67	101	0.093	0.02
	Formulation (textile, pesticide)	0.13	7.97	0.008	0.0055
	Emulsion polymerisation	0.012	0.72	0.006	0.005
	Textiles	0.30	18.1	0.020	0.008
	Paint formulation	0.086	5.2	0.018	0.008
	Paint application	0.009	0.54	0.005	0.005
	Ether sulphate production	0.59	35.5	0.081	0.02

Data on measured concentrations in marine biota are given in Section 3.3.4.2.1.

A2.2 Derivation of marine PNECs

A2.2.1 PNEC for water

The main report provides full details of the available toxicity data and their interpretation. For 4-*tert*-octylphenol there are valid acute saltwater data for invertebrates and fish, the values being:

- 48-hour LC_{50} (lethality) for adult *A. tonsa* (copepod) = 0.42 mg/L;
- 96-hour LC_{50} (lethality) for larval *F. heteroclitus* (estuarine fish) = 0.28-0.34 mg/L;
- 96-hour LC_{50} (lethality) for *C. variegatus* (estuarine fish) = 0.72 mg/L.

However, no long-term NOEC values are available for saltwater species. The lowest valid long-term NOEC for a freshwater species is 6.1 µg/L for the growth of rainbow trout (*On. mykiss*). Long-term NOECs are also available for algae (based on growth in *S. subspicatus* and *Se. capricornutum*) and invertebrates (juvenile production in *D. magna*), but these values are higher.

The database has no representatives from additional marine taxonomic groups. As there are long-term NOEC values for three different taxonomic groups, a factor of 100 could be considered. The arguments regarding the sensitivity of invertebrates not represented in the long-term data presented for the freshwater PNEC (Section 4.1.8.1) are also applicable here. In particular, for nonylphenol there are results with saltwater invertebrates that show lower effect levels. From the tests to look at endocrine-disrupting

effects, there are NOEC values for the sheepshead minnow of 11.5 µg/L, and for the sand goby of 7 µg/L. The saltwater copepod *Tigriopus* showed effects at 1 µg/L in a long-term study, although the authors considered that exposure at the levels tested (up to 10 µg/L) would have little impact on the population. The data on saltwater organisms is limited, and is not considered sufficient to allow a different interpretation to that for freshwater. As a result, the assessment factor is increased to 500 in the same way as that for freshwater was increased from 10 to 50. This may be considered to be a conservative approach. The resulting PNEC is 0.0122 µg/L.

A2.2.2 PNEC for sediment

The $PNEC_{sediment}$ for the marine environment can be estimated from the $PNEC_{saltwater}$ using the equilibrium partitioning method, since no marine or freshwater sediment toxicity data are available for 4-*tert*-octylphenol. Since the log K_{ow} for 4-*tert*-octylphenol is <5 it is not considered likely that significant uptake may occur via ingestion of sediment. Therefore, the saltwater $PNEC_{sediment}$ can be derived by equilibrium partitioning from the $PNEC_{saltwater}$ using the appropriate equation in Section 8.2.3 of the revised TGD, resulting in a value of 0.74 µg/kg.

A2.2.3 PNEC for predators

The PNEC for secondary poisoning ($PNEC_{oral}$) is 10.0 mg/kg, based on a two-generation feeding study in rats with a NOEL of 15 mg/kg bw/day.

A2.3 Risk characterisation for the marine environment

The PEC/PNEC ratios for water and predators/top predators are shown in *Table A2.2*. Since the sediment PNEC and PECs were estimated using equilibrium partitioning, the PEC/PNEC ratios for local sediment will be similar to those for local seawater and so are not included in the table.

All ratios for water (and sediment) are above one with the exception of the use of ethoxylates in emulsion polymerisation and the application of paints that contain OPEs. The ratios for the uses that involve resins are similar to those for freshwater, slightly higher as a result of the assumption of no wastewater treatment (increased dilution is cancelled out by a lower PNEC). The ratios for production and manufacture of resins and ethoxylates are significantly higher than the freshwater equivalents; this results from the use of a higher dilution in the freshwater calculations for these scenarios,

There are no risks for predators or top predators in any of the scenarios.

Table A2.2 Estimated PEC/PNEC ratios for 4-*tert*-octylphenol for the local marine risk assessment

Life-cycle stage		Seawater/sediment	Predator	Top predator
Production of 4-*tert*-octylphenol		**237**	0.08	0.02
Phenol–formaldehyde resins	Resin manufacture	**246**	0.08	0.02
	Resins in rubber formulation	**5.16**	<0.01	<0.01
	Resins in varnishes	**2.16**	<0.01	<0.01
	Resins in printing inks	**86.3**	0.03	<0.01
	Ethoxylated resin production	**108**	<0.01	<0.01
	Marine paint formulation	**1.29**	<0.01	<0.01
	Marine paint application	**88.8**	0.03	<0.01
	Marine paint disposal	**158**	0.05	0.01
Octylphenol ethoxylates	Production	**137**	<0.01	<0.01
	Formulation (textile, pesticide)	**10.8**	<0.01	<0.01
	Emulsion polymerisation	0.97	<0.01	<0.01
	Textiles	**24.6**	<0.01	<0.01
	Paint formulation	**7.01**	<0.01	<0.01
	Paint application	0.74	<0.01	<0.01
	Ether sulphate production	**48.3**	<0.01	<0.01

A2.5 PBT assessment

The nature of the open sea is such that a PEC/PNEC comparison is not appropriate for risk assessment of this environmental compartment. An assessment of persistence (P), bioaccumulation potential (B) and toxicity (T) has therefore been developed to take into account the unacceptably high uncertainty in predicting reliable exposure and/or effect concentrations that hampers quantitative risk assessment. The PBT and 'vPvB' assessment criteria included in the revised TGD are shown in *Table A2.3*.

Table A2.3 Criteria for identification of PBT and vPvB substances

Criterion	PBT criteria	vPvB criteria
P	Half-life >60 days in marine water or >40 days in freshwater* or half-life >180 days in marine sediment or >120 days in freshwater sediment*	Half-life >60 days in marine- or freshwater or >180 days in marine or freshwater sediment
B	BCF >2000	BCF >5000
T	Chronic NOEC <0.01 mg/L or CMR or endocrine disrupting effects	Not applicable

* For the purpose of marine environment risk assessment half-life data in freshwater and freshwater sediment can be overruled by data obtained under marine conditions.

A2.5.1 Persistence (P)

The degradation of 4-*tert*-octylphenol is discussed in Section 3.2.2 of the main report, where it is concluded that, although it is inherently biodegradable, it is not readily biodegradable. No marine or freshwater standard simulation test data are available, but there are some non-standard simulation test data for degradation in river waters and river sediments (Johnson *et al.,* 2000). The freshwater half-lives varied from 7 to 50 days in laboratory microcosms and no degradation over 83 days in spiked sediments incubated under anaerobic conditions. Ying and Kookana (2003) found relatively rapid removal of 4-*tert*-octylphenol from seawater after an extended lag period, with an effective half-life of around 30 days. They also found similar results for marine aerobic sediment, with a shorter effective half-life of ~8 days after a 20-day lag period.

The data suggest that 4-*tert*-octylphenol probably fulfils the screening persistence criterion, but probably does not fulfil the very persistent criterion (half-life >60 days in marine- or freshwater or >180 days in marine or freshwater sediment).

A2.5.2 Bioaccumulation (B)

Bioaccumulation data are discussed in Section 3.2.9. *In vivo* BCFs ranged from 46 to 297 and BCFs estimated from models ranged from 634-3300. A conservative value of 634 estimated from a BCF model was preferred for use in the risk assessment and that value is below the bioaccumulation criterion (BCF >2000). The substance is unlikely to bioaccumulate in mammalian species (oral bioavailability is low and it is rapidly metabolised and eliminated) (Certa *et al*., 1996).

A2.5.3 Toxicity (T)

The toxicity of 4-*tert*-octylphenol is discussed in detail in Section 4 and briefly in Section A2.2. The lowest chronic NOEC (for growth) was 6.1 µg/L from the 60-day post-hatch early life stage toxicity study with rainbow trout (*On. mykiss*). This value exceeds the toxicity criterion (chronic NOEC <0.01 mg/L). In addition, adverse effects that are related to endocrine disruption may occur at lower concentrations.

A2.5.4 Summary

4-*tert*-Octylphenol exceeds the screening P and the T criterion, but does not exceed the B criterion. Hence it can be considered to be potentially persistent and toxic. As it meets two of the criteria, the degree to which the third one is missed should be considered according to the TGD. In this case the measured BCFs are only just over 10% of the threshold, so no further studies are required and the substance is not a PBT or vPvB substance.

Note: The substance still meets the UK Government Chemicals Stakeholder Forum's PBT criteria for substances of concern, since their bioaccumulation criterion is slightly less stringent (log K_{ow} >4 or BCF >500 where data are available).

A2.6 Overall conclusions of the marine risk assessment

The provisional risk assessment indicates a potential risk to marine water (and sediment) organisms from a number of applications for this substance. No risks to predators or top predators are indicated. The substance does not meet the EU PBT criteria, because of its low bioaccumulation potential.

The comments on the results for freshwater are also largely applicable here. There is further scope for refining the PNEC value for the marine assessment, through long-term testing on marine species, but it is suggested that this is not pursued until further efforts to improve the emission and concentration estimates have been made. Improving the freshwater database as indicated in Section 5.1.1.2 could lead to a reduction in the assessment factor to 10, which with the current data would remove the concern for resins in varnishes and the formulation of marine paints.

APPENDIX 3 EUSES LIFE-CYCLE STAGES

These tables relate the EUSES model stages and use patterns to the descriptors of the life-cycle of 4-*tert*-octylphenol used in the report. Three separate EUSES files have been used, with the regional and continental tonnages from the three combined and used to over-write the total release estimates in each file. Note that the local emissions in file 3 are estimated as ethoxylate emissions, and are converted into 4-*tert*-octylphenol emissions at the WWTP stage.

EUSES file	EUSES Use Pattern	EUSES Model Stage	Scenario
1	1	Production	Production of 4-*tert*-octylphenol
	3	Industrial use	Production of phenolic resins
	2	Industrial use	Production of octylphenol ethoxylates (octylphenol emissions)
2	1	Formulation	Production of rubber formulations for tyres
		Private use	Releases from tyres in use
	2	Industrial use	Use of phenolic resins in electrical insulating varnishes
	3	Formulation	Use of phenolic resins in printing inks
	4	Industrial use	Use of phenolic resins in ethoxylated resins
	5	Formulation	Formulation of marine paints containing resins
		Industrial use	Application of marine paints
		Private use	Lifetime releases from marine paints
		Waste treatment	Removal and disposal of marine paints
3	1	Production	Production of octylphenol ethoxylates (ethoxylate emissions)
	2	Formulation	General formulation (textiles, pesticides)
	3	Industrial use	Use of ethoxylates in emulsion polymerisation
	4	Industrial use	Use of ethoxylates in textiles
	5	Formulation	Formulation of paints with ethoxylates
		Industrial use	Use of paints with ethoxylates
	6	Industrial use	Use of pesticide formulation with ethoxylates
	7	Industrial use	Production of ether sulphates

The EUSES files are available from the project manager on request.

APPENDIX 4 THE HYPOTHETICAL REPLACEMENT OF NONYLPHENOL BY 4-*TERT*-OCTYLPHENOL

A4.1 INTRODUCTION

The main report (which contains the references cited in this Appendix) considers risks arising from the current use pattern of 4-*tert*-octylphenol. The risk-reduction strategy for nonylphenol identified other alkylphenols (particularly octylphenol) as the only alternative to nonylphenol where they are used as an intermediate in the formation of derivatives other than ethoxylates (e.g., phenol–formaldehyde resins, phenolic oximes and plastic stabilisers). In addition, although OPEs were not identified as a likely substitute for NPEs, their similar chemical nature does not preclude the possibility. Indeed, nonylphenol can be substituted by 4-*tert*-octylphenol in most ethoxylate uses (as occurs in the USA).

It is therefore necessary to investigate whether use of octylphenol as a substitute for nonylphenol is likely to give rise to a similar level of risk. This Appendix considers a hypothetical complete substitution scenario. The data and assumptions for the production capacity, use pattern and emissions to the environment are the same as those used in the EU risk assessment report for nonylphenol. This allows a direct comparison between the two alkylphenols if they were to be used in the same way. It is recognised that the tonnage data were derived from 1997 figures, and the current consumption of nonylphenol is likely to be lower. In addition, whereas nonylphenol is a liquid, 4-*tert*-octylphenol is a solid at room temperature and therefore pumping is only possible at temperatures around 90°C. *Engineering controls and emissions could therefore be significantly different*, but this has not been considered. The efficacy of any octylphenol derivative might not be suitable for the intended use, and this has also not been considered. *The assessment ought, therefore, to be viewed as an illustration only*.

Basic data for 4-*tert*-octylphenol are summarised and discussed in the main assessment, but for convenience a summary of the main physico-chemical and biological properties of this substance and nonylphenol are given in *Table A4.1*.

Table A4.1 Comparison of properties of 4-*tert*-octylphenol and nonylphenol

	4-*tert*-Octylphenol	**4-Nonylphenol (branched)**
Physico-chemical properties		
Physical state at n.t.p.	Solid	Liquid
Molecular weight	206.33 g $mole^{-1}$	220 g $mole^{-1}$
Vapour pressure	1 Pa	0.3 Pa
Water solubility	19 mg/L	6 mg/L
n-Octanol–water partition coefficient	4.12	4.48
Henry's Law constant	10.86 $Pa.m^3.mol^{-1}$	11.02 $m^3.mol^{-1}$
Fate and behaviour		
Atmospheric degradation – estimated half-life for reaction with hydroxyl radicals	0.25 days	0.3 days

Table A4.1 continued

	4-*tert*-Octylphenol	4-Nonylphenol (branched)
Fate and behaviour (continued)		
Aquatic degradation	Photodegradation and hydrolysis generally negligible. Inherently biodegradable, possibly needing a period of adaptation	Photodegradation and hydrolysis generally negligible. Inherently biodegradable, possibly needing a period of adaptation
Degradation in soil	No data	300 days
Elimination in WWTP	No data	0.1 hr^{-1}
BCF	634 (calculated)	1280
K_{oc}	2740 L/kg	5360 L/kg
Kp_{susp}	274 L/kg	536 L/kg
Kp_{sed}	137 L/kg	268 L/kg
Kp_{soil}	54.8 L/kg	107 L/kg
$K_{soil\text{-}water}$	82.4	161
$K_{susp\text{-}water}$	69.4	135
$K_{sed\text{-}water}$	69.3	135
PNECs		
Surface water	0.122 µg/L (AF of 50 on chronic data)	0.33 µg/L (AF of 10 on chronic data)
Sediment	0.0074 mg/kg wwt (equilibrium partitioning)	0.039 mg/kg wwt (equilibrium partitioning)
WWTP	>0.1 mg/L	9.5 mg/L
Soil	0.0059 mg/kg wwt (equilibrium partitioning)	0.3 mg/kg wwt (AF of 10 on chronic data)
Secondary poisoning	10 mg/kg food	10 mg/kg food

A4.2 ENVIRONMENTAL EXPOSURE

The life-cycles of nonylphenol and its major products, especially the ethoxylates, were considered in detail in the nonylphenol risk assessment report to estimate direct and indirect releases to the environment (ECB, 1999). The basic data and assumptions supporting that assessment are not repeated in this appendix, and interested readers should refer to the nonylphenol risk assessment report for full details. Appropriate summary data are, however, included.

A4.2.1 Production

4-*tert*-Octylphenol is considered to be produced at four sites (I-IV) in the EU for this assessment. The production volume in the EU is assumed to be 73,500 tonnes. Export and import tonnages are assumed to be the same as for nonylphenol (see *Figure A4.1*).

A4.2.1.1 Releases during production

Site I For modelling purposes in EUSES, the yearly emission from the site based on measured data was used (11.8 kg/year).
Site II The total amount released to receiving waters would be <23.04 kg/year.
Site III Reported emissions were zero.
Site IV A daily release rate to wastewater of 12.3 kg would be calculated.

Regional and continental emissions

A release rate to air of 0.45 kg/day (site A) and 2.15 kg/day to water (site D) were used for regional releases, as these were the highest values. The sum of the emissions to air and water from all four sites minus the regional emissions was used to estimate the continental releases of 0 to air and 0.1 kg/day to water. The emissions from production are summarised in *Table A4.2*.

Table A4.2 Summary of emissions from octylphenol production sites

Site	Releases to air	Releases to water	Notes
A	52 g/year (0.00016 kg/day) from OP production. 90 kg/year OP (0.45 kg/day) from OPE production.	475 kg/year (1.4 kg/day) from OP production before WWTP 90 tonnes/year (450 kg/day) from OPE production before WWTP 11.8 kg/year (0.04 kg/day) after WWTP based upon measured level of OP in outflow from plant	OP production for 330 days/year. OPE production 2400 hours/year (200 days assuming 12 hours/day). Dried sludges from the plant are incinerated
B	None	<23.04 kg/year (0.06 kg/day) after WWTP	Based upon company estimations. Sludges from WWTP applied to agricultural land
C	None	None	Waste gases and wastewaters are incinerated
D	None	12.3 kg/day to WWTP 2.15 kg/day after WWTP	Waste gases incinerated. Default release estimation for removal in WWTP used to give release after WTTP. Sludges disposed of to authorised disposal sites
Total	Regional 0.45 kg/day Continental 0	Regional 2.15 kg/day Continental 0.10 kg/day	

A4.2.2 Uses

Figure A4.1 represents the 4-*tert*-octylphenol life-cycle derived using the production and use data from nonylphenol.

As a direct replacement of nonylphenol, 4-*tert*-octylphenol would be used within the EU in the production of:

- Ethoxylates (the main use);
- Resins, plastics, stabilisers, etc.;
- Phenolic oximes.

The ethoxylates are a source of alkylphenols in the environment (see Appendix 1). There are assumed to be seven companies in the EU involved with the manufacture of the ethoxylates. *Table A4.3* lists the industrial use categories for ethoxylates in the EU.

Figure A4.1 4-*tert*-Octylphenol life-cycle (all figures refer to the quantity of nonylphenol as of 1997)

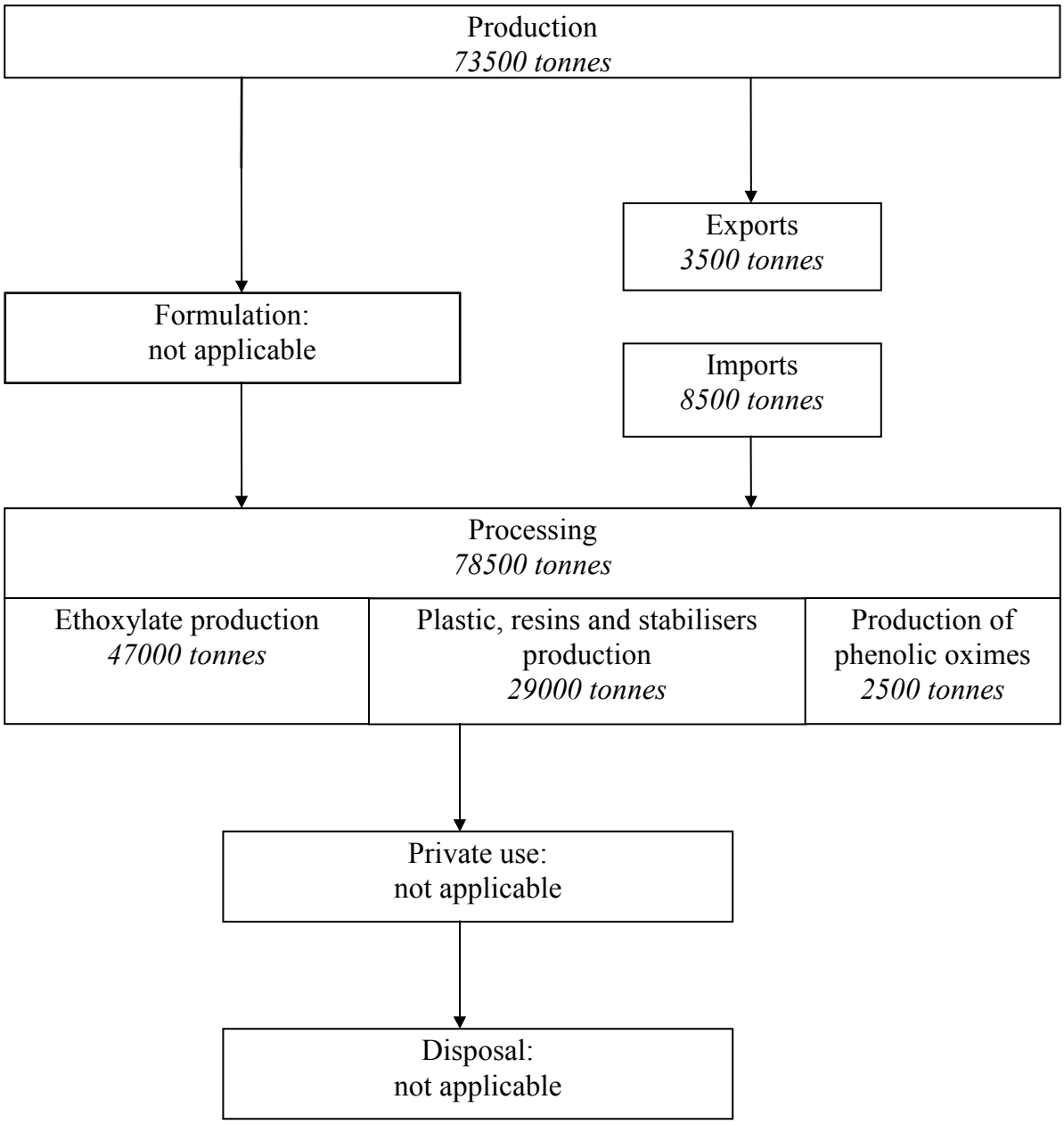

Table A4.3 Use categories for octylphenol ethoxylates

Industrial use of ethoxylates	Main category
Agricultural industry (pesticide application)	4
Captive use by the chemicals industry	(1)/2
Electrical/electronic engineering industry	3/2
Industrial and institutional cleaning	
Leather processing industry	2/3
Metal extraction, refining and processing industry	2/3
Mineral oil and fuel industry	3/4
Photographic industry	2/3
Polymers industry	2
Pulp, paper and board industry	2/3
Textile processing industry	2/3
Paints, lacquers and varnishes industry	2/3/4
Engineering industry, civil and mechanical	

The same tonnage and use pattern has been used for 4-*tert*-octylphenol as for nonylphenol, with the exception of the agricultural and the mineral oil and fuel industries (as scenarios for these were not included in the EUSES model produced for nonylphenol). Predicted releases may therefore be an underestimate, since releases from use in agriculture through formulation and application of pesticides or through use in fuel oil may add to the amounts of ethoxylate entering the environment, and hence to the environmental levels of 4-*tert*-octylphenol subsequently formed.

A4.2.2.1 Releases from production of octylphenol ethoxylates

The releases from the various companies and sites taken from the nonylphenol assessment are summarised in *Table A4.4*. The estimated emissions from ethoxylate production have been used unchanged.

A4.2.2.2 Releases from uses of octylphenol ethoxylates

The ethoxylates have a wide range of uses and extensive data and calculations were made to estimate emissions from these within the nonylphenol assessment. Summaries of the emissions from these uses are provided in *Tables A4.5* and *A4.6* to show the emissions used in the EUSES modelling scenario applied to 4-*tert*-octylphenol.

Table A4.4 Summary of releases from octylphenol ethoxylate production plants

	Release to air	Release to water	Other information
Company A	90 kg/year OP 0.45 kg/day OP	0.04 kg/day OP 0.07 kg/day OPE1+OPE2	Alkylphenol production and processing site. Releases to water after WWTP.
Company B	146 kg/year (0.49 kg/day) OP	46 t/year OP+OPE *(153 kg/day)*	Releases direct to receiving waters
Company C Sites 1+2	2.2 kg/year (0.1 kg/day) OP	*15.4 t/year (70 kg/day)* OP *16.5 t/year (75 kg/day)* OPE	220 days production a year Releases to on-site treatment plant then municipal WWTP.
Company C Site 3	28 kg/year (0.56 kg/day) OP	850 kg/year (17 kg/day) OP+OPE	50 days production a year. Release to industrial WWTP.
Company D Site 1	35 kg/year (0.12 kg/day) OP	360 kg/year *(1.2 kg/day)* OPE	Releases to on-site treatment plant then municipal WWTP.
Company D Site 2	0.2 kg/year (0.007 kg/day) OP	10 kg/year (0.33 kg/day) OPE	30 days production a year Release to on-site WWTP
Company E	1.17 kg/year (0.03 kg/day) OP	0	40 days production a year. Wastewaters incinerated
Company F	160 kg/year (0.8 kg/day) OP	0	200 days production a year. OPE washwaters concentrated then incinerated.
Company G	0	0	50 weeks production a year. Polluted wastewater incinerated on-site
Total	Total 463 kg/year (2.56 kg/d) OP	153 kg/day OP (surface water) 0.07 kg/day OPE (surface water) 87 kg/day OP (WWTP) 76.5 kg/day OPE (WWTP)	

Table A4.5 Summary of regional emissions

Life-cycle stage	Regional OP emission (kg/day) (surface water)	Regional OPE emission (kg/day) (wastewater)	As % of OP burden	As % of OPE burden
Direct release of octylphenol				
Production	2.15		0.7	
Production of ethoxylates (OP release)	15.3 *3.05* (8.7 wastewater)		4.8 0.9	
Alkylphenol–formaldehyde resin production	*0.002* (0.06 wastewater)		0.0006	
Tris(alkylphenyl)phosphite (TAPP) production	0		0	
Epoxy resin manufacture	*0.01* (0.04 wastewater)		0.003	
Production of other plastic stabilisers	*0.05* (0.14 wastewater)		0.016	
Phenolic oxime production	0.26		0.08	
Sub Total	20.8		6.5	
Indirect release of octylphenol from ethoxylates				
Ethoxylate production (OPE release)	*0.19*	7.7	0.06	0.06
Formulation of pesticides	*1.48*	59.2	0.46	0.5
Agricultural use	*3.43*	137	1.1	1.1
Captive use by the chemical industry	*0.34*	13.4	0.1	0.1
Use in electrical engineering	*0.003*	0.13	0.0009	0.001
Industrial and institutional cleaning	*142*	5,671	44.4	47.4
Use in leather processing	*19.4*	774	6.1	6.5
Use in metal processing and extraction	*3.9*	156	1.2	1.3
Use in fuel and oil industry (manufacture and blending)	*0.025*	1	0.008	0.008
Use in the photographic industry	*0.5*	20	0.16	0.2
Use in polymer production	*0.006*	0.25	0.002	0.002
Use in the pulp and paper industry	*5.48*	219	1.7	1.8
Use in textile processing	*46.6*	1,863	14.6	15.6
Use in paints	*0.14* (manufacture) *0.45* (use)	5.5 (manufacture) 17.8 (use)	0.04 0.14	0.05 0.15
Use in civil/mechanical engineering	*0.06*	2.55	0.02	0.02
Other applications	*75.2* (incl. Import tonnage)	3,008 (incl. import tonnage)	23.5	25.2
Sub Total	299.2	11,955	93.2	100
Total	320		100	

OP, octylphenol; OPE, octylphenol ethoxylate. Where a release value is given as OP+OPE the whole release is taken as OP (as a worst case). Figures in italics are generated by default values or estimated on the basis of available data using the following methods:

a) For octylphenol life-cycle stages in which a release is given to WWTP, the surface water release is calculated by multiplying the release by the Fstp (Fraction of emission directed to surface waters after wastewater treatment); for octylphenol this is 0.35 (see main report for further details). For some life-cycle stages two emissions are given; this is where data about direct emissions to surface and to WWTPs are known.

b) The emission of ethoxylates to the WWTP is multiplied by 0.025 to give the resultant emission of octylphenol to surface waters (see Appendix 1 for further details).

Table A4.6 Summary of continental emissions

Life-cycle stage	Continental OP emission (kg/day) (surface water)	Continental OPE emission (kg/day) (wastewater)	As % of OP burden	As % of OPE burden
Direct release of octylphenol				
Production	0.1		0.003	
Production of ethoxylates (OP release)	138 *27.4* (78.3 wastewater)		4.8 0.96	
Alkylphenol–formaldehyde resin production	*0.20* (0.56 wastewater)		0.007	
Tris(alkylphenyl)phosphite (TAPP) production	0		0	
Epoxy resin manufacture	*0.12* (0.36 wastewater)		0.004	
Production of other plastic stabilisers	*0.43* (1.23 wastewater)		0.02	
Phenolic oxime production	0		0	
Sub Total	166		5.8	
Indirect release of octylphenol from ethoxylates				
Ethoxylate production (OPE release)	*1.72*	68.9	0.06	0.06
Formulation of pesticides	*13.3*	533	0.47	0.49
Agricultural use	*30.8*	1233	1.08	1.15
Captive use by the chemical industry	*3*	120	0.10	0.11
Use in electrical engineering	*0.03*	1.15	0.001	0.001
Industrial and institutional cleaning	*1276*	51,041	44.7	47.4
Use in leather processing	*174*	6962	6.09	6.47
Use in metal processing and extraction	*35*	1,402	1.22	1.30
Use in fuel and oil industry (manufacture and blending)	*0.25*	10	0.008	0.009
Use in the photographic industry	*4.58*	183	0.16	0.17
Use in polymer production	*0.06*	2.22	0.002	0.002
Use in the pulp and paper industry	*49.3*	1,973	1.72	1.83
Use in textile processing	*419*	16,767	14.1	15.6
Use in paints	*1.23* *4*	49.3 (manufacture) 160 (use)	0.04 0.14	0.05 0.15
Use in civil/mechanical engineering	*0.57*	22.9	0.02	0.02
Other applications	*677*	27,074	22.7	25.1
Sub Total	2,690	107,602	94.2	100
Total	2,856		100	

See notes to Table A4.5 for further explanation.

A4.2.3 Predicted environmental concentrations (PECs)

Table A4.7 provides a summary of the PECs for the aquatic compartment (including sediment and WWTP), terrestrial compartment and secondary poisoning scenarios.

The regional and continental aquatic PECs include contributions from both direct releases of 4-*tert*-octylphenol and indirect releases from environmental degradation of OPEs. The $PEC_{sediment}$ is calculated from the $PEC_{local(water)}$ using the equilibrium partitioning approach.

Direct releases to the terrestrial compartment are unlikely to occur, except for the use of ethoxylates in agriculture. The major route to soil is via sewage-sludge spreading for processes that discharge aqueous effluent containing the alkylphenol or its derivatives. Any 4-*tert*-octylphenol released to soil either directly or indirectly will be strongly bound to the soil. It is therefore unlikely to enter groundwater or be transported a considerable distance.

Emissions to air are likely to be small both in the vapour and aerosol phases, and since the half-life in air is short, atmospheric concentrations are predicted to be very low and are not reported here.

Table A4.7 Predicted environmental concentrations (PECs)

Scenario	Aquatic			Terrestrial	Secondary poisoning	
	PEC for WWTP organisms (µg/L)	PEC for water (µg/L)	PEC for sediment (mg/kg wwt)	PEC local $_{agri,soil}$ (mg/kg wwt) (averaged over 30 days)	PECs for fish eaten by predators (mg/kg)	PECs for worms eaten by predators (mg/kg)
Direct release of octylphenol						
Production:						
Site I	0.6	0.686	0.0414	0.0143	0.413	1.47
Site II	4	0.647	0.039	0.0151	0.403	1.46
Site III	0	0.626	0.0378	0.0000127	0.397	1.39
Site IV	1.18	0.649	0.0392	0.000375	0.403	1.39
Production of ethoxylates:						
Company B	76,500	3.63	0.219	0.000204	1.18	1.39
Company C	7290; 3490	0.626; 348	0.0378; 21	386; 11.3	0.397; 90.9	1760; 52.7
Company D	15; 13.8	2.12; 2	0.128; 0.12	0.795, 0.728	0.786; 0.754	5.01; 4.71
Company E	0	0.626	0.0378	0.0000244	0.397	1.39
Company F	0	0.626	0.0378	0.000325	0.397	1.39
Company G	0	0.626	0.0378	0.0000127	0.397	1.39
Alkylphenol–formaldehyde resin production	30.8	3.69	0.223	0.0994	1.2	1.84
Tris(alkylphenyl)phosphite production	0	0.626	0.0378	0.0000127	0.397	1.39
Epoxy resin manufacture	0.5	0.68	0.041	0.00174	0.411	1.4
Production of other plastic stabilisers	32.8	3.9	0.235	0.106	1.25	1.87
Phenolic oxime production	372	0.631	0.0381	0.0000225	0.398	1.39

Table A4.7 continued

Scenario	Aquatic			Terrestrial	Secondary poisoning	
	PEC for WWTP organisms (µg/L)	PEC for water (µg/L)	PEC for sediment (mg/kg wwt)	PEC local $_{agri,soil}$ (mg/kg wwt) (averaged over 30 days)	PECs for fish eaten by predators (mg/kg)	PECs for worms eaten by predators (mg/kg)
Indirect release of octylphenol from ethoxylates						
Formulation of pesticides	125; 31.2; 12.5	13.1; 3.74; 1.87	0.789; 0.226; 0.113	6.64; 1.67; 0.681	3.64; 1.21; 0.721	31.7; 9.03; 4.51
Use in agriculture (pesticide application and veterinary medicine use)	-	-	-	-	-	-
Captive use by the chemical industry	51	0.646	0.039	2.7	0.402	13.7
Use in electrical engineering	30.8	3.69	0.223	1.63	1.19	8.81
Industrial and institutional cleaning	259	26.4	1.59	13.7	7.11	63.9
Use in leather processing	844-169	84.7-17.4	5.11-1.05	44.7-8.94	22.3; 4.77	205; 42.1
Use in metal processing and extraction	1,430	143	8.6	75.5	37.4	345
Use in fuel and oil industry	-	-	-	-	-	-
Use in the photographic industry	15.5-0.1	2.17-0.637	0.131-0.0384	0.821-0.00564	0.799; 0.4	5.13; 1.41
Use in polymer production	12.5	1.87	0.113	0.662	0.721	4.41
Use in the pulp and paper industry	156	16.2	0.976	8.28	4.45	39.1
Use in textile processing	3500	349	21.1	185	91.2	846
Use in paints: - Production - Domestic use - Industrial use	 50 0.1 0.125	 5.61 0.636 0.639	 0.338 0.0384 0.0385	 2.65 0.00531 0.00556	 1.69 0.4 0.4	 13.5 1.41 1.42
Use in civil/mechanical engineering	310	31.5	1.9	16.4	8.44	76.2
Regional and continental PECs from direct emissions and the breakdown of ethoxylates						
Regional	-	0.629	0.0524	0.255	-	-
Continental	-	0.075	0.00673	-	-	-

A4.3 ENVIRONMENTAL RISK CHARACTERISATION

The risk characterisation is performed by dividing the PECs by the derived PNECs (see main report) to obtain a RCR. These are summarised in *Table A4.8*. An RCR above one suggests a concern (these are highlighted in bold in the table). A ratio less than one indicates an acceptable level of risk.

A4.3.1 Aquatic compartment

There may be a significant level of risk to the aquatic compartment associated with the complete substitution of nonylphenol by 4-*tert*-octylphenol. All of the RCR values are considerably greater than one, both for the local (and therefore worst-case) situation and also for the regional situation. As the regional concentration gives an RCR above one, the RCR for the local PECs will inevitably be greater than one, since the regional concentration is added to the local concentrations to take account of background levels.

Note that all the local RCRs are significantly greater than those produced for nonylphenol (in many cases an order of magnitude greater). While 4-*tert*-octylphenol and nonylphenol have similar PECs (e.g., regional values of 0.629 and 0.60 µg/L, respectively) the difference in ratio mainly results from the different PNEC values (0.122 and 0.33 µg/L for 4-*tert*-octyl- and nonylphenol, respectively). This may be an artificial distinction because of the use of different assessment factors to reflect the difference in data availability, although the toxicity profiles appear generally comparable. Note, however, that the PNEC for 4-*tert*-octylphenol at least might be lower still, and further studies are needed to clarify this (see main report).

Adverse effects may also occur on sediment-dwelling species, although the PNEC is only provisional. Since both the PECs and PNEC for sediment are derived assuming equilibrium partitioning, the RCRs will be similar to those for water and are not reported separately.

For WWTP, an RCR greater than one is obtained for the following uses:

- Ethoxylate production (two sites);
- Phenolic oxime production;
- Ethoxylate used in:
  - industrial and institutional cleaning,
  - leather processing,
  - metal processing and extraction,
  - pulp, paper and board industry,
  - textile processing,
  - civil/mechanical engineering.

A concern is suggested for a larger number of uses than was the case for nonylphenol. This largely results from the different PNEC values (100 µg/L and >1000 µg/L for 4-*tert*-octylphenol and nonylphenol, respectively). This may be an artificial distinction because of the use of different assessment factors to reflect the difference in data availability. Further toxicity data could refine the PNEC.

Table A4.8 Risk characterisation ratios (RCRs) for the environment

Scenario	Aquatic		Terrestrial	Secondary poisoning	
	RCRs for WWTP organisms	RCRs for surface water	RCRs for soil	RCRs for fish food chain	RCRs for earthworm food chain
Direct release of octylphenol					
Production:					
Site I	<0.006	**6**	**2**	0.04	0.15
Site II	<0.04	**5**	**3**	0.04	0.15
Site III	0	**5**	0.03	0.04	0.14
Site IV	<0.020	**5**	0.06	0.04	0.14
Production of ethoxylates:					
Company B	**765**	**30**	0.03	0.11	0.14
Company C	**72.9; 34.9**	**5; 2852**	**65,313; 1912**	0.04; **9.1**	**176; 5.27**
Company D	0.15; 0.14	**17; 16**	**135; 123**	0.08; 0.08	0.50; 0.47
Company E	0	**5**	0.004	0.04	0.14
Company F	0	**5**	0.05	0.04	0.14
Company G	0	**5**	0.002	0.04	0.14
Alkylphenol–formaldehyde resin production	0.3	**30**	**17**	0.12	0.18
Tris(alkylphenyl)phosphite (TAPP) production	0	**5**	0.002	0.04	0.14
Epoxy resin manufacture	0.005	**6**	0.3	0.04	0.14
Production of other plastic stabilisers	0.33	**32**	**18**	0.13	0.19
Phenolic oxime production	**4**	**5**	0.004	0.04	0.14

Table A4.8 continued

Scenario	Aquatic		Terrestrial	Secondary poisoning	
	RCRs for WWTP organisms	RCRs for surface water	RCRs for soil	RCRs for fish food chain	RCRs for earthworm food chain
Indirect release of octylphenol from ethoxylates					
Formulation of pesticides	**1**; 0.3; 0.1	**107; 31; 15**	**1123; 283; 115**	0.36; 0.12; 0.07	**3.17**; 0.90; 0.45
Use in agriculture (pesticide application and veterinary medicine use)	-	-	-	-	-
Captive use by the chemical industry	0.5	**5**	**457**	0.04	**1.4**
Use in electrical engineering	0	**30**	**276**	0.12	0.88
Industrial and institutional cleaning	**3**	**216**	**2318**	0.71	**6.4**
Use in leather processing	**8-2**	**694-143**	**7563-1513**	**2.2**; 0.48	**20.5; 4.2**
Use in metal processing and extraction	**14**	**1172**	**12,775**	**3.7**	**34.5**
Use in fuel and oil industry	-	**-**	**-**	-	-
Use in the photographic industry	0.15-0.001	**17-5**	**139-1**	0.08; 0.04	0.51; 0.14
Use in polymer production	0.12	**15**	**112**	0.07	0.44
Use in the pulp and paper industry	**2**	**133**	**1401**	0.45	**3.9**
Use in textile processing	**35**	**2861**	**31,303**	**9.1**	**84.6**
Use in paints: - Production - Domestic use - Industrial use	 0.5 0.001 0.001	 **46** **5** **5**	 **448** **1** **1**	 0.17 0.04 0.04	 **1.4** 0.14 0.14
Use in civil/mechanical engineering	**3**	**258**	**2775**	0.84	**7.6**
Regional and continental PECs from direct emissions and the breakdown of ethoxylates					
Regional	-	5	**43**	-	-

$PNEC_{aquatic}$ 0.122 µg/L
$PNEC_{sediment}$ 0.0065 mg/kg (provisional)
$PNEC_{micro\text{-}organisms}$ 100 µg/L
$PNEC_{terrestrial}$ = 0.00591 mg/kg (provisional)
$PNEC_{oral}$ = 10 mg/kg food
See the main report for details of the derivation of these values and the underlying uncertainties.

A4.3.2 Terrestrial compartment

An RCR greater than one is obtained for the following uses:

- Production (two sites);
- Ethoxylate production (two sites);
- Production of octylphenol–formaldehyde resins;
- Production of other plastic stabilisers;
- Ethoxylates used in the following:
  - pesticide formulation,
  - captive use in the chemical industry,
  - electronic engineering,
  - industrial and institutional cleaning,
  - leather processing,
  - metal extraction,
  - photographic industry,
  - polymer industry,
  - pulp, paper and board industry,
  - textile processing,
  - paints, lacquers, etc.,
  - civil/mechanical engineering.

As with the aquatic compartment, there may be a significant level of risk to soil organisms associated with the use of 4-*tert*-octylphenol using the scenario assumed in this assessment. The majority of the RCR values are considerably greater than one, both for the local (and therefore worst-case) situation and also for the regional situation. This might be expected since the soil PNEC is based on that for the aquatic compartment because of the lack of soil-specific toxicity data. Terrestrial toxicity data were available for nonylphenol, and the corresponding PNEC is two orders of magnitude higher than that for 4-*tert*-octylphenol (see main report). Further toxicity data could therefore refine the PNEC. This is unlikely to affect the conclusion for most scenarios, however.

A4.3.3 Atmospheric compartment

Risks are not expected in view of the low concentrations expected in the atmosphere. See the main report for a fuller discussion.

A4.3.4 Non-compartment specific exposure relevant for the food chain (secondary poisoning)

A number of scenarios show a risk to predators through exposure via the food chain. For exposure through the aquatic food chain, risks are indicated for the production of ethoxylates, and their use in leather processing, in metal processing and in textile processing. For exposure through the terrestrial food chain, in addition to the scenarios above, risks arise through the use of ethoxylates in the chemical industry, in industrial and institutional cleaning, the pulp and paper industry and civil and mechanical engineering. The PNEC for 4-*tert*-octylphenol is based on a full chronic study and so is unlikely to be revised.

A4.4 SUMMARY OF CONCLUSIONS

The partial EU ban of several uses of nonylphenol will have a significant impact on the use of that substance. There could be pressure to replace nonylphenol with 4-*tert*-octylphenol for the affected uses. The emission scenario used in this Appendix considers the hypothetical complete replacement of nonylphenol by 4-*tert*-octylphenol. The data and assumptions for the production capacity, use pattern and emissions to the environment are the same as those used in the EU risk assessment report for nonylphenol. This allows a direct comparison between the two alkylphenols if they were to be used in the same way.

The results suggest a possibility of adverse environmental impact for all the endpoints considered, with similar risks as predicted for nonylphenol. This is to be expected in view of the similarities in environmental fate, behaviour and toxicity of the two substances. However, the assessment is simplistic and should be viewed as an illustration only. This is because:

- A number of PNECs could be refined;
- The efficacy of any octylphenol derivative might not be suitable for the intended use, and this has not been considered;
- Given the hypothetical nature of this assessment no real data are available to allow any refinement of the exposure assessment. Should 4-*tert*-octylphenol ever replace nonylphenol in some or all of the uses assumed here, there may be differences in the volumes, use patterns and releases (e.g., through differences in engineering controls arising from the different physical state of the two substances), which cannot be predicted in this assessment.

Ethoxylate production and use was one of the principal concerns identified in the nonylphenol risk assessment. Nonylphenol can be substituted by 4-*tert*-octylphenol in most ethoxylate uses, and 4-*tert*-octylphenol is widely used in the USA for their production. However, similar use in Europe is limited to specialist applications at present, mainly because of the high price of 4-*tert*-octylphenol (arising from the limited availability of octene feedstock) and difficulties in handling (nonylphenol is a liquid, whereas 4-*tert*-octylphenol is a solid at room temperature and therefore pumping of the material is only possible at temperatures around 90°C). In addition, many uses of NPEs can be substituted by the use of fatty alcohol ethoxylates. These are significantly cheaper than OPEs (but slightly more expensive than NPEs) (CEPAD, 2002).

APPENDIX 5 POTENTIAL RISKS ARISING FROM THE USE OF NONYLPHENOL

Environmental exposure to branched octylphenol isomers can arise from releases during the production and use of nonylphenol, since they can be present as an impurity in that substance. To investigate the significance of this source, PECs have been calculated using EUSES assuming a typical impurity level of 5% (probably a worst case), and the production and use scenarios (as existed in 1997) from the nonylphenol risk-assessment report (ECB, 1999). The tonnage of nonylphenol is likely to have changed, and will change further once risk reduction measures and voluntary agreements are introduced. In addition, it is recognised that the isomers involved might not actually include the 4-*tert*-octylphenol structure. However, they would be expected to behave in a similar way and so the properties of the latter substance have been used in this assessment. *The results should therefore be considered as illustrative only*.

The PECs are presented in *Table A5.1* (see Appendix 4 for an explanation of the number of values given for each use). RCRs are shown in *Table A5.2*.

Conclusion

Branched octylphenol isomers similar to 4-*tert*-octylphenol can be a significant impurity in commercial nonylphenol. Potential risks from this source have been predicted for several uses of nonylphenol. Risk reduction measures are being put in place for nonylphenol, so this source should decline in importance. Nevertheless, the consequences of co-release of nonylphenol and 4-*tert*-octylphenol should be considered where they are used in the same processes, for example in site-specific assessments.

Table A5.1 PECs related to unintentional releases of 4-*tert*-octylphenol from the production and use of nonylphenol

Scenario	Aquatic			Terrestrial	Secondary poisoning	
	PEC for WWTP organisms (µg/L)	PEC for water (µg/L)	PEC for sediment (mg/kg wwt)	PEC local $_{agri,soil}$ (mg/kg wwt) (averaged over 30 days)	PECs for fish eaten by predators (mg/kg)	PECs for worms eaten by predators (mg/kg)
Direct release of octylphenol						
Nonylphenol production:						
Site I	0.03	0.0343	0.00212	0.00074	0.020	0.074
Site II	0.2	0.0324	0.0020	0.00076	0.020	0.073
Site III	0	0.0313	0.00189	0.00000064	0.020	0.07
Site IV	0.059	0.0325	0.00196	0.0000188	0.020	0.07
Production of nonylphenol ethoxylates:						
Company B	3825	0.182	0.011	0.00001	0.059	0.07
Company C	362; 175	0.0323; 17.4	0.0019; 1.05	19.3; 0.57	0.02; 4.5	88; 2.6
Company D	0.75; 0.69	0.11; 0.1	0.0064; 0.006	0.04, 0.35	0.039; 0.0375	0.25; 0.24
Company E	0	0.0323	0.0019	0.0000012	0.02	0.07
Company F	0	0.0323	0.0019	0.000016	0.02	0.07
Company G	0	0.0323	0.0019	0.00000063	0.02	0.07
Nonylphenol–formaldehyde resin production	1.54	0.18	0.011	0.005	0.06	0.092
Tris(4-nonylphenyl)phosphite (TNPP) production	0	0.0323	0.0019	0.0000006	0.02	0.07
Epoxy resin manufacture	0.025	0.034	0.002	0.00008	0.02	0.07
Production of other plastic stabilisers	1.64	0.2	0.012	0.005	0.063	0.094
Phenolic oxime production	18.6	0.032	0.0019	0.0000011	0.02	0.07
Indirect release of octylphenol from ethoxylates						
Formulation of pesticides	6.3; 1.56; 0.63	0.65; 0.17; 0.094	0.0395; 0.0113; 0.0056	0332; 0.084; 0.034	0.18; 0.06; 0.036	1.6; 045; 0.23
Use in agriculture (pesticide and veterinary medicine use)	-	-	-	-	-	-
Captive use by the chemical industry	2.6	0.0323	0.002	0.14	0.020	0.69
Use in electrical engineering	1.54	0.18	0.011	0.082	0.06	0.44
Industrial and institutional cleaning	13	1.32	0.08	0.69	0.35	3.2
Use in leather processing	42.2-8.5	4.2-0.87	0.26-0.053	2.25-0.45	1.1; 0.24	10.3; 2.1
Use in metal processing and extraction	72	7.2	0.43	3.8	1.9	17.3

Table A5.1 continued

Scenario	Aquatic			Terrestrial	Secondary poisoning	
	PEC for WWTP organisms (µg/L)	PEC for water (µg/L)	PEC for sediment (mg/kg wwt)	PEClocal $_{agri,soil}$ (mg/kg wwt) (averaged over 30 days)	PECs for fish eaten by predators (mg/kg)	PECs for worms eaten by predators (mg/kg)
Use in fuel and oil industry	-	-	-	-	-	-
Use in the photographic industry	0.78-0.005	0.11-0.032	0.066-0.0019	0.041-0.00028	0.04; 0.02	0.26; 0.07
Use in polymer production	0.63	0.094	0.0057	0.033	0.036	0.22
Use in the pulp and paper industry	7.8	0.81	0.049	0.41	0.22	1.95
Use in textile processing	175	17.5	1.06	9.3	4.6	42.3
Use in paints:						
- Production	2.5	0.28	0.017	0.13	0.085	0.68
- Domestic use	0.05	0.0323	0.0019	0.00026	0.02	0.07
- Industrial use	0.0063	0.032	0.0019	0.00028	0.02	0.071
Use in civil/mechanical engineering	15.5	1.58	0.095	0.82	0.42	3.8
Regional and continental PECs from direct emissions and the breakdown of ethoxylates						
Regional	-	0.031	0.0026	0.013	-	-
Continental	-	0.004	0.00034	-		

Table A5.2 RCRs related to unintentional releases of 4-*tert*-octylphenol from the production and use of nonylphenol

Scenario	Aquatic		Terrestrial	Secondary poisoning	
	RCRs for WWTP organisms	RCRs for surface water	RCRs for soil	RCRs for fish food chain	RCRs for earthworm food chain
Direct release of octylphenol					
Nonylphenol production: Site I Site II Site III Site IV	 0.0003 0.002 0 0.001	 0.3 0.25 0.25 0.25	 0.1 0.15 0.0001 0.003	 <0.01 <0.01 <0.01 <0.01	 <0.01 <0.01 <0.01 <0.01
Production of nonylphenol ethoxylates: Company B Company C Company D Company E Company F Company G	 **3.8** 0.37; 0.18 0.01; 0.005 0 0 0	 **1.5** 0.25; **143** 0.85**;** 0.8 0.25 0.25 0.25	 0.00075 **3266; 96** **7.2; 59** 0.0002 0.0025 0.0001	 <0.01 <0.01; 0.45 <0.01; <0.01 <0.01 <0.01 <0.01	 <0.01 **8.8;** 0.26 0.03; 0.02 <0.01 <0.01 <0.01
Nonylphenol–formaldehyde resin production	0.015	**1.5**	0.85	<0.01	<0.01
Tris(4-nonylphenyl)phosphite (TNPP) production	0	0.25	0.0001	<0.01	<0.01
Epoxy resin manufacture	0.000025	0.3	0.015	<0.01	<0.01
Production of other plastic stabilisers	0.015	**1.6**	0.9	<0.01	<0.01
Phenolic oxime production	0.2	0.25	0.0002	<0.01	<0.01
Indirect release of octylphenol from ethoxylates					
Formulation of pesticides	0.06; 0.015; 0.005	**5.4; 1.6;** 0.75	**56; 14; 5.8**	0.02; <0.01; <0.01	0.16; 0.05; 0.02
Use in agriculture (pesticide application and veterinary medicine use)	-	-	-	-	-
Captive use by the chemical industry	0.025	0.25	**23**	<0.01	0.07
Use in electrical engineering	0.015	**1.5**	**13.8**	<0.01	0.04
Industrial and institutional cleaning	0.15	**10.8**	**116**	0.04	0.32
Use in leather processing	0.1-0.45	**34.7-7.4**	**76-378**	0.11; 0.02	**1.03;** 0.21
Use in metal processing and extraction	0.7	**58.6**	**639**	0.19	**1.7**
Use in fuel and oil industry	-	-	-	-	-
Use in the photographic industry	0.005	0.8-0.25	0.05-**7.0**	<0.01; <0.01	0.03; <0.01
Use in polymer production	0.005	0.75	**5.6**	<0.01	0.02
Use in the pulp and paper industry	0.1	**6.7**	**70**	0.02	0.20
Use in textile processing	**1.8**	**143**	**1565**	0.46	**4.2**
Use in paints: - Production - Domestic use - Industrial use	 0.025 0.00005 0.00005	 **2.3** 0.25 0.25	 **22.4** 0.045 0.045	 <0.01 <0.01 <0.01	 0.07 <0.01 <0.01
Use in civil/mechanical engineering	0.15	**13**	0.002	0.04	**0.38**
Regional PECs from direct emissions and the breakdown of ethoxylates					
Regional	-	0.25	**2.15**	-	-

APPENDIX 6 EMISSION SCENARIO FOR CARBONLESS COPY PAPER

As described in Section 2.2.2.3.6, the European Phenolic Resins Association has confirmed that there is no record of 4-*tert*-octylphenol having been used in Europe for CCP manufacture – resins based on nonylphenol are used instead. Accordingly this use is not considered in the main assessment. However, an emission scenario has been included here in case it is of use for assessments in other parts of the world.

Paper production

For paper coating, releases can be considered from the coating process itself and from the recycling of paper coated with the substance. Considering the coating process, the resin is applied to the surface of the paper after the paper has been produced, and so the process can be considered to be more like printing than papermaking. Accordingly, the resin has been considered to be a colouring agent for the purposes of the calculations (it is actually part of the latent colouring system of the paper).

There is no specific information on releases from this use. The default emission factors from the TGD are zero to air and 5×10^{-4} to water. The default fraction used on a site is 3% of the EU total. This seems low for a relatively specialist product. For the assessment it is assumed that 25%, or 100 tonnes of resin, are used on one site. The resin makes up 12% of the coating formulation and the coating makes up 15-21% of the paper; hence the 100 tonnes of resin make up 4000-5500 tonnes of paper. The number of emission days is 300. The estimated emissions are:

Wastewater: local 5×10^{-3} kg/day; regional 1.5 kg/year; continental 4.5 kg/year.

Paper recycling

Releases from the paper in use are expected to be negligible. However, releases are possible from paper recycling. The following estimates are based on the similar estimates for the risk assessment of medium-chain chlorinated paraffins, which are also used in CCP (EA, 2004). The medium-chain chlorinated paraffins assessment was, in turn, based largely on the ESD for the pulp, paper and board industry in the TGD (Chapter 7).

The components of the colour former, which include the resin, are expected to be released from the paper during alkaline pulping into water. As only a small fraction of the paper surface is actually used, it is assumed that the amount released is the amount coated on to the paper at production. Poorly soluble components are removed from the water through at least primary sedimentation processes, which are expected to remove 90% of the substance. This removal rate is assumed for 4-*tert*-octylphenol. Hence the amount of 4-*tert*-octylphenol released to water is 10% of that on paper entering a recycling site.

A paper recycling rate of 50% is suggested in the absence of more specific information. This is an approximate figure for all types of paper, some of which are recycled to a much higher degree than others. CCP is used in offices and a certain proportion is expected to be retained in filing systems or incinerated. For example, a mixed office waste recovery

rate of 22% has been estimated for London (Davis, 2002). As there are no specific figures for CCP the default rate of 50% is used, recognising that this may be an over-estimate.

The default site in the ESD is assumed to recycle 10% of the paper in the EU, and operates for 250 days. Combining this size of site with the recycling rate of 50%, the amount of CCP going to the site is 5% of that produced. The amount of resin used is 400 tonnes, so the amount of 4-*tert*-octylphenol is 12 tonnes. Hence, the amount going to the recycling site is 600 kg per year. From above, 10% of this is released to water, and hence 60 kg/year to water. The daily emission is 0.24 kg/day. The regional emission is the same as the local site emission. The estimated emissions from paper recycling are:

Wastewater: local 0.24 kg/day; regional 60 kg/year; continental 540 kg/year.

References

David Davies Associates (2002). *Fibre Flows in London*. Final draft. A report for London Remade Ltd., January 2002.

EA, 2004 European Union Risk Assessment Report: medium-chain chlorinated paraffins. Draft, November 2004.

APPENDIX 7 DATA COLLECTION AND PEER REVIEW PROCESS

This report has been produced using publicly available data gathered and assessed by the contractor for the Environment Agency. Additional information has been submitted voluntarily by member companies of the Conseil Européen des Phénols Alkylés et Derivés (CEPAD), working with the European Phenolic Resins Association (EPRA). Additional useful information has also kindly been supplied by the Danish Environmental Protection Agency (information from their product register), the German and Austrian Federal Environment Agencies (environmental monitoring data) and OSPAR contracting parties (notably Norway).

The Environment Agency has been keen to ensure that the data used in this report are as complete and accurate as possible. Original reports and literature articles were retrieved and assessed for reliability wherever possible (it is clearly indicated where this was not the case). Information that has been cited in the OECD SIDS assessment (SIDS, 1994) is assumed to be acceptable because these studies will already have undergone validation and peer review. Data obtained from the OECD assessment are clearly indicated in the text, as are data from other studies for which a full report has not been obtained (e.g., by indicating that it is a cited study).

As a consequence of its known endocrine-disrupting potential, this substance is a common test compound within academic circles, and new scientific information is produced frequently. *The scientific literature was last searched in November 2004.*

Drafts of this report have been circulated to key stakeholders in UK and European Industry for comment, although some were only consulted at a late stage (the final opportunity for comment closed in March 2005). All comments received have been addressed in the final report where appropriate. A full list of consultees is included at the end of this Appendix.

In addition, certain technical aspects of the report were peer-reviewed by an independent expert group set up by the Environment Agency for this purpose in October 2003. Again, this report addresses those comments. The experts were:

Dr Mark Crane, Crane Consultants;
Dr Stuart Dobson, Centre for Ecology and Hydrology (Monks Wood);
Dr Susan Jobling, Brunel University.

Their comments have not been published but are available on request.

LIST OF KEY ORGANISATIONS CONSULTED DURING THE PREPARATION OF THIS REPORT

INDUSTRIAL ORGANISATIONS

Technical Committee of Petroleum Additive Manufacturers in Europe ("Additives Technical Committee")
Alkylphenols and Ethoxylates Research Council, USA
American Chemistry Council
Asahi Glass Fluoropolymers UK Ltd
British Association for Chemical Specialities
British Chambers of Commerce
British Chemical Distributors and Traders Association
British Coatings Federation Ltd
British Fragrance Association
British Rubber Manufacturers Association
Bureau de Liaison des Industries du Caoutchouc (BLIC) (European Association of the Rubber Industry)
Chemical Industries Association
Cleaning & Hygiene Suppliers Association
Confederation of National Associations of Tanners and Dressers of the European Community (Cotance)
Confederation of Paper Industries Ltd
Conseil Européen des Phénols Alkylés et Derivés (CEPAD)
Cosmetic, Toiletry and Perfumery Association
Crop Protection Association
European Paper Chemicals Group
European Phenolic Resins Association
European Polymer Dispersion and Latex Association
European Rubber Chemicals Association
International Institute of Synthetic Rubber Producers
Polimeri Europa
Rohm & Haas (UK) Ltd
Surface Engineering Association
Textile Finishers Association
United Kingdom Cleaning Products Industry Association
United Kingdom Lubricants Association Ltd
Uniqema

UK GOVERNMENT BODIES

Department of the Environment, Food and Rural Affairs
Department of the Environment, Northern Ireland
Department of Health
Department of Trade and Industry
English Nature
Food Standards Agency
Health and Safety Executive
Pesticides Safety Directorate

Scottish Environment Protection Agency
Scottish Executive
Welsh Assembly
Veterinary Medicines Directorate

EUROPEAN REGULATORY AUTHORITIES

Existing Substances Regulation environmental technical meeting participants
OSPAR Contracting Parties

We welcome views from our users, stakeholders and the public, including comments about the content and presentation of this report. If you are happy with our service, please tell us about it. It helps us to identify good practice and rewards our staff. If you are unhappy with our service, please let us know how we can improve it.

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