Proposed EQS for Water Framework Directive Annex VIII substances: benzyl butyl phthalate *(For consultation)*

by
Water Framework Directive - United Kingdom Technical Advisory Group (WFD-UKTAG)

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Use of this report

The development of UK-wide classification methods and environmental standards that aim to meet the requirements of the Water Framework Directive (WFD) is being sponsored by the UK Technical Advisory Group (UKTAG) for WFD on behalf of its members and partners.

This technical document has been developed through a project managed by the Environment Agency and has involved members and partners of UKTAG. It provides background information to support the ongoing development of the standards and classification methods.

While this report is considered to represent the best available scientific information and expert opinion available at the time of its completion, it does not necessarily represent the final or policy positions of the Environment Agency, UKTAG or any of its partner agencies.

Executive summary

The UK Technical Advisory Group (UKTAG) has commissioned a programme of work to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). This report proposes predicted no-effect concentrations (PNECs) for benzyl butyl phthalate (BBP) using the methodology described in Annex V of the Directive. There are existing non-statutory EQSs for BBP, but the method used to derive these is not considered to comply with the requirements of Annex V and so is unsuitable for deriving Annex VIII EQSs.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for BBP, along with any data that relate impacts under field conditions to exposure concentrations. The data have been subjected to rigorous quality assessment such that decisions are based only on scientifically sound data. Following consultation with an independent peer review group, critical data have been identified and assessment factors selected in accordance with the guidance given in Annex V of the WFD.

A draft EU Risk Assessment Report (RAR) has been compiled for BBP. The UK has already committed to the use of RAR PNECs for the derivation of the Water Framework Directive Annex X EQSs. Consequently, in this document, RAR PNECs are recommended as the proposed long term PNECs for freshwater and saltwaters. Short term PNECs have been derived based on the available data.

If they were to be adopted as EQSs, the long-term PNEC would normally be expressed as an annual average concentration and the short-term PNEC as a 95th percentile concentration.

At this stage, no consideration has been taken of the feasibility of implementing these PNECS as EQSs, but that would be an essential step before a regulatory EQS can be recommended.

Properties and fate in water

Few data are available on the mode of toxic action of BBP in aquatic organisms. However, polar narcosis is generally accepted as the primary mode of action.

BBP has a relatively low water solubility (2.8 mg l^{-1}) and based on its log Kow of 4.8 and log Koc of 10500 is expected to partition to sediments, suspended matter and biota in the aqueous environment. Hydrolysis and photolysis are not expected to be major fate processes for BBP and based on its Henry's law constant (0.176 $Pa.m^3/mol$) volatilisation from water surfaces is likely to be insignificant. Biodegradation is the rate controlling process for environmental degradation of BBP. Aerobic degradation is rapid in natural water and sewage systems with degradation rates of >80% reported after 14 days and 2 days in domestic sewage and river water, respectively. BBP also degrades rapidly under anaerobic conditions with 90% degradation after 8-days in municipal sewage sludge. The major degradation products of BBP are monobutylphthalate, monobenzylphthalate and phthalic acid.

Availability of data

Long-term freshwater toxicity data are available for five taxonomic groups including algae, bacteria, ciliates, crustaceans and fish. Short-term freshwater toxicity data are available for seven taxonomic groups including algae, bacteria, crustaceans, fish,

hydroids, insects and molluscs. Long-term saltwater toxicity data are available for three taxonomic groups: algae, crustaceans and fish. Short-term saltwater toxicity data are available for five different taxonomic groups: algae, annelids, crustaceans, fish and molluscs. Based on the available data there appears to be little difference between trophic levels in the sensitivity of organisms to BBP. In addition there are no significant differences in the sensitivity of freshwater or saltwater species of the same taxonomic group. These conclusions are in line with the non-specific mode of action of BBP, which is generally accepted to be a polar narcotic.

There were no fresh or saltwater field or mesocosm data available for BBP.

BBP may have both oestrogenic and anti-androgenic effects. However, effects *in vivo* tend to occur at higher concentrations at which effects such as mortality may also be observed in some species using standard tests. In addition, there is still some uncertainty about long-term effects and the issue of parent offspring transfer. Until these issues are resolved PNECs for BBP should be set using standard toxicity endpoints.

Derivation of PNECs

Long-term PNEC for freshwaters

The lowest valid long-term freshwater data point was a 30-day NOEC (for growth and length) of 0.14 mg l^{-1} for the fathead minnow (*Pimephales promelas*). However, given the similarity in effects in both fresh and saltwater the EU RAR combined the two data sets. In saltwater a 28-day NOEC (reproduction/growth) of 0.075 mg l^{-1} was reported for the mysid shrimp (*Mysidopsis bahia*). This datum was generated in a GLP study under flow through conditions and was regarded by the RAR as fully valid for PNEC derivation.

The long-term freshwater PNEC in the EU RAR for BBP was therefore based on the 28-day NOEC (for reproduction and growth) of 0.075 mg l^{-1} reported for the mysid shrimp (*Mysidopsis bahia*) and an assessment factor of 10 applied, because of the availability of long-term data for three trophic levels and the similarity in sensitivity of trophic levels, resulting in a **$PNEC_{freshwater\_lt}$ of 0.0075 mg l^{-1} (7.5 µg l^{-1}) BBP.**

This value is slightly lower than the existing EQS of 20 µg l^{-1} derived by applying an extrapolation factor of 50 (10 to account for extrapolation from acute effects to acute no effects and a factor of 5 to account for extrapolation to chronic no effects) to the lowest observed acute toxicity values for fish and invertebrates available at the time the EQS was derived, namely a 96-hour LC50 of 0.82 mg l^{-1} for rainbow trout (*Oncorhynchus mykiss*) and a 48-hour EC50 of 1.0 mg l^{-1} for *Daphnia magna*.

Short-term PNEC for freshwaters

The lowest valid short-term freshwater data point was a 72-hour EC50 (growth rate) of 0.64 mg l^{-1} for the alga *Navicula pelliculosa*. However, given the similarity in effects in both fresh and saltwaters the EU RAR combined the two data sets. In saltwater a 96-hour LC50 of 0.51 mg l^{-1} was reported for the shiner perch (*Cymastogaster aggregata*). This datum was generated under flow-through conditions with measured exposure concentrations and was regarded, by the RAR, as fully valid for PNEC derivation.

Although short-term critical data were identified in the RAR, EU RARs do not usually derive intermittent (short-term) PNECs. Consequently, no short-term RAR PNEC was available to be adopted as the EQS. Therefore, a short-term PNEC was derived in this report by applying an assessment factor of 10 to the lowest available datum, because

of the availability of reliable short-term data for at least three trophic levels and the similarity in sensitivity of trophic levels resulting in a **$PNEC_{freshwater\_st}$ of 0.051 mg l^{-1} (51 µg l^{-1}) BBP.**

This value is slightly lower than the existing maximum allowable concentration (MAC) of 100 µg l^{-1} proposed to protect freshwater life from episodic exposure to BBP. The MAC was derived by applying an assessment factor of 10 to the lowest acute data (highlighted in the above long-term section) to account for extrapolation from acute effects to acute no effects.

Long-term PNEC for saltwaters

Long-term saltwater data for BBP were available for the 'base set' of organisms (algae, invertebrates and fish). The long-term saltwater PNEC in the EU RAR for BBP was based on the 28-day NOEC (reproduction/growth) of 0.075 mg l^{-1} reported for the mysid shrimp (*Mysidopsis bahia*). An assessment factor of 100 was proposed by the EU RAR. An additional assessment factor of 10 was used than for the freshwater PNEC as no chronic effect data were available for additional marine taxonomic groups such as echinoderms or molluscs, resulting in a **$PNEC_{saltwater\_lt}$ of 0.00075 mg l^{-1} (0.75 µg l^{-1}) BBP.**

This value is lower than the existing EQS of 20 µg l^{-1}, which was 'read-across' from the freshwater long-term value.

Short-term PNEC for saltwaters

No short-term saltwater PNEC was derived in the EU RAR for BBP. However, the lowest short-term critical data were identified in the RAR. The lowest reliable data point in the combined data set was the 96-hour LC50 of 0.51 mg l^{-1} reported for the shiner perch. An assessment factor of 50 is proposed because in addition to reliable short-term data for at least three trophic levels, there are short-term data for an additional marine taxonomic group (molluscs). The latter was found to be no more sensitive than algae, crustaceans and fish. The use of such an assessment factor with the short-term saltwater data is in line with the guidance within the EU TGD (ECB 2003). This results in a **$PNEC_{saltwater\_st}$ of 0.010 mg l^{-1} (10 µg l^{-1}) BBP.**

This value is lower than the existing maximum allowable concentration (MAC) of 100 µg l^{-1}, which was 'read-across' from the freshwater short-term value.

PNECs for sediment

BBP has a log Kow value of 4.84 which is above the TGD trigger level of 3. As such sediment standards for BBP should be derived. However, it was not possible to locate data on the direct toxicity of BBP to sediment-dwelling organisms. Consequently, it was not possible, at this time, to derive a sediment PNEC.

PNECs for secondary poisoning

The draft RAR identified the NOAEL of 50 mg/kg body weight from a rat reproduction toxicity study as most suitable for derivation of $PNEC_{secpois.biota}$. The appropriate assessment factors to derive a PNEC based on a chronic $NOAEL_{food}$ from a mammalian study are a conversion factor of 20 and an assessment factor of 30 resulting in a **$PNEC_{secpois.biota}$ of 33.3 mg/kg BBP in food.**

Reported BCF values for whole fish range from 188 to 663. However, the draft RAR identified a BCF of 449 in Bluegill sunfish (*Lepomis macrochirus*) as the most suitable

for the estimation of secondary poisoning. Consequently, the concentration in water preventing bioaccumulation in prey to levels >$PNEC_{secpois.biota}$ is a **$PNEC_{secpois.water}$ of 0.074 mg l^{-1} (74 µg l^{-1}) BBP.**

This concentration is higher than the proposed long-term PNECs for the protection of pelagic communities in both inland and marine water bodies. Therefore, if quality standards are set on the basis of the freshwater and saltwater PNECs, the protection of predators from secondary poisoning will be covered. (The same conclusion is drawn if the worst-case BCF of 663 is used.)

Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC (µg l^{-1})	Existing EQS (µg l^{-1})
Freshwater/long-term	7.5	20 (AA)
Freshwater/short-term	51	100 (MAC)
Saltwater/long-term	0.75	20 (AA)
Saltwater/short-term	10	100 (MAC)
Sediment	-	–
Secondary poisoning	74	–

AA = Annual Average
MAC =Maximum Allowable Concentration

Analysis

BBP may be analysed by gas chromatography/mass spectrometry and by high-performance liquid chromatography.

Proposed PNECs derived for BBP range from 0.75 to 51 µg l^{-1} in environmental waters. The data quality requirements are that at a third of the EQS total error of measurement should not exceed 50 per cent. Using this criterion, current analytical methodologies should offer adequate performance to analyse for BBP.

Implementation issues

Based on consideration of the information collated within the report and the proposed PNECs the following comments are made re: implementation:-

- The current analytical capability should be adequate for compliance assessment
- The proposed PNECs are consistent with those proposed in the EU Risk Assessment Report.
- The PNECs are suitable for use as EQSs as they are not subject to excessive uncertainty.

Contents

1 Introduction

The UK Technical Advisory Group (UKTAG) supporting the implementation of the Water Framework Directive (2000/60/EC)[1] is a partnership of UK environmental and conservation agencies. It also includes partners from the Republic of Ireland. UKTAG has commissioned a programme of work to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). This report proposes predicted no-effect concentrations (PNECs) for benzyl butyl phthalate (BBP) using the methodology described in Annex V of the Directive. There are existing non-statutory EQSs for BBP, but the method used to derive these is not considered to comply with the requirements of Annex V and so is unsuitable for deriving Annex VIII EQSs.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for BBP, along with any data that relate impacts under field conditions to exposure concentrations. The data have been subjected to rigorous quality assessment such that decisions are based only on scientifically sound data[2]. Following consultation with an independent peer review group, critical data have been identified and assessment factors selected in accordance with the guidance given in Annex V of the WFD.

A draft EU Risk Assessment Report (RAR) has been compiled for BBP, and the UK has already committed to the use of RAR PNECs for the derivation of the WFD Annex X EQSs. Consequently, in this document, RAR PNECs are recommended as the proposed PNECs. At this stage, no consideration has been taken of the feasibility of implementing these PNECS as EQSs but that would be an essential step before regulatory EQSs can be recommended.

1.1 Properties and fate in water

BBP is a clear oily liquid that is used as a plasticizer mainly in polyvinyl chloride for vinyl floor tiles, vinyl foams and carpet backing (HSDB 2007).

Few data are available on the mode of toxic action of BBP in aquatic organisms. However, polar narcosis is generally accepted as the primary mode of action (Verhaar *et al.* 1992 and Russom *et al.* 1997).

BBP has a relatively low water solubility (2.8 mg l^{-1}) and based on its log Kow of 4.8 and Koc of 10500 is expected to partition to sediments, suspended matter and biota in the aqueous environment. Hydrolysis and photolysis are not expected to be major fate processes for BBP and based on its Henry's law constant (0.176 $Pa.m^3/mol$) volatilisation from water surfaces is likely to be insignificant. Biodegradation is the rate controlling process for environmental degradation of BBP. Aerobic degradation is rapid in natural water and sewage systems with degradation rates of >80% reported after 14 days and 2 days in domestic sewage and river water, respectively. BBP also degrades rapidly under anaerobic conditions with 90% degradation after 8 days in municipal sewage sludge. The major degradation products of BBP are butyl phthalate, benzyl phthalate and phthalic acid.

BCF values for BBP and its metabolites (principally monobutyl phthalate and monobenzyl phthalate) in whole fish range from 188 to 663, indicating a strong potential to bioconcentrate (see Section 3.5.2). However, the BCF values for the parent compound alone are much lower with a whole fish BCF of 12 reported for BBP alone. Given the metabolism of BBP and the potential for toxic effects of the mono-esters (albeit in mammalian studies)

[1] *Official Journal of the European Communities*, **L327**, 1–72 (22/12/2000). Can be downloaded from http://www.eu.int/comm/environment/water/water-framework/index_en.html

[2] Data quality assessment sheets are provided in Annex 1.

the draft RAR proposed to use BCF values relating to both, the BBP parent compound and metabolites rather than the parent compound alone.

2 Results and observations

2.1 Identity of substance

Table 2.1 gives the chemical name and Chemical Abstracts Service (CAS) number for BBP.

Table 2.1 Chemical species covered by this report

Name	CAS Number
Benzyl butyl phthalate (BBP)	85-68-7

2.2 PNECs proposed for derivation of quality standards

Table 2.2 lists proposed PNECs obtained using the methodology described in the Technical Guidance Document (TGD) issued by the European Chemicals Bureau (ECB) on risk assessment of chemical substances (ECB 2003). *(NB: EU technical guidance for the derivation of EQSs (2011) has been produced since the finalisation of this report but used the same principles as the TGD)*

Section 2.6 summarises the effects data identified from the literature for BBP. The use of these data to derive the values given in Table 2.2 is explained in Section 3.

Table 2.2 Proposed overall PNECs as basis for quality standard setting

PNEC	TGD deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS
Freshwater short-term	51 µg l^{-1}	Insufficient data	100 µg l^{-1} (MAC)
Freshwater long-term	7.5 µg l^{-1}	Insufficient data	20 µg l^{-1} (AA)
Saltwater short-term	10 µg l^{-1}	Insufficient data	100 µg l^{-1} (MAC)
Saltwater long-term	0.75 µg l^{-1}	Insufficient data	20 µg l^{-1} (AA)
Sediment	Insufficient data	Insufficient data	-
Secondary poisoning	74 µg l^{-1}	–	-

AA = Annual Average
AF = Assessment Factor
MAC = Maximum Allowable Concentration
SSD = Species Sensitivity Distribution

2.3 Hazard classification

Table 2.3 gives the R-phrases (Risk-phrases) and labelling for the species of interest.

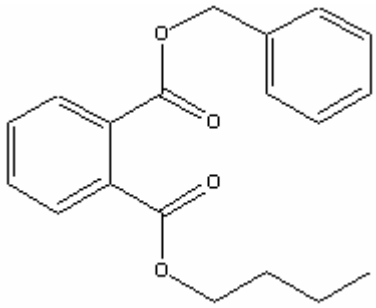
Table 2.3 Hazard classification

R-Phrases and Labelling	Reference:
R61, R62, T N, R50-53	ECB 2005

2.4 Physical and chemical properties

Table 2.4 summarises the physical and chemical properties of the species of interest.

Table 2.4 Physical and chemical properties of benzyl butyl phthalate

Property	Value	Ref.
CAS Number	85-68-7	ECB 2005
Substance name	Benzyl butyl phthalate	ECB 2005
Molecular formula	$C_{19}H_{20}O_4$	ECB 2005
Molecular structure		ECB 2005
Molecular weight	312.35	ECB 2005
Appearance	Clear oily liquid	ECB 2005
Melting point (°C)	<-35°C	ECB 2005
Boiling point (°C)	370°C at 10.10 hPa	ECB 2005
Vapour pressure	0.00112 Pa at 20°C	ECB 2005
Henry's law constant	0.176 $Pa.m^3/mol$	ECB 2005
Water solubility (g l^{-1})	2.8 mg l^{-1} at 20-25°C	ECB 2005
Octanol-water partition coefficient (log K_{ow})	3.57-5.8 4.84 (mean of above values used in RAR)	ECB 2005

2.5 Environmental fate and partitioning

Table 2.5 summarises the information obtained from the literature on the environmental fate and partitioning of BBP.

Table 2.5 Environmental fate and partitioning of benzyl butyl phthalate

Property	Value:	Ref.
Hydrolytic stability (DT_{50})	Hydrolysis of BBP is expected to be an insignificant fate pathway.	ECB 2005
Photostability	In a study in sunlight, 0% degradation occurred after 10 days, 43% after 28 days. In two studies, <5% photodegradation occurred after 28 days. The half-life was estimated to be >100 days.	ECB 2005 ECB 2005

Property	Value:	Ref.
Volatility	Based on a Henry's law constant of 0.176 $Pa.m^3/mol$ volatilisation from the aquatic compartment is not expected.	ECB 2005
Biodegradability: Aerobic	BBP is regarded as readily biodegradable. 14 day MITI-I (OECD 301C) domestic sewage biodegradation test: 81% (ready) degradation (OECD 301F) domestic sewage biodegradation test: 86% (14 days) and 88% (28 days) (ready) degradation Inherent degradation study, semi-continuous activated sludge (SCAS) using a high (22 mg/24 h) and low (5 mg / 24 h) additional rate of BBP, gave a primary degradation rate of 93 and 99%, respectively. No study duration reported. A half-life (primary degradation) of 1-3 days has been reported in a river die-away test.	ECB 2005 ECB 2005 ECB 2005 ECB 2005 ECB 2005 ECB 2005
Anaerobic	32-day anaerobic test, without shaking at 37°C with 90% (4 mg l^{-1}) after 8 days primary degradation. 365-day anaerobic test without shaking at 30°C using inoculates from a freshwater lake sediment, a saltwater marsh sediment, municipal digester or a lab-scale landfill digester. Primary degradation half-lives were: FW sediment: 15 days SW sediment: 10 days Municipal digester: 63 days Landfill digester: no degradation	ECB 2005 ECB 2005
Degradation in Water/sediment -DT_{50} water - DT_{50} whole system	1-3 days (50-500 µg l^{-1}, Mississippi river water) 0.5-2 days (1 mg l^{-1}, Mississippi river water) ≤ 2 days (10-100 µg l^{-1}, freshwater/sediment mesocosm) 1.4 days (12-1000 µg l^{-1} lake water/sediment mesocosm)	ECB 2005 ECB 2005
Mineralisation	$t_{1/2}$ 8-13 days (complete degradation to CO_2)	ECB 2005
Bound residue	-	
Distribution in water / sediment systems	Fugacity modelling predicts BBP will be distributed 65% to water and 35% to sediment.	WHO 1999
Residues relevant to the aquatic environment	Monobutyl phthalate and monobenzyl phthalate, phthalic acid.	ECB 2005

Property	Value:	Ref.
Degradation in soil	Primary degradation of 65-75% has been measured in artificial compost mixtures over 7-30 days. Half-lives of 59.2 and 178.2 days have been measured in soils inoculated with wood preservative sludge containing BBP. The longer half-lives are believed to be due to the antimicrobial nature of the inoculum. A DT_{50} of 59.2 days has been reported in laboratory soil containing 15% clay, 63% silt and 22% sand.	ECB 2005 ECB 2005 IUCLID 2000
Partition coefficient (log K_{OW}) pKa Koc Sediment – water Suspended matter – water	3.57-5.8 4.84 (mean of above values used in RAR) - 9000-17000 (10500 used in RAR) - -	ECB 2005 ECB 2005
Bioconcentration factor (BCF)	Bluegill sunfish (*Lepomis macrochirus*) = 663 (parent compound and metabolites) Bluegill sunfish (*Lepomis macrochirus*) = 188 (parent compound and metabolites) Bluegill sunfish (*Lepomis macrochirus*) = 449 (parent compound and metabolites) 12 (parent compound) Eastern Oyster (*Crassostrea virginica*) = 135 (parent compound and metabolites)	IUCLID 2000 ECB 2005 ECB 2005 ECB 2005

BBP is a clear oily liquid that is used as a plasticizer mainly in polyvinyl chloride for vinyl floor tiles, vinyl foams and carpet backing. It is also used in the production of cellulose plastics and polyurethane (HSDB 2007). The use of BBP as a chemical intermediate means that it is likely to be released to the environment through various waste streams.

On release to water, the low solubility (2.8 mg l^{-1} at 20-25°C) and high log Kow value (4.84) of BBP result in it partitioning from the water phase to suspended matter, sediments and biota (ECB 2005). Abiotic degradation of BBP is expected to be minimal. Hydrolysis and photolysis of BBP are likely to be insignificant and the low vapour pressure (0.00112 pa at 20°C) and Henry's law constant (0.176 $Pa.m^3/mol$) indicate that volatilisation from water will also be low (ECB 2005).

Biodegradation is likely to be the primary fate process for BBP in aquatic and terrestrial environments. Ready biodegradation tests with domestic sewage indicate that BBP is readily biodegradable with 86% (OECD 301) and 81% (MITI 1) degradation of 100 mg l^{-1} BBP after 14 days (ECB 2005). BBP has also been shown to degrade rapidly in natural waters. River die-away tests reported 80% primary degradation of 1.0 mg l^{-1} BBP in Mississippi River water after only 2 days. Similar degradation rates have been reported in Mississippi River water when the tests were run in the dark (ECB 2005). However, no degradation was seen in river water samples that had been autoclaved (ECB 2005), supporting the assumption that biodegradation is the primary removal process for BBP in water.

BBP also degrades rapidly under anaerobic conditions. ECB (2005) reported the following primary degradation half-lives from four distinct anaerobic environments carried out using a 365-day anaerobic test without shaking at 30°C;

- freshwater lake sediment = 15 days
- salt marsh sediment = 10 days
- municipal digester sludge = 63 days
- anaerobic leachate = no degradation observed

The lower degradation observed in the digester sludge compared to the fresh and saltwater sediments was attributed to potential toxicity of BBP breakdown products or lower bioavailability in the sludge system compared to the sediment systems. The lack of degradation in the landfill digester was attributed to the inability of the resident bacteria to support the bioconversion of the phthalate. It should be noted that other studies have reported higher rates of anaerobic degradation of BBP in sewage sludge (e.g. 90% primary degradation after 8 days) (ECB 2005). Differences in test set up i.e. initial inoculum density and species composition and test concentration may explain some of the differences in the results of these studies.

The metabolic pathway of aerobic and anaerobic biodegradation of BBP is: BBP → monobutyl/monobenzyl phthalate → phthalic acid → 4,5 dihydroxyphthalic acid→ oxalic acid → formic acid → CO_2 (ECB 2005).

BCF values for BBP and its metabolites (principally monobutyl phthalate and monobenzyl phthalate) in whole fish range from 188 to 663, indicating a strong potential to bioconcentrate (see Section 3.5.2). However, the BCF values for the parent compound alone are much lower with a whole fish BCF of 12 reported for BBP alone (Carr 1992 cited in ECB 2005). Given the metabolism of BBP and the potential for toxic effects of the mono-esters (albeit in mammalian studies) the draft RAR proposed to use BCF values relating to both, the BBP parent compound and metabolites rather than the parent compound alone.

2.6 Effects data

A summary of the mode of action of this substance can be found in Section 2.6.5.

Data collation followed a tiered approach. Critical data on freshwater and marine organisms were collected from the existing EQS documents (Lewis *et al.* 1998) as well as from the EU Risk Assessment Report (RAR) for BBP (ECB 2005). Further data were then retrieved from the US Environmental Protection Agency (US EPA) ECOTOX database[3].

Further data sources used included:

- ScienceDirect®;[4]
- Hazardous Substances Data Bank (HSDB®) of the US National Library of Medicine;[5]
- WHO CICAD document on BBP (WHO 1999).

Toxicity data and other information on the inherent properties of BBP taken from the draft EU risk assessment report were not subjected to additional quality assessment in this data sheet as

[3] http://www.epa.gov/ecotox/
[4] http://www.sciencedirect.com/
[5] http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB

the data were already subjected to quality assessment by the authors of the risk assessment. Where such data have been used in this report the quality criteria assigned by the RAR document have been reported and prefixed by the word RAR to identify it as an RAR assigned quality criteria. Studies identified in this report that were not covered by the RAR have been subject to quality assessment and suitable quality criteria assigned.

2.6.1 Toxicity to freshwater organisms

Freshwater toxicity data on BBP are available for various taxonomic groups including algae, invertebrates and fish as required for the application of the approach specified in the EU Technical Guidance Document (TGD) (ECB 2003). Long-term data are available for five taxonomic groups including algae, bacteria, ciliates, crustaceans and fish. Freshwater short-term toxicity data are available for seven taxonomic groups including algae, bacteria, crustaceans, fish, hydroids and insects and molluscs.

Diagrammatic representations of the available freshwater data (cumulative distribution functions) for BBP are presented in Figures 2.1 and 2.2. These diagrams include all data regardless of quality and provide an overview of the spread of the available data. These diagrams are not species sensitivity distributions and have not been used to set the BBP PNECs. The lowest critical freshwater data for BBP are presented in Tables 2.6 and 2.7.

Figure 2.1 Cumulative distribution function of freshwater long-term data (mg l^{-1}) for benzyl butyl phthalate

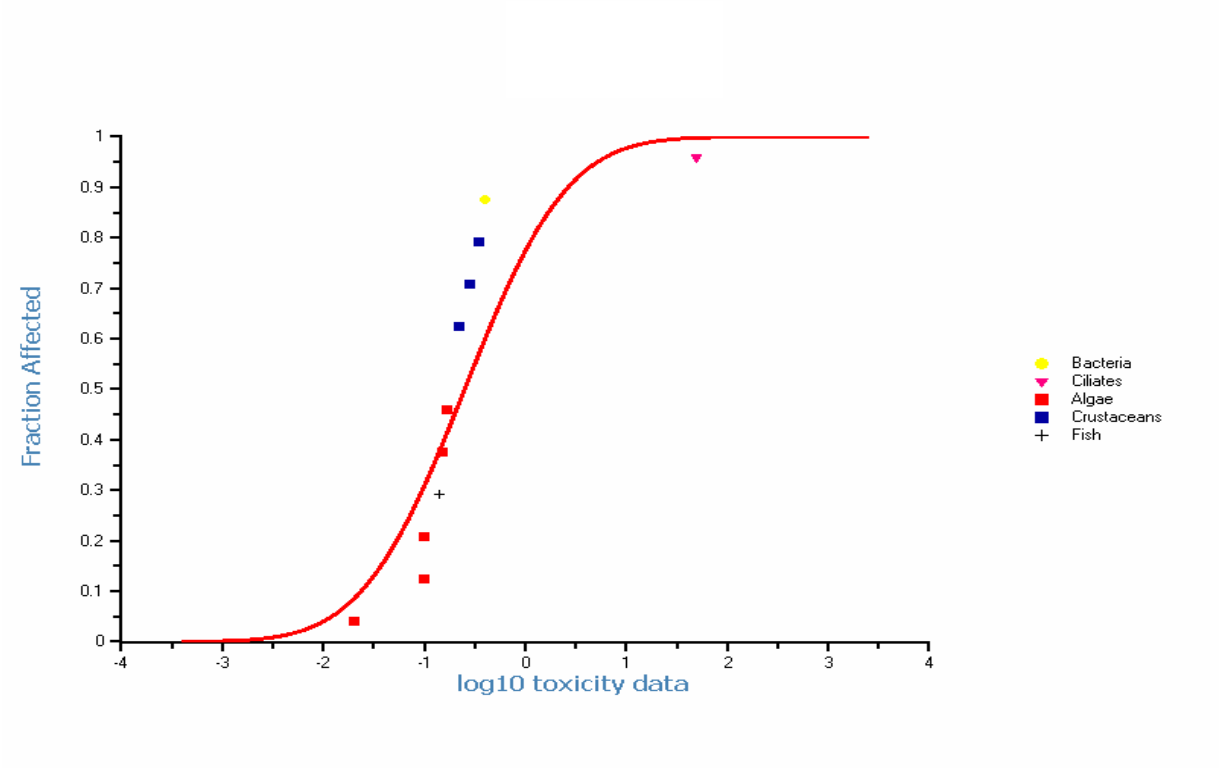


Figure 2.2 Cumulative distribution function of freshwater short-term data (mg l^{-1}) for benzyl butyl phthalate

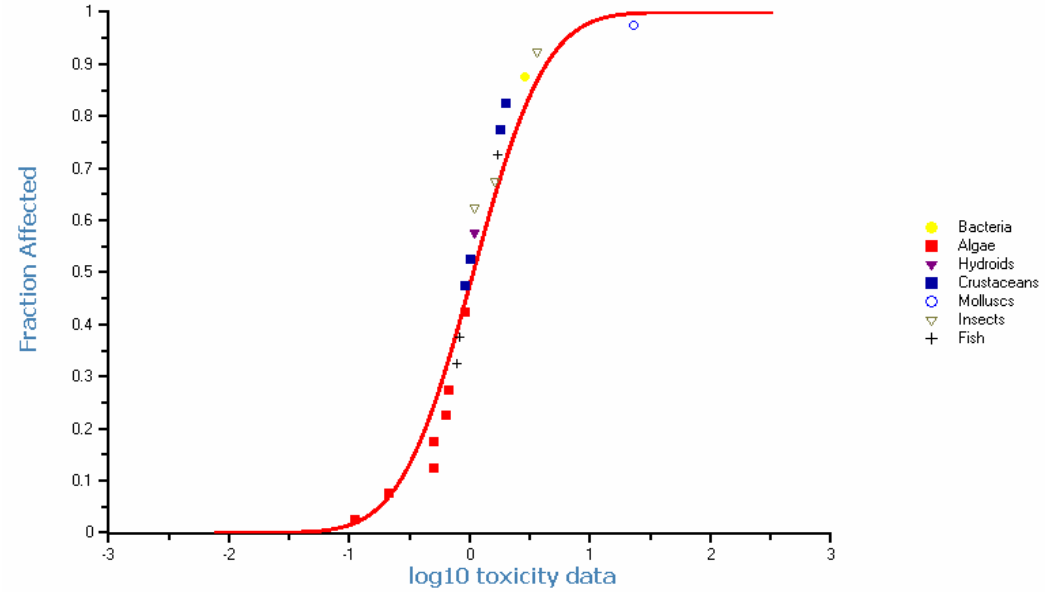


Table 2.6 Lowest available benzyl butyl phthalate long-term aquatic toxicity data of freshwater organisms

Scientific Name	Common Name	Taxon. Grp.	End-point	Effect	Test Duration (hours)	Conc. (mg l^{-1})	Environmental conditions	Exposure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code)*	Reference
Bacteria												
	Activated sludge	BAC	NOEC	O_2 consumption	0.5	0.4		s	n		NA	Yoshizaea *et al.* 1977
Ciliate												
Tetrahymena pyriformis	Ciliate	CIL	NOEC	Growth inhibition	24	50	DO monitored and reaeration performed when the DO dropped below 40% saturation. pH = 6.7-7.3, temp. = 24-26°C.	s	n	Value above the limit of solubility.	NA	Volskay *et al.* 1988
Tetrahymena pyriformis	Ciliate	CIL	LOEC	Growth inhibition	24	100	DO monitored and reaeration performed when the DO dropped below 40% saturation. pH = 6.7-7.3, temp. = 24-26°C.	s	n	Value above the limit of solubility.	NA	Volskay *et al.* 1988
Algae												
Navicula pelliculosa	Diatom	ALG	NOEC	Growth rate	72	0.17		s	y		RAR (valid)	HLS SLU 004/002301 2000 (Cited in ECB 2005)
Navicula pelliculosa	Diatom	ALG	EC10	Growth rate	72	0.2		s	y		RAR (valid)	Carolina Ecotox 14-01-1 1995 (Cited in ECB 2005)
Scenedesmus subspicatus	Green alga	ALG	NOEC	Growth rate	72	0.15		s	y		RAR (valid)	HLS SLU 005/002302 2000 (Cited in ECB 2005)
Scenedesmus subspicatus	Green alga	ALG	EC10	Growth rate	72	0.31		s	y		RAR (valid)	Carolina Ecotox 14-01-2 1995 (Cited in ECB 2005)
Selenastrum capricornutum	Green alga	ALG	NOEC	Cell count	96	0.1		s	n		RAR (use with care)	EG&G Bionomics 78-9-148 1978 (Cited in ECB 2005)
Selenastrum capricornutum	Green alga	ALG	NOEC	Chlorophyll a	96	<0.07		s	n	Range = 0.02-0.28	RAR (not valid)	Sugatt and Foote 1981 (Cited in Staples *et al.* 1997)
Selenastrum capricornutum	Green alga	ALG	NOEC	Biomass	14 day	<0.02	Temp. = 24°C, 4000 lumens	s	n	Tests were carried out with test material that is no longer representative of current production.	RAR (not valid)	Monsanto report SR-81-0252 1983 (Cited in ECB 2005)
Selenastrum capricornutum	Green alga	ALG	NOEC	Cell number	6 day	<0.10	Hardness = 25-50 mg l^{-1}, alkalinity = 25-50 mg l^{-1}, temp. = 22-24°C, light intensity = 60-70.5 µE/m2	s	y		3	Adams *et al.* 1995
Crustaceans												
Daphnia magna	Waterflea	CRU	NOEC	Reproduction	21 day	0.22		ss	y	Renewal 3 times/week. GLP	RAR (valid)	Monsanto report ES-82-SS103 1983 (Cited in ECB 2005)

Scientific Name	Common Name	Taxon. Grp.	End-point	Effect	Test Duration (hours)	Conc. (mg l^{-1})	Environmental conditions	Exposure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code)*	Reference
Daphnia magna	Waterflea	CRU	MATC	Reproduction	21 day	0.22-0.35	Well water	ss	y	Renewal 3 times/week. GLP	RAR (valid)	Monsanto report ES-82-SS103 1983 (Cited in ECB 2005)
Daphnia magna	Waterflea	CRU	NOEC	Survival/ reproduction	21 day	0.28	Hardness to 150-180 mg l^{-1}. Alkalinity = 100-130 mg l^{-1}. pH = 7.9-8.3. Temp. = 21 +/- 2°C. DO = 60% of saturation, conductivity = 400-600 µmho/cm.	f	y	Life stage = <24 hours. GLP	RAR (valid)	Rhodes *et al.* 1995 (Cited in ECB 2005)
Daphnia magna	Waterflea	CRU	LOEC	Survival/ reproduction	21 day	1.4	Hardness to 150-180 mg l^{-1}. Alkalinity = 100-130 mg l^{-1}. pH = 7.9-8.3. Temp. = 21 +/- 2°C, DO = 60% of saturation, conductivity = 400-600 µmho/cm.	f	y	Life stage = <24 hours. GLP	RAR (valid)	Rhodes *et al.* 1995 (Cited in ECB 2005)
Daphnia magna	Waterflea	CRU	NOEC	Growth/ reproduction	21 day	0.35		s	y		1	Adams *et al.*1995
Daphnia magna	Waterflea	CRU	LOEC	Growth/ reproduction	21 day	0.7		s	y		1	Adams *et al.* 1995
Fish												
Pimephales promelas	Fathead minnow	FIS	NOEC	Length	30 day	0.14		f	y	Newly hatched embryo and larval fish	RAR (valid)	EG&G Bionomics 1981 (Cited in ECB 2005)
Pimephales promelas	Fathead minnow	FIS	LOEC	Length	30 day	0.36		f	y	Newly hatched embryo and larval fish	RAR (valid)	EG&G Bionomics 1981 (Cited in ECB 2005)
Oncorhynchus mykiss	Rainbow trout	FIS	NOEC	Growth	109 days	>0.2	Hardness = 225-275 mg l^{-1}. Alkalinity = 325-375 mg l^{-1}. Temp. = 12 +/- 2°C. 14:10 hour light:dark photoperiod	f	y	GLP Total study length = 124 days (109 days post-hatch). No-effects at highest conc. tested	1	Rhodes *et al.* 1995
Oncorhynchus mykiss	Rainbow trout	FIS	LOEC	Growth	109 days	>0.2	Hardness = 225-275 mg l^{-1}. Alkalinity = 325-375 mg l^{-1}. Temp. = 12 +/- 2°C. 14:10 hour light:dark photoperiod	f	y	GLP Total study length = 124 days (109 days post-hatch). No-effects at highest conc. tested	1	Rhodes *et al.*1995

* See Annex 1.
[1] Exposure: s = static; ss = semi-static; f = flow-through
[2] Toxicant analysis: y = measured; n = not measured
ALG = alga, BAC = bacteria, CIL = ciliate, CRU = crustacean, FIS = fish,
NOEC = no observed effect concentration
LOEC – lowest observed effect concentration
MATC = maximum allowable toxicant concentration
EC10 = effective concentration for a 10% effect
NA = As the data was not critical for PNEC derivation the reliability has not been assessed
RAR indicates that the respective study was already quality assessed in the EU Risk Assessment on BBP.

Table 2.7 Lowest available butyl benzyl phthalate short-term aquatic toxicity data of freshwater organisms

Scientific Name	Common Name	Taxon Grp	Endpoint	Effect	Test Duration (hours)	Conc. mg l^{-1}	Environmental conditions	Exposure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code)*	Reference
Bacteria												
Activated sludge		BAC	EC50	Respiration	0.5	2.9		s			NA	IUCLID 2000
Algae												
Selenastrum capricornutum	Green alga	ALG	EC50	Cell count	96	0.5		s	n		RAR (use with care)	EG&G Bionomics 78-9-148 1978 (Cited in ECB 2005)
Selenastrum capricornutum	Green alga	ALG	EC50	Chlorophyll a	96	0.11		s	n	Range = 0.02-0.28	RAR (not valid)	Sugatt and Foote 1981 (Cited in Staples *et al.* 1997)
Selenastrum capricornutum	Green alga	ALG	EC50	Biomass	14 day	0.5	Temp. = 24°C, 4000 lumens, 'cool' white lights, algal assay media.	s	n	Tests were carried out with test material that is no longer representative of current production.	RAR (not valid)	Monsanto report SR-81-0252 1980 (Cited in ECB 2005)
Selenastrum capricornutum	Green alga	ALG	EC50	Cell number	6 day	<0.21	Hardness = 25-50 mg l^{-1}, alkalinity = 25-50 mg l^{-1}, temperature = 22-24°C, light intensity = 60-70.5 µE/m2	s	y		3	Adams *et al.* 1995
Selenastrum capricornutum	Green alga	ALG	EC50	Biomass	96	0.52		s	n		RAR (use with care)	Monsanto report 86-9076 1985 (Cited in ECB 2005)
Pseudokirchneriella subcapitata	Green alga	ALG	EC50	Chlorophyll	96	0.1		s	n	Minimum concentration = 0.02 mg l^{-1}, maximum concentration = 0.25 mg l^{-1}	4	U.S.EPA 1978
Pseudokirchneriella subcapitata	Green alga	ALG	EC50	Population growth	96	0.12		s	n	Minimum concentration = 0.02 mg l^{-1}, maximum concentration = 0.33 mg l^{-1}	4	U.S.EPA 1978
Scenedesmus subspicatus	Green alga	ALG	EC50	Growth rate	72	0.92		s	y		RAR (valid)	Carolina Ecotox 14-01-2 1995 (Cited in ECB 2005)
Navicula pelliculosa	Diatom	ALG	EC50	Growth rate	72	0.66		s	y		RAR (valid)	HLS SLU 004/002301 2000 (Cited in ECB 2005)
Navicula pelliculosa	Diatom	ALG	EC50	Growth rate	72	0.64		s	y		RAR (valid)	Carolina Ecotox 14-01-1 1995 (Cited in ECB 2005)
Hydroids												
Hydra littoralis	Hydra	HYD	LC50	Mortality	96	1.1	0.5-2.0	f	y		NA	Danish EPA 1998
Crustaceans												

Scientific Name	Common Name	Taxon Grp	Endpoint	Effect	Test Duration (hours)	Conc. mg l^{-1}	Environmental conditions	Exposure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code)*	Reference
Daphnia magna	Waterflea	CRU	EC50	Immobilsation	48	2.2	Temperature = 22°C, hardness = 241 mg l^{-1}.	s	n	Tested using triethylene glycol as the solvent	RAR (valid)	Barera and Adams 1981 (Cited in ECB 2005)
Daphnia magna	Waterflea	CRU	EC50	Immobilisation	48	1.8	Temperature = 22°C, hardness = 241 mg l^{-1}.	s	n	Tested using dimethylformide as the solvent	RAR (valid)	Barera and Adams 1981 (Cited in ECB 2005)
Daphnia magna	Waterflea	CRU	NOEC	Immobilisation	48	0.62	Temp. = 22°C, pH = 7.8. DO = 7.3 mg l^{-1}, alkalinity = 231 mg l^{-1}, hardness = 241 mg l^{-1}, conductivity = 866 µmhos	s	n	Life stage = <24 hours. Unfed *Daphnia*. Tested using dimethylformide as the solvent	RAR (valid)	Barera and Adams 1981 (Cited in ECB 2005)
Daphnia magna	Waterflea	CRU	EC50	Immobilsation	48	>0.92	Temp = 20°C, pH = 7.9-9, alkalinity = 25-50 mg l^{-1} and hardness = 25-50 mg l^{-1}.	s	y	GLP	1	Adams *et al.* 1995
Daphnia magna	Waterflea	CRU	EC50	Immobilisation	48	1.8	Temp. = 22°C, hardness = 136-2621 mg l^{-1} .	s	n		RAR (Valid)	Monsanto 85-9180 9184 (Cited in ECB 2005)
Daphnia magna	Waterflea	CRU	EC50	Not stated	48	1.0	Temp. = 22°C, hardness = 241 mg l^{-1}.		n	No solvent	RAR (valid)	Barera and Adams 1981 (Cited in ECB 2005)
Molluscs												
Physa heterostropha	Pond snail	MOL	LC50	Mortality	96	23		s	n	Formulation. Min conc. = 17.2 mg l^{-1}, max conc. = 30.8 mg l^{-1}	NA	Horne and Oblad 1983
Insects												
Chironomus tentans	Midge	INS	EC50	Not stated	48	1.6	Temp. = 22°C, well water	s	n		RAR (valid)	Monsanto report MO-85-9180 1984 (Cited in ECB 2005)
Chironomus tentans	Midge	INS	NOEC	Not stated	48	1.25		s	n	GLP	RAR (valid)	Monsanto report MO-85-9165 1982 (Cited in ECB 2005)
Hexagenia limbata	Mayfly	INS	LC50	Mortality	96	1.1		f	y		RAR (not assignable)	Monsanto Study 34173C 1986 (Cited in Staples *et al.* 1997 and ECB 2005)
Paratanytarsus parthenogenetica	Midge	INS	LC50	Mortality	96	>3.6		s	y	GLP	1	Adams *et al.* 1995
Fish												
Oncorhynchus mykiss	Rainbow trout	FIS	LC50	Mortality	96	0.82	Hardness = 25-50 mg l^{-1}, alkalinity = 25-50 mg l^{-1}, temp. = 22°C.	f	y	Fish were acclimatised and observed for 14 days prior to testing. Size = 39-62 mm.	RAR (valid)	Adams *et al.* 1995 (Cited in ECB 2005)
Pimephales promelas	Fathead minnow	FIS	LC50	Mortality	96	>0.78	Hardness = 25-50 mg l^{-1}, alkalinity = 25-50 mg l^{-1}, temp. = 22°C.	s	y	Fish were acclimatised and observed for 14 days prior to testing.	RAR (valid)	Adams *et al.* 1995 (Cited in ECB 2005)

Scientific Name	Common Name	Taxon Grp	Endpoint	Effect	Test Duration (hours)	Conc. mg l^{-1}	Environmental conditions	Exposure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code)*	Reference
Lepomis macrochirus	Bluegill sunfish	FIS	NOEC	Mortality	96	0.36	Hardness = 25-50 mg l^{-1}, alkalinity = 25-50 mg l^{-1}, temp. = 22°C.	s	y	10% mortality or less occurred at the highest concentration tested.	RAR (valid)	Adams *et al.* 1995 (Cited in ECB 2005)
Lepomis macrochirus	Bluegill sunfish	FIS	LC50		96	1.7	Hardness = 28-44 mg l^{-1}, Temp. = 22°C	s	n		RAR (use with care)	Monsanto Study 82-0015 1979 (Cited in ECB 2005)

* See Annex 1.
[1] Exposure: s = static; f = flow-through
[2] Toxicant analysis: y = measured; n = not measured
ALG = alga, BAC = bacteria, CRU = crustacean, FIS = fish, HYD = hydroid, INS = insect, MOL = mollusc
EC50 = effective concentration for a 50% effect
LC50 = lethal concentration for a 50% effect
NOEC = no observed effect concentration
NA = As the data was not critical for PNEC derivation the reliability has not been assessed
RAR indicates that the respective study was already quality assessed in the EU Risk Assessment on BBP.

2.6.2 Toxicity to saltwater organisms

Saltwater toxicity data for BBP are available for various taxonomic groups including algae, invertebrates and fish. Long-term toxicity data are available for three taxonomic groups: algae, crustaceans and fish. Short-term saltwater toxicity data are available for five different taxonomic groups: algae, annelids, crustaceans, fish and molluscs.

Diagrammatic representations of all the available long and short-term saltwater data (cumulative distribution function) for BBP are presented in Figures 2.3 and 2.4, respectively. These diagrams include all data regardless of quality and provide an overview of the spread of the available data. The diagrams are not species sensitivity distributions and have not been used to set the BBP PNECs.

Long-term toxicity data for marine species are summarised in Table 2.8 and short-term toxicity data are summarised in Table 2.9.

Figure 2.3 Cumulative distribution function of saltwater long-term data (mg l^{-1}) for benzyl butyl phthalate

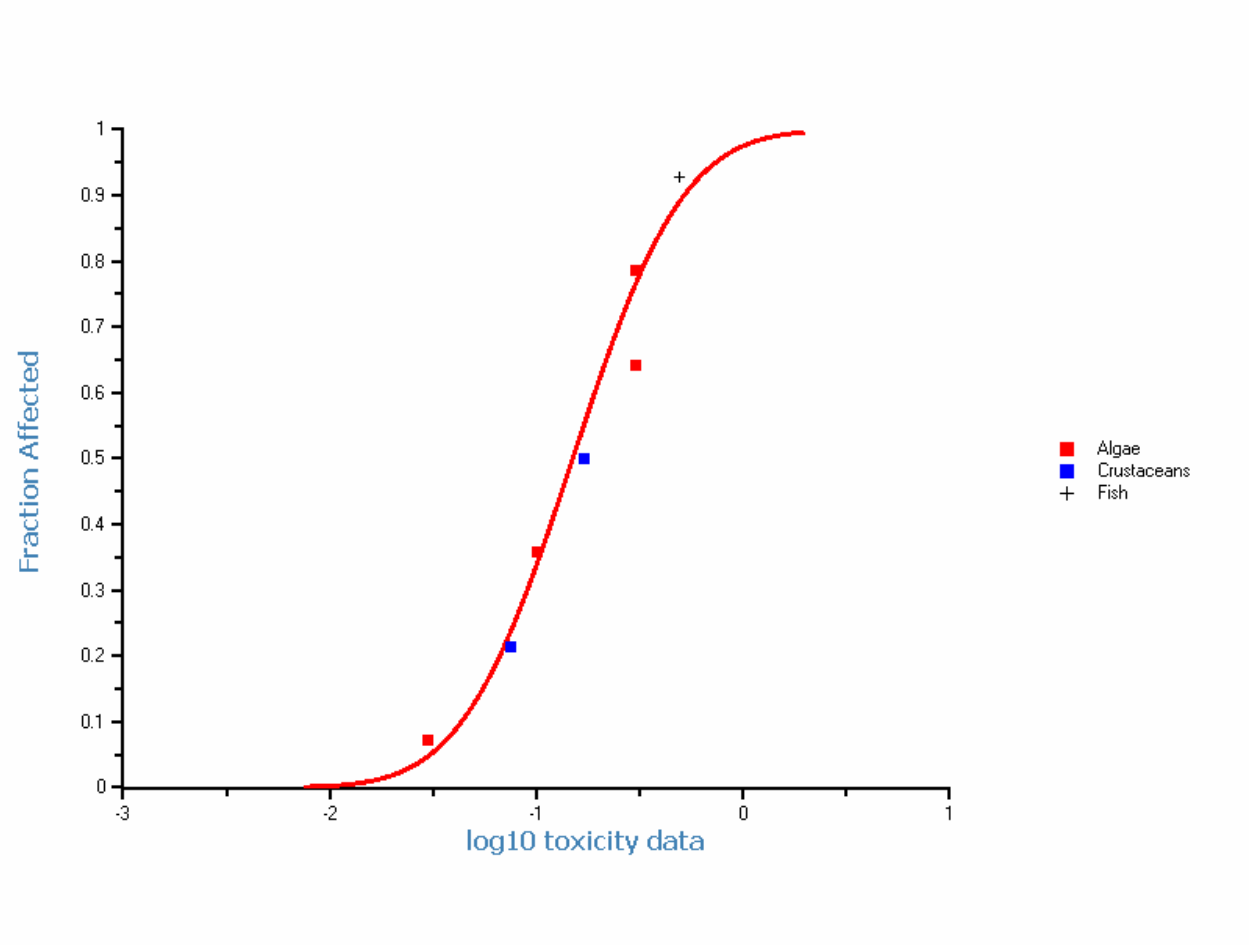


Figure 2.4 Cumulative distribution function of saltwater short-term data (mg l^{-1}) for benzyl butyl phthalate

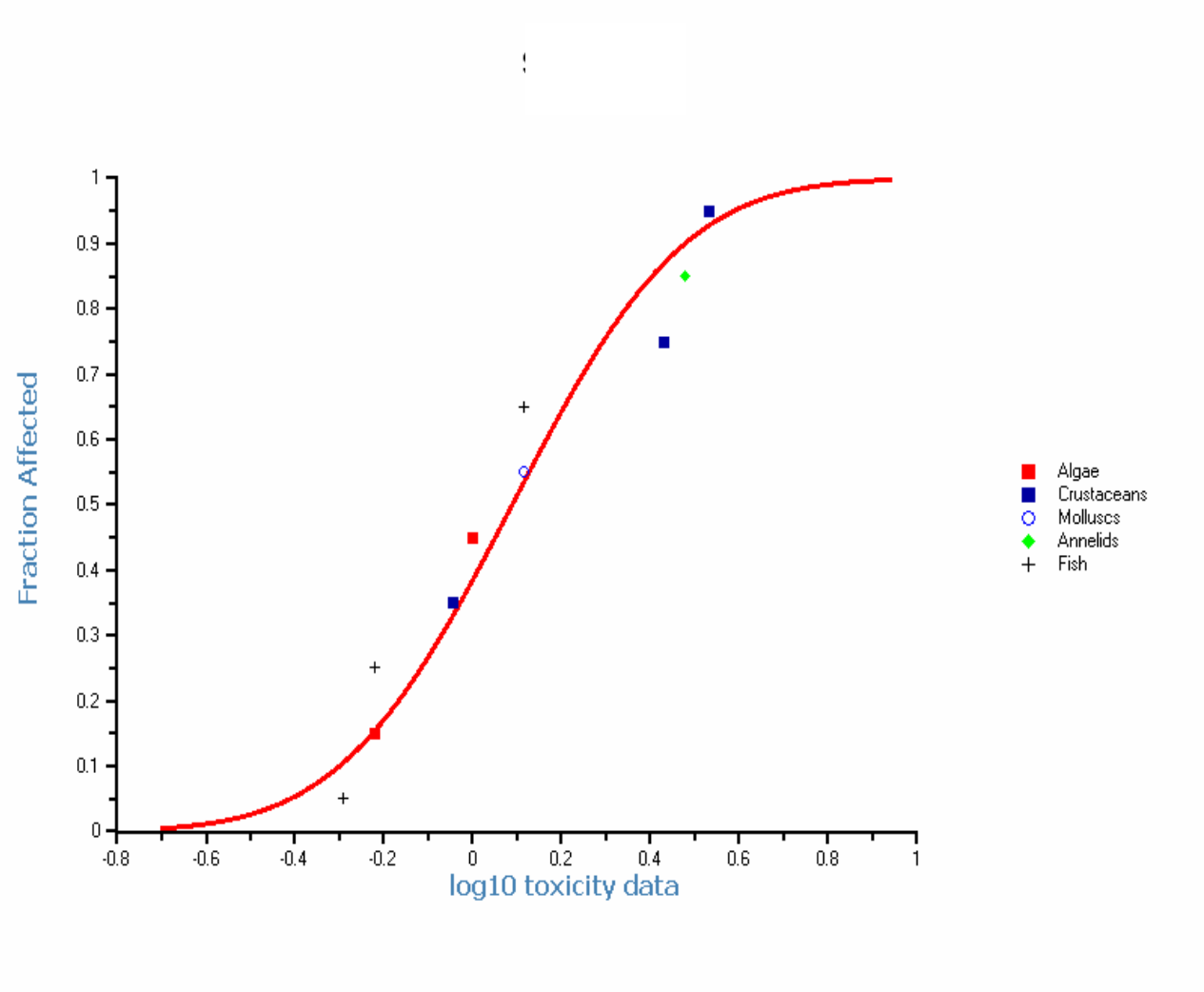


Table 2.8 Lowest available butyl benzyl phthalate long-term toxicity data of marine organisms

Scientific Name	Common Name	Taxon Grp	Endpoint	Effect	Test Duration (hours)	Conc. mg l^{-1}	Environmental conditions	Expo-sure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code)*	Reference
Algae												
Skeletonema costatum	Diatom	ALG	NOEC	Chlorophyll a	96	<0.03		s	n		RAR (not valid)	Sugatt and Foote 1981 (Cited in ECB 2005)
Skeletonema costatum	Diatom	ALG	LOEC	Cell count	96	0.1	Temp. = 20°C, salinity = 30 ppt, 2000 lux	s	n	Acetone carrier.	RAR (use with care)	EG&G Bionomics 78-9-148 1978 (Cited in ECB 2005)
Skeletonema costatum	Diatom	ALG	NOEC /EC10	Growth rate	96	0.3		s	n		RAR (use with care)	EG&G Bionomics 78-9-148 1978 (Cited in ECB 2005)
Dunaliella tertiolecta	Alga	ALG	NOEC /EC10		96	0.3		s	n		RAR (use with care)	EG&G Bionomics 78-9-148 1978 (Cited in ECB 2005)
Crustaceans												
Mysidopsis (Americamysis) bahia	Mysid shrimp	CRU	NOEC	Reproduction/ growth	28 day	0.075		f	y	GLP	RAR (valid)	Monsanto 86-7-2074 (Cited in ECB 2005)
Mysidopsis (Americamysis) bahia	Mysid shrimp	CRU	NOEC	Survival	28 day	0.17		f	y	GLP	RAR (valid)	Monsanto 86-7-2074 (Cited in ECB 2005)
Fish												
Cymastogaster aggregata	Shiner perch	CRU	LC50	Mortality	8 day	0.49	DO = >80%, 16h/8h light/dark cycle, Salinity 30-33 ppt. Temp = 12 +/- 1°C.	f	y	Confidence interval = 0.45-0.56	RAR (valid)	Ozretich *et al.* 1983 (Cited in ECB 2005)

* See Annex 1.
[1] Exposure: s = static; f = flow-through
[2] Toxicant analysis: y = measured; n = not measured
ALG = alga, CRU = crustacean, FIS = fish
NOEC = no observed effect concentration
LOEC – lowest observed effect concentration
EC10 = effective concentration for a 10% effect
LC50 = lethal concentration for a 50% effect
RAR indicates that the respective study was already quality assessed in the EU Risk Assessment on BBP.

Table 2.9 Lowest available benzyl butyl phthalate short-term toxicity data of marine organisms

Scientific Name	Common Name	Taxon Grp	Endpoint	Effect	Test Duration	Concentration mg l^{-1}	Environmental conditions	Expo-sure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code)*	Reference
Algae												
Skeletonema costatum	Diatom	ALG	EC50	Cell number	96	0.19		s	n	Confidence interval = 0.08-0.36	RAR (not valid)	Sugatt and Foote 1981 (Cited in ECB 2005)
Skeletonema costatum	Diatom	ALG	EC50	Growth rate	96	0.17		s	n		RAR (not valid)	Sugatt and Foote 1981(Cited in ECB 2005)
Skeletonema costatum	Diatom	ALG	EC50	Growth rate	96	0.6		s	n		RAR (use with care)	EG&G Bionomics 78-9-148 1978 (Cited in ECB 2005)
Dunaliella tertiolecta	Alga	ALG	EC50		96	1		s	n		RAR (use with care)	EG&G Bionomics 78-9-148 1978 (Cited in ECB 2005)
Crustaceans												
Mysidopsis (Americamysis) bahia	Mysid shrimp	CRU	NOEC	Mortality	96	0.4	Temp. = 20°C, salinity = 18 g l^{-1}, natural seawater		y		2	Gledhill *et al.*1980
Mysidopsis (Americamysis) bahia	Mysid shrimp	CRU	LC50	Mortality	96	0.9	Temp. = 20°C, salinity = 18 g l^{-1}, natural seawater		y		2	Gledhill *et al.*1980
Mysidopsis (Americamysis) bahia	Mysid shrimp	CRU	EC50	Not stated	96	>0.74	Temp. = 22°C, salinity = 30 ppt	f	y	GLP	RAR (valid)	Springborn 87-10-2525 1986 (Cited in ECB 2005)
Paleomonetes vulgaris	Shrimp	CRU	EC50		96	>2.7	Temp. = 20°C, salinity = 32 ppt	f	y	GLP	RAR (valid)	Springborn 86-7-2087 1986 (Cited in ECB 2005)
Molluscs												
Crassostrea virginica	American or Virginia oyster	MOL	EC50	Shell deposition	96	1.3	Salinity 32 ppt	f	y	GLP	RAR (valid)	Springborn 86-7-2083 1986 (Cited in ECB 2005)
Annelid												
Nereis virens	Annelid worm	ANN	LC50	Mortality	96	>3	Salinity 32 ppt	f	y	GLP	RAR (valid)	Springborn 86-7-2094 1986 (Cited in ECB 2005)
Fish												
Cymastogaster aggregata	Shiner perch	FIS	LC50	Mortality	96	0.51	DO = >80%, 16h/8h light/dark cycle, Salinity = 30-34 ppt. Temp. = 12 +/- 1°C.	f	y	95% CI = 0.46-0.56.	RAR (valid)	Ozretich *et al.* 1983 (Cited in ECB 2005)

Scientific Name	Common Name	Taxon Grp	Endpoint	Effect	Test Duration	Concentration mg l^{-1}	Environmental conditions	Expo-sure[1]	Toxicant analysis[2]	Comment	Reliability (Klimisch code)*	Reference
Parophrys vetulus	English sole	FIS	LC50	Mortality	96	0.55	Temp. = 12 +/- 1°C, 16/8 hour light/dark cycle DO = 6.61 mg l^{-1}. pH = 7.3. Salinity = 31.4 ppt.	f	y	CI = 0.48-0.64 mg l^{-1}. Concentrations measured but results reported as nominal	RAR (use with care)	Randall *et al.* 1983 (Cited in ECB 2005)
Parophrys vetulus	English sole	FIS	LC50	Mortality	96	0.66	Temp. = 12 +/- 1°C, 16/8 hour light/dark cycle, DO = 6.61 mg l^{-1}. pH = 7.3. Salinity = 31.4 ppt.	s	y	CI = 0.48-0.64 mg l^{-1}. Concentrations measured but results reported as nominal	RAR (use with care)	Randall *et al.* 1983 (Cited in ECB 2005)
Cyprinodon variegatus	Sheepshead minnow	FIS	NOEC	Mortality	96	>0.68	Hardness = 25-50 mg l^{-1}. Alkalinity = 25-50 mg l^{-1}. pH = 7.6-7.9.	s	y	Fish were observed for 14 days prior to exposure. Size = 6-17 mm.	1	Adams *et al.* 1995

* See Annex 1.
[1] Exposure: s = static; f = flow-through
[2] Toxicant analysis: y = measured; n = not measured
ALG = alga, ANN = annelid, CRU = crustacean, FIS = fish, MOL = mollusc
EC50 = effective concentration for a 50% effect
LC50 = lethal concentration for a 50% effect
NOEC = no observed effect concentration
RAR indicates that the respective study was already quality assessed in the EU Risk Assessment on BBP.

2.6.3 Toxicity to sediment dwelling organisms

Data on direct toxicity to sediment-dwelling organisms were not found.

2.6.4 Endocrine-disrupting effects

BBP has been shown to have endocrine disrupting effects in wildlife. BBP is suggested to cause both oestrogenic and anti-androgenic effects.

Oestrogenic effects

Various studies have investigated the oestrogenic effects of BBP on fish. The results are mixed, but in general BBP appears to be oestrogenic *in vivo* only at concentrations higher than 0.1 mg l^{-1}. Concentrations of BBP ranging from 0.01 to 10 mg l^{-1} have been shown to reduce the *in-vitro* binding of 17β-estradiol by 40-60% in trout oestrogen receptors (Jobling *et al.* 1995). The report states that BBP acts as an estrogen agonist, but does not appear to act as an anti-oestrogen at any concentration in the active range. The results were based on nominal exposure concentrations. In contrast, Harries *et al.* (2000) (cited in ECB 2005) found no effect on the number of spawnings, number of eggs or the size of eggs, plasma vitellogenin or the gonadosomatic index of breeding pairs of fathead minnow (*Pimephales promelas*) exposed to 0.1 mg l^{-1} BBP for three weeks. Similarly, in a study by the Japanese Ministry of the Environment significant effects on vitellogenin production in Japanese medaka (*Oryzias latipes*) occurred only at exposure concentrations of >1 mg l^{-1} (ECB 2005). A BBP concentration of 0.3 mg l^{-1} had no effect. However, the reporting and methodological details for this study were incomplete and as a result the draft RAR stated that no firm conclusion could be drawn from the study. In an unpublished life cycle test with Japanese medaka carried out by the Japanese government no effects on reproduction, ovotestis or vitellogenin induction were found at concentrations up to 0.1 mg l^{-1}.

In tests where BBP was administered to rainbow trout (*Oncorhynchus mykiss*) intraperitoneally significant induction of vitellogenin has been observed (Christiansen *et al.* 2000 cited in ECB 2005). Injection of 5 and 50 mg/kg BBP to juvenile rainbow trout has also been shown to significantly lower plasma levels of eggshell proteins (Knudsen *et al.* 1998 cited in ECB 2005). However, exposure to 50 mg/kg had no effect on the induction of the oestrogen receptor.

Anti-androgenic effects

BBP has also been reported to have anti-androgenic effects in mammalian *in-vivo* studies. In an *in-vitro* study (recombinant yeast assay), BBP has been found to be as potent an anti-androgen as the model anti-androgen substance flutamide (ECB 2005). Anti-androgenicity is more difficult to establish in fish due to the lack of a standardised model. Ankley *et al.* (2004) (cited in ECB 2005) found anti-androgenic effects *in-vitro* (in terms of effects on competitive binding) using COS cells into which the fathead minnow (*Pimephales promelas*) androgen receptor had been cloned. However, such effects have yet to be established *in-vivo*.

Parent to offspring transfer

The draft RAR for BBP (ECB 2005) states that the issue with much of the data for endocrine effects is that the studies have not incorporated aspects of parent to offspring transfer. Consequently, an agreement was reached with industry to perform a partial life cycle study with breeding pairs of fathead minnow where egg production and

hatchability would be assessed along with the sexual differentiation of the offspring (continued exposure of the eggs). However, there were significant problems in maintaining the exposure concentrations in the test systems due to the rapid biodegradation of BBP. Consequently, it has not been possible, as yet, to perform these tests successfully.

The results of tests on the endocrine effects of BBP suggest that this chemical may have both estrogenic and anti-androgenic effects. However, there are issues with using the available endocrine data to set the overall PNECs. In the first instance all of the tests reporting endocrine disrupting effects at concentrations less than 0.1 mg l^{-1} are *in vitro* exposures. Endocrine disrupting effects *in vivo* tend to occur at higher concentrations, within a range where effects such as mortality have also been observed in some species using standard aquatic ecotoxicity tests. In addition there is still some uncertainty about long-term consequences of the observed endocrine disrupting effects and whether there is any evidence of parent-offspring transfer. Until these issues are resolved it is not possible to incorporate ED effects into the PNEC derivation. The same conclusion was reached in the draft RAR for BBP.

The effects observed in aquatic organisms should also be considered in the context of the available mammalian data (see Table 3.1) which show that whilst there are established ED effects, these typically occur at high exposure concentrations.

2.6.5 Mode of action of benzyl butyl phthalate

Few data are available on the mode of toxic action of BBP in aquatic organisms. Ozretich *et al.* (1983) reported that the reduced brain levels of epinephrine found in shiner perch (*Cymastogaster aggregata*), surviving acute exposures to BBP, indicated that the mode of acute toxicity for this species may be through its effects on the catecholamines of the central adrenergic nervous system. However, in general terms polar narcosis, with BBP acting as a reactive electrophile/proelectrophile, is accepted as the primary mode of action of this chemical (Verhaar *et. al*. 1992 and Russom *et al.* 1997).

In addition to polar narcosis, BBP may also have specific endocrine modes of action, as highlighted above.

2.6.6 Mesocosm and field studies

Freshwater mesocosm and field studies

No data from mesocosm or field studies using freshwater organisms were found.

Saltwater mesocosm and field studies

No data from mesocosm or field studies using saltwater organisms were found.

3 Calculation of PNECs as a basis for the derivation of quality standards

3.1 Derivation of PNECs by the TGD deterministic approach (AF method)

Based on the available data there appear to be no significant differences in the sensitivity of freshwater or saltwater species of the same taxonomic group. This may reflect the mode of toxic action of BBP as a polar narcotic. Consequently the draft RAR for BBP combined freshwater and saltwater datasets for the derivation of both the freshwater and saltwater PNECs. The same approach has been adopted for the derivation of PNECs in this report.

3.1.1 PNECs for freshwaters

PNEC accounting for the annual average concentration

Freshwater long-term data are available for five taxonomic groups including algae, bacteria, ciliates, crustaceans and fish. Consequently, data are available for the 'base set' of toxicity tests (i.e. tests with algae, crustaceans and fish) and the EU TGD assessment factor (AF) method can be applied (ECB 2003). There appears to be little difference between trophic levels in the sensitivity of organisms to BBP (see Table 2.6).

Studies are available for various freshwater algal species. Comparisons of the available NOEC/EC10 data indicate similar sensitivities of the species tested. The lowest data available are NOECs of <0.07 and <0.02 mg l^{-1} for changes in biomass and chlorophyll-*a*, respectively of *Selenastrum capricornutum* (Monsanto SR-81-0252, Sugatt and Foote 1981 cited in Staples *et al.* 1997). However, both studies were regarded by the EU RAR as unsuitable for PNEC derivation due to the study durations (4 and 14 days, respectively), which were greater than the standard OECD 201 method of 72 hours. Both studies also lacked measured exposure concentrations. Six day and 4 day NOECs of <0.1 and 0.1 mg l^{-1} (for cell number/count) in the same species have been reported by Adams *et al.* (1995) and EG&G Bionomics (cited in ECB 2005). However, both these studies should be treated with caution due to the study durations and the endpoints reported (cell count), which again do not comply with the OECD 201 methodology.

A number of algal studies with *Navicula pelliculosa* and *Scenedesmus subspicatus* are available that were conducted in line with the OECD 201 methodology (i.e. 72 hour exposure measuring algal growth rate) (HLS 004/002301, HLS 005/002302, Carolina Ecotox 14-01-1 and Carolina Ecotox 14-01-2 all cited in ECB 2005). The results of these studies are similar with NOEC/EC10s in the two species ranging from 0.15 to 0.31 mg l^{-1}. All studies were regarded as suitable for PNEC derivation. However, an EC10 of 0.2 mg l^{-1} was reported by the EU RAR as the critical algal datum as the

endpoint was statistically derived (i.e. EC10) and not a NOEC as reported in the other studies.

Long-term freshwater data for crustaceans are available primarily for *Daphnia magna*. The lowest data available are reported in a number of GLP studies with measured exposure concentrations (Monsanto ES-82-SS103, cited in ECB 2005 and Rhodes *et al.* 1975). Twenty one day NOECs of 0.22 and 0.28 mg l^{-1} (LOECs of 0.35 and 1.4 mg l^{-1}, respectively) were reported in these studies. Both values would be regarded as suitable for PNEC derivation.

The lowest available long-term freshwater fish study was a 30-day NOEC (for growth and length) of 0.14 mg l^{-1} in the fathead minnow (*Pimephales promelas*) (EG&G Bionomics, 1981 cited in ECB, 2005). This study was conducted with continuous exposure and measured exposure concentrations. It was regarded by the EU RAR to be fully valid for PNEC derivation.

Toxicity data for bacteria and ciliates indicate lower sensitivity in these organisms than reported in algae, crustaceans or fish.

The lowest valid long-term freshwater data point was the 30-day NOEC (growth/length) of 0.14 mg l^{-1} in the fathead minnow (*Pimephales promelas*) (EG&G Bionomics, 1981 cited in ECB, 2005) However, given the similarity in effects in both fresh and saltwater the EU RAR combined the two data sets. In saltwater a 28-day NOEC (reproduction/growth) of 0.075 mg l^{-1} was reported for the mysid shrimp (*Mysidopsis bahia*) (Monsanto 86-7-2074 cited in ECB, 2005). This datum was generated in a GLP study under flow through conditions with measured exposure concentrations. Consequently, it was regarded by the RAR as fully valid for PNEC derivation.

The long-term freshwater PNEC in the EU RAR for BBP was therefore based on the 28 day NOEC (reproduction/growth) of 0.075 mg l^{-1} reported for the mysid shrimp (*Mysidopsis bahia*) and an assessment factor of 10 was applied because of the availability of long-term data for three trophic levels and the similarity in sensitivity of trophic levels. This resulted in:

$PNEC_{freshwater\_lt}$ = 0.075 mg l^{-1} /AF (10) = 0.0075 mg l^{-1} (7.5 µg l^{-1})

PNEC accounting for a maximum allowable concentration

Freshwater short-term toxicity data are available for seven taxonomic groups including algae, bacteria, crustaceans, fish, insects, hydra and molluscs (see Table 2.7). Consequently, data are available for the 'base set' of organisms and the EU TGD assessment factor (AF) method can be applied (ECB 2003). As with the long-term data the difference in sensitivity of organisms from different trophic levels is small.

There are a number of short-term studies with algae. The lowest available value was a 96-hour EC50 (chlorophyll) of 0.1 mg l^{-1} in *Pseudokirchneriella subcapitata* (US EPA 1978). There were few details available with which to assess the quality of this study. However, the 96 hour duration means that it is not comparable with the standard OECD 201 methodology and as such it should be treated with caution. There are a number of relatively low EC50 values for *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata* that have been generated using non standard methods (i.e. longer exposure durations than the 72 hour recommended by OECD 201 and/or reporting non-standard endpoints such as cell count) and/or without measured exposure concentrations (EG&G Bionomics 78-9-148, Sugatt and Foote 1981, Monsanto SR-81-0252, Adams *et al.* 1995 and Monsanto 86-9076). These results are either unsuitable for PNEC derivation or should be treated with caution. The lowest

reliable short-term freshwater algal data point was a 72-hour EC50 (for growth rate) of 0.64 mg l^{-1} for *Navicula peilliculosa* (Carolina Ecotox 14-01-1 cited in ECB 2005). This study was based on measured exposure concentrations and was regarded by the EU RAR as valid.

As with long-term exposures, data for crustaceans are available primarily for *Daphnia magna.* The range of 48-hour EC50 values from different studies are comparable, with values ranging from ~1-2 mg l^{-1}. The lowest reliable EC50 identified by the EU RAR was a 48-hour EC50 of 1 mg l^{-1} (Barera and Adams, 1981). This study was based on nominal concentrations, but was reported to be valid by the RAR.

Short-term freshwater data are also available for insects. The lowest reported value is a 96-hour LC50 of 1.1 mg l^{-1} in the mayfly (*Hexagenia limbata*) (Monsanto 34173C, 1986 cited in Staples *et al.* 1997 and ECB 2005). As this study was reported in a review article few details were available with which to assess its quality. As such its quality cannot be assigned. The lowest reliable EC50 identified by the EU RAR was 48 -hour EC50 of 1.6 mg l^{-1} in the midge *Chironomus tentans* (Monsanto MO-85-9180 cited in ECB 2005). This result was based on nominal concentrations and as such should be treated with caution.

Short-term freshwater fish data were available for various species. The lowest LC50 of 0.82 mg l^{-1} for rainbow trout (*Oncorhynchus mykiss*) was generated in a flow-through study with measured exposure concentrations (Adams *et al.* 1995). Consequently it was regarded as valid for PNEC derivation.

The available data for hydra and molluscs indicate that these species are of similar or lower sensitivity than freshwater algae, crustaceans, insects and fish.

The lowest valid short-term freshwater data point was the 72-hour EC50 (growth rate) of 0.64 mg l^{-1} for *Navicula pelliculosa* (Carolina Ecotox 14-01-1 cited in ECB 2005) However, given the similarity in effects in both fresh and saltwaters the EU RAR combined the two datasets. In saltwater a 96-hour LC50 of 0.51 mg l^{-1} was reported for the shiner perch (*Cymatogaster aggregata*) (Ozretich *et al.* 1983 cited in ECB 2005). This datum was generated under flow-through conditions with measured exposure concentrations. Consequently, it was regarded, by the RAR, as fully valid for PNEC derivation.

No short-term freshwater PNEC was derived in the EU RAR for BBP. However, the lowest short-term critical data were identified in the RAR, as described above. Applying an assessment factor of 10 to the 96-hour LC50 of 0.51 mg l^{-1} reported for the shiner perch is proposed because of the availability of reliable short-term data for at least three trophic levels and the similarity in sensitivity of trophic levels. This results in:

$PNEC_{freshwater\_st}$ = 0.51 mg l^{-1} /AF (10) = 0.051 mg l^{-1} (51 µg l^{-1})

3.1.2 PNECs for saltwaters

The effects database for marine species is smaller than that for freshwater organisms. Long-term toxicity data are available for three taxonomic groups: algae, crustaceans and fish (see Table 2.8). Short-term saltwater toxicity data are available for five different taxonomic groups: algae, annelids, crustaceans, fish and molluscs (see Table 2.9). Both data sets include data for the base set of organisms (i.e. algae, crustaceans and fish) and so the EU TGD assessment factor approach can be applied.

Based on the available data there appear to be no significant differences in the sensitivity of freshwater or saltwater species of the same taxonomic group. This may reflect the mode of toxic action of BBP as a polar narcotic. Consequently the draft RAR for BBP combined freshwater and saltwater datasets for the derivation of both the freshwater and saltwater PNECs. The same approach has been adopted for the derivation of PNECs in this report.

PNEC accounting for the annual average concentration

Only limited saltwater toxicity data were available. The lowest long-term algal data point was a 96-hour NOEC of <0.03 mg l^{-1} in *Skeletonema costatum* (Sugatt and Foote, 1981 cited in ECB 2005). This study was deemed unreliable by the EU RAR due to the study duration and the fact that the test methodology was inadequate compared to current standards. The only other available NOECs were 96 hour values of 0.3 mg l^{-1} reported for *Skeletonema costatum* and *Dunaliella tertiolecta* (EG&G Bionomics 98-9-148 cited in ECB 2005). Due to the study duration and nominal concentrations the RAR reported that these studies should be treated with care.

The lowest available saltwater long-term crustacean datum was a 28-day NOEC (for reproduction and growth) of 0.075 mg l^{-1} reported for the mysid shrimp (*Mysidopsis bahia*) (Monsanto 86-7-2074 cited in ECB, 2005). This datum was generated in a GLP study under flow through conditions with measured exposure concentrations. Consequently, it was regarded by the RAR as fully valid for PNEC derivation.

The only longer-term saltwater fish value available was an 8-day LC50 of 0.49 mg l^{-1} for the shiner perch (*Cymastogaster aggregata*) (Ozretich *et al.* 1983 cited in ECB 2005). This datum was generated under flow through conditions with measured exposure concentrations. Consequently, it was regarded by the RAR as fully valid for PNEC derivation.

The long-term saltwater PNEC in the EU RAR for BBP was based on the 28-day NOEC (for reproduction and growth) of 0.075 mg l^{-1} reported for the mysid shrimp (*Mysidopsis bahia*). An assessment factor of 100 was proposed by the EU RAR. A larger assessment factor (than used for the freshwater PNEC) was adopted due to a lack of long-term data for additional marine taxa such as echinoderms or molluscs. Such an approach is line with the EU TGD (ECB 2003). This resulted in:

$PNEC_{Saltwater\_lt}$ = 0.075 mg l^{-1} /AF (100) = 0.00075 mg l^{-1} (0.75 µg l^{-1})

PNEC accounting for a maximum allowable concentration

The lowest short-term saltwater algal data are the 96-hour EC50 (for cell number and growth rate) values of 0.17 and 0.19 mg l^{-1} for *Skeletonema costatum* (Sugatt and Foote, 1981 cited in ECB 2005). This study was deemed unreliable by the EU RAR due to the study duration and the fact that the test methodology was inadequate compared to current standards. The only other available EC50s were 96-hour values of 0.6 and 1 mg l^{-1} reported for *Skeletonema costatum* and *Dunaliella tertiolecta*, respectively (EG&G Bionomics 98-9-148 cited in ECB 2005). Due to the study duration and nominal concentrations the RAR reported that these studies should be treated with care.

Short-term saltwater crustacean data were available for mysid shrimp (*Mysidopsis bahia*) and grass shrimp (*Paleomonetes vulgaris*). The lowest value was a 96-hour LC50 of 0.9 mg l^{-1} for *Mysidopsis bahia* (Gledhill *et al.* 1980). This study was generated in a static system using nominal exposure concentrations. As such it should be treated

with caution. In a GLP study using flow-through procedures and measured exposures concentrations a 96 hour EC50 of >0.74 mg l^{-1} (the highest concentration tested) was reported for the same species (Springborn 87-10-2525, cited in ECB 2005).

Short-term saltwater fish data were available for various species. The lowest LC50 was a 96 hour value of 0.51 mg l^{-1} for the shiner perch (*Cymatogaster aggregata*) (Ozretich *et al.* 1983 cited in ECB 2005). This value was generated in a flow-through study with measured exposure concentrations. Consequently it was regarded as valid for PNEC derivation. This value is supported by a 96-hour LC50 value of 0.55 mg l^{-1} for the English sole (*Parophrys vetulus*), which was also conducted under flow-through conditions with measured exposure concentrations (Randall *et al.* 1983, cited in ECB 2005).

The available data for annelids and molluscs indicate that these species are of similar or lower sensitivity than saltwater algae, crustaceans and fish.

No short-term saltwater PNEC was derived in the EU RAR for BBP. However, the lowest short-term critical data were identified in the RAR. The lowest reliable data point in the combined data set was the 96-hour LC50 of 0.51 mg l^{-1} reported for the shiner perch. An assessment factor of 50 is proposed, because in addition to reliable short-term data for at least three trophic levels, there are short-term data to show that one additional marine taxonomic group (molluscs) is no more sensitive than algae, crustaceans and fish. The use of such an assessment factor with the short-term saltwater data is in line with the guidance within the EU TGD (ECB 2003). This results in:

$PNEC_{saltwater\_st}$ = 0.51 mg l^{-1} /AF (50) = 0.010 mg l^{-1} (10 µg l^{-1})

3.2 Derivation of PNECs by the TGD probabilistic approach (SSD method)

The minimum number of long-term toxicity data (at least 10 NOECs from eight taxonomic groups) is not available. Therefore, the species sensitivity distribution (SSD) approach cannot be used for PNEC derivation.

3.3 Derivation of existing EQSs

A number of short and long-term EQS values were derived for BBP by Lewis *et al.* (1998). An Annual Average (AA) EQS of 20 µg l^{-1} was proposed for freshwaters. This EQS was derived by applying an extrapolation factor of 50 to the lowest observed acute toxicity values for fish and invertebrates (96-h LC50 of 820 µg l^{-1} for rainbow trout (*Oncorhynchus mykiss*) and a 48-h EC50 of 1000 µg l^{-1} for *Daphnia magna*).

The extrapolation factor of 50 comprised a factor of 10 (to account for extrapolation from acute effects to acute no effects) and a factor of 5 (to account for extrapolation to chronic no effects). The extrapolation factor of five was felt justified because the available toxicity data indicated that the acute/chronic ratio was small.

A maximum allowable concentration (MAC) of 100 µg l^{-1} was also proposed to protect freshwater life from episodic exposure to BBP. This appeared to be derived by applying an assessment factor of 10 to the lowest acute data (highlighted above) to account for extrapolation from acute effects to acute no effects as with the AA EQS above. The

value was derived due to uncertainty about the sensitivity of fish eggs. It was suggested that the standard be revisited when additional data were available.

The freshwater AA and MAC were also adopted as the EQSs for saltwaters.

3.4 Derivation of PNECs for sediment

BBP has a log Kow value of 4.84 which is above the TGD trigger level of 3. As such sediment standards for BBP should be derived. However, it was not possible to locate data on direct toxicity to sediment-dwelling organisms. Consequently, it was not possible, at this time, to derive a sediment PNEC.

3.5 Derivation of PNECs for secondary poisoning of predators

3.5.1 Mammalian and avian toxicity data

Several reviews have been published regarding BBP (US EPA, 1993; IARC, 1982; IARC, 1999; WHO, 1999; IUCLID, 2000; ECB, 2005; the starred studies indicate the key studies in the EU RAR). Being the most recent review available, the EU RAR has been assumed to contain the most sound and complete mammalian data. For this reason, this was the primary source used. However, the other reviews were also consulted. Additional literature searches were performed from 2004 (the date the human health section of the RAR was completed) to the present day to locate any lower effect data since 2004.

For avian data, due to the lack of relevant data in the EU RAR, the other reviews were also consulted. A comprehensive literature search was also performed from 2004 to the present day to locate any lower effect data since 2004. However, only one study was located.

Table 3.1 Most sensitive mammalian and bird oral toxicity data relevant for the assessment of secondary poisoning

Type of study, reference & result	Details
Sub-chronic toxicity to mammals	
NTP (1982) and Hammond *et al.* (1987) Cited in ECB (2005), IUCLID (2000) and WHO (1999) **Sub-chronic LOEL = 240 mg/kg bw/day (male)** **Sub-chronic NOEL = 946 mg/kg bw/day (female)**	Male and female B6C3F1 mice (number of animals per group was not stated) received BBP orally via their diet for 90 days at doses of 0, 240, 464, 946, 1875 and 3750 mg/kg bw/day. No adverse toxicological effects were observed at any dose level. However, decreased body weight gain was observed at all doses in males and at the highest dose in females. Thus the LOEL and NOEL were based on decreased body weight gain.
Piersma *et al.* (2000) Cited in ECB (2005) **Sub-chronic NOAEL = 580 mg/kg bw/day**	Young male Cpb-WU rats (3/sex/group) received BBP orally via gavage for 28 days at doses of 0, 270, 350, 450, 580, 750, 970, 1250, 1600 or 1200 mg/kg bw/day. The NOAEL was based on increased liver weight.
Hammond *et al.* (1987) Cited in ECB (2005), IUCLID (2000) and WHO (1999) **Sub-chronic NOAELs = 750 and 375 mg/kg bw/day for males and females, respectively**	Male and female Sprague-Dawley rats (10/sex/group) received BBP orally via their diet for 90 days at doses of 0 to 20 000 mg/kg diet (stated to be approximately 0, 188, 375, 750, 1125 and 1500 mg/kg bw/day). The NOAELs were based on increased liver weight.
*Hammond *et al.* (1987) Cited in ECB (2005), IUCLID (2000) and WHO (1999) **Sub-chronic NOAEL = 151 and 171 mg/kg bw/day for males and females, respectively**	Male and female Wistar rats (27-45/sex/group) received BBP orally via their diet for 90 days at doses of 0 to 12 000 mg/kg diet (stated to be approximately 0, 151, 381 and 960 mg/kg bw/day). The NOAELs were based on histopathological changes in the pancreas (cell vacuolisation, peri-islet congestion and occasionally peri-islet inflammatory cell infiltration with slight fibrosis, pycnotic nuclei, acinar atrophy and periacinar inflammatory cell infiltration), gross pathological changes in the liver (small areas of cellular necrosis) and significantly increased relative kidney weight.
Hammond *et al.* (1987) Cited in ECB (2005), IUCLID (2000) and WHO (1999) **Sub-chronic NOAELs = 1852 and 1973 mg/kg bw/day for males and females, respectively**	Male and female Beagle dogs (3/sex/group) received BBP orally via their diet for 90 days at doses of 0 to 50 000 mg/kg diet (stated to be approximately 0, 400, 1000 and 1852 mg/kg bw/day for males and 0, 700, 1270 and 1973 mg/kg bw/day for females). NB In the IUCLID, these doses are stated to be 0, 250, 500 and 1250 mg/kg bw/day. However, due to poor palatability, the high dose animals received the compound via oral capsules from day 39, with the same resultant doses. The NOAELs were the highest doses tested, due to the absence of adverse effects and the poor palatability of the diet. These higher NOAELs (as compared to those of rats and mice) could be due to the decreased absorption that has been reported in Beagle dogs.

Chronic toxicity to mammals	
NTP (1982) Cited in ECB (2005), IUCLID (2000) and WHO (1999) **Carcinogenic NOAEL = 1680 mg/kg bw/day**	Male and female B6C3F1 mice (50/sex/group) received BBP orally via their diet for 2 years at doses of 0 to 12 000 mg/kg diet (stated to be 0, 840 and 1680 mg/kg bw/day). No increased incidences of any type of cancer were observed.
NTP (1997) Cited in ECB (2005) and WHO (1999) **Systemic NOAEL = 240 mg/kg bw/day (males)** **Systemic LOAEL = 300 mg/kg bw/day (females**)	Male and female F344 rats (60/sex/group) received BBP orally via their diet for 2 years at doses of 0 to 12 000 mg/kg diet (stated to be 0, 120, 240 and 500 mg/kg bw/day) for males and 0 to 24 000 mg/kg diet (stated to be 0, 300, 600 and 1200 mg/kg bw/day) for females. NTP concluded that there was some evidence of carcinogenicity in males (due to increased incidences of pancreatic acinar cell adenoma and combined acinar call adenoma and carcinoma) and equivocal evidence of carcinogenicity in females (due to slightly increased incidences of pancreatic acinar adenoma and transitional epithelial papilloma of the urinary bladder). The systemic NOAEL was based on significantly increased relative kidney weight, while the systemic LOAEL was based on nephropathy.
NTP (1982) Cited in ECB (2005) and IUCLID (2000) **Systemic LOAEL = 360 mg/kg bw/day**	Male and female F344 rats (50/sex/group) received BBP orally via their diet for 30 weeks or 2 years at doses of 0 to 12 000 mg/kg diet (stated to be 0, 360 and 720 mg/kg bw/day). However, a significant number of males died due to unexplained internal bleeding after week 14 and so all remaining males were killed by week 30. Thus carcinogenicity could only be assessed adequately in females. The systemic LOAEL was based on decreased body weight gain in females. However, at the high doses in females, a statistically significant increased number of mononuclear cell leukaemias were observed.
A large number of studies have found that BBP is unlikely to be genotoxic (EU RAR, 2005; IUCLID, 2000).	

Effects on fertility of mammals	
*Tyl *et al.* (2004) Cited in ECB (2005) **Parental systemic NOAEL = 250 mg/kg bw/day** **Offspring reproductive NOAEL = 50 mg/kg bw/day** **Fertility NOAEL = 250 mg/kg bw/day**	In a 2 generation study, male and female CD (Sprague-Dawley) rats (40-45 days old; 30 F0 animals/sex/group) received BBP orally via their diet for 10 weeks at doses of 0, 50, 250 or 750 mg/kg bw/day. Within this period, animals were mated within dose groups for a two week period to produce the F1 offspring. F0 and F1 males were terminated following delivery of the F1 animals. Selected F1 offspring (30/sex/dose) were administered the same doses stated above for 10 weeks and then mated within dose groups for a 2 week period. Animals were terminated after delivery of the F2 generation. The parental systemic NOAEL was based on organ weight changes (increased absolute and relative liver weight and relative kidney weight) and unspecified histopathological changes in the liver. The reproductive NOAEL was based on reduced anogenital distance in the F1 and F2 generations. The fertility NOAEL was based on statistically significant reduced mating and fertility indices in the F1 parents. This study complied with GLP and the US EPA OPPTS Testing Guideline.
*Nagao *et al.* (2000) Cited in ECB (2005) **Reproductive NOAEL = 100 mg/kg bw/day (males)** **Developmental NOAEL = 20 mg/kg bw/day (both sexes)**	In a 2 generation study, male and female Sprague-Dawley rats (8 weeks old; 25 F0 animals/sex/group) received BBP orally via gavage at doses of 0, 20, 100 or 500 mg/kg bw/day. F0 males were dosed for 12 weeks before a 2 week mating period and then terminated. F0 females were dosed for 2 weeks before the 2 week mating period and terminated on post partum day 21. F1 animals were dosed after weaning (post partum day 22) until termination following delivery of F2 animals. F2 animals were terminated on post natal day 21. The reproductive NOAEL in males was based on atrophy of the testis, epididymis and seminal vesicle at 10 or 18 weeks old and reduced reproductive organ weight in F1 animals. The development NOAEL was based on reduced bodyweight in F1 offspring.
NTP (1997) Cited in ECB (2005) and WHO (1999) **Reproductive NOAEL = 20 mg/kg bw/day** **Fertility NOAEL = 200 mg/kg bw/day**	As part of the 2 year F344 rat study detailed in the chronic section, a modified mating study was also performed. Male F344 rats (15/group) received BBP orally via their diet for 10 weeks at doses of 0, 20, 200 or 2200 mg/kg bw/day. Following this exposure, two days of recovery was initiated, followed by a 7 day mating period. After mating, animals were terminated. The reproductive NOAEL was based on reduced epididymal spermatozoa concentration, while the NOAEL for fertility was based on no pregnant females following mating with the high dose males. This study complied with GLP.
Piersma *et al.* (1995) Cited in ECB (2005) and WHO (1999) **Parental reproductive NOAEL = 500 mg/kg bw/day** **Offspring NOAEL = 250 mg/kg bw/day**	Male and female RIVM bred WU rats (10/sex/group) received BBP orally via gavage for 14 days pre-mating followed by a 14 day mating period, at doses of 0, 250, 500 or 1000 mg/kg bw/day. Males were then dosed for a further day and then terminated, while females were dosed until post partum day 6 and then terminated. The reproductive

	NOAEL was based on reduced pregnancy rate, testis and epididymis weight, testicular degeneration (as well as interstitial, Leydig, cell hyperplasia) and increased cellular debris, time to conception and post implantation loss. The offspring NOAEL was based on reduced pup weight. This study complied with OECD 421 Test Guideline.
Monsanto (1993) Cited in ECB (2005) and WHO (1999) **Parental NOAEL = 206 mg/kg bw/day (males) and 217 mg/kg bw/day (females)** **Offspring reproductive & developmental NOAEL = 418 mg/kg bw/day (males) and 446 mg/kg bw/day (females**)	In a one generation study, male and female Wistar (Crl:WI (WU) BR) rats (12-24/sex/group) received BBP orally via their diet for one generation (producing 2 litters) at doses of 0, 0.2, 0.4 or 0.8% diet (the conversions were not stated). The parental NOAELs were based on increased liver weight. The offspring NOAELs were based on the absence of adverse effects and were the highest doses tested. This study complied with GLP, the EEC Annex 5 Directive 79/831/EEC and OECD Guidelines No. 415.
Effects on reproduction of mammals	
Agarwal *et al.* (1985) Cited in ECB (2005), IUCLID (2000) and WHO (1999) **Reproductive NOAEL = 625 mg/kg bw/day** **Systemic LOAEL = 312 mg/kg bw/day**	Male Fisher F344 rats (unknown group size) received BBP orally via their diet for 14 days at doses of 0, 312, 625, 1250 or 2500 mg/kg bw/day. The reproductive NOAEL was based on effects on male reproductive organs (including reduced absolute thymus, testis, epididymis, prostate and seminal vesicle weights, atrophy of the testis, prostate and seminal vesicles, immature sperm cells in the tubular lumens, necrosis of the tubular epithelium in the caput epididymis and increased follicle stimulating hormone and luteinising hormone plasma concentrations). The systemic LOAEL was based on increased relative liver and kidney weights.
Piersma *et al.* (2000) Cited in ECB (2005) **Reproductive NOAEL = 750 mg/kg bw/day** **Systemic NOAEL = 580 mg/kg bw/day**	As part of the 28 day fertility study detailed above, male Cpb-WU rats (28 days old; 3/group) received BBP orally via gavage for 28 days at doses of 0, 270, 350, 450, 580, 750, 970, 1250, 1600 or 2100 mg/kg bw/day. The reproductive NOAEL was based on effects on the reproductive organs (critical effect was testicular atrophy). The systemic NOAEL was based on increased liver weight.
NTP (1997) Cited in ECB (2005) **Systemic NOAEL = 180 mg/kg bw/day** **Fertility NOAEL = 550 mg/kg bw/day**	Male Fisher F344 rats (15/group) received BBP orally via their diet for 6 months at doses of 0, 30, 180, 550 or 1660 mg/kg bw/day. This study complied with GLP. The systemic NOAEL was based on increased cell haemoglobin concentration (possibly associated with the observed anaemia at the highest dose) and increased relative liver weight. The fertility NOAEL was based on decreased pregnancy rate and reduced epididymal spermatozoa concentration.
Aso *et al.* (2005) Cited in ECB (2005) **Parental NOAEL = <100 mg/kg bw/day** **Developmental NOAEL = <100 mg/kg bw/day**	Male and female Crj:CD (SD) IGS rats (24/group) received BBP orally via gavage for 2 generations (exact exposure period unstated) at doses of 0, 100, 200 or 400 mg/kg bw/day. The basis of the parental NOAEL was unstated. The developmental NOAEL was based on softening of the testes, diffuse atrophy of testicular seminiferous tubules,

	decreased spermatozoa and residual germ cells in the epididymal lumina.
Developmental toxicity	
NTP (1990) Cited in ECB (2005) and WHO (1999) **Maternal & developmental NOAEL = 182 mg/kg bw/day**	Timed-pregnant Swiss DC-1 mice (27-30 dams/group) received BBP orally via their diet between gestation days 6 and 15 at doses of 0, 182, 910, 2330 or 4121 mg/kg bw/day. All animals were terminated on gestation day 17. The NOAEL was based on reduced maternal body weight gain, increased foetal death and increased number of malformed foetuses.
NTP (1989) Cited in ECB (2005) **Maternal & developmental NOAEL = 419 mg/kg bw/day**	Timed-pregnant Sprague-Dawley rats (27-30 dams/group) received BBP orally via their diet between gestation days 6 and 15 at doses of 0, 419, 1102 or 1641 mg/kg bw/day. All animals were terminated on gestation day 20. The NOAEL was based on reduced maternal body weight gain and increased number of malformed foetuses.
Piersma *et al.* (2000) Cited in ECB (2005) **Maternal NOAEL = 450 mg/kg bw/day** **Developmental NOAEL = 270 mg/kg bw/day**	Pregnant and non-pregnant Cpb-WU rats (8 weeks old) received BBP orally via gavage between either gestation days 6 and 15 or gestation days 6 and 20 for pregnant animals or for 10 or 15 days for non-pregnant animals. Doses were 0, 270, 350, 450, 580, 750, 970, 1250, 1600 or 2100 mg/kg bw/day. For the pregnant animals 25 animals per group were used for doses 450, 750 and 1250 mg/kg bw/day, while 10 animals per group were used for the remaining doses. Three non-pregnant animals were used for each dose. All animals were terminated on gestation day 21. The maternal NOAEL was based on increased liver weight. The developmental NOAEL was based on reduced foetal weight.
Ema *et al.* (1991) Cited in ECB (2005) and WHO (1999) **Maternal NOAEL = 375 mg/kg bw/day** **Developmental NOAEL = 185 mg/kg bw/day**	Pregnant rats (species unspecified; 13-17 dams/group) received BBP orally via their diet between gestation days 0 and 20 at doses of 0, 185, 375, 654 or 974 mg/kg bw/day. All animals were terminated on gestation day 20. The maternal NOAEL was based on reduced maternal body weight gain. The developmental NOAEL was based on reduced number of live foetuses per litter.
Ema *et al.* (1992) Cited in ECB (2005) and IUCLID (2000) **Maternal LOAEL = 500 mg/kg bw/day** **Developmental NOAEL = 500 mg/kg bw/day**	Pregnant Wistar rats (10 dams/group) received BBP orally via gavage in corn oil between gestation days 7 and 15 at doses of 0, 500, 750 or 1000 mg/kg bw/day. All animals were terminated on gestation day 20. The maternal LOAEL was based on reduced maternal body weight gain and food consumption. The developmental NOAEL was based on decreased foetal weight, reduced number of live foetuses per litter and increased incidence of foetal malformations.
Ema *et al.* (1998) Cited in ECB (2005) Maternal LOAEL = 250 mg/kg bw/day **Developmental NOAEL = 250**	Pregnant and pseudopregnant Wistar rats (10-14 dams/group) received BBP orally via gavage at doses of 0, 250, 500, 750 or 1000 mg/kg bw/day. Pregnant animals were dosed between gestation days 0 and 8 and terminated

mg/kg bw/day **Pseudopregnant rat NOAEL = 250 mg/kg bw/day**	on gestation day 20. Pseudopregnant rats were dosed on the same gestation days and terminated on gestation day 9. The maternal LOAEL was based on reduced maternal body weight gain and food consumption. The developmental NOAEL was based on decreased foetal weight. The pseudopregnant rat NOAEL was based on decreased bodyweight gain.
Endocrine Disruption	
Several *in vitro* and *in vivo* oestrogenicity studies have been conducted on BBP. Only weak estrogenic activity at high concentrations has been reported in *in vitro* tests and no activity in *in vivo* tests. *In vitro* and *in vivo* assays have also been performed to assess anti-androgenic activity of BBP. *In vitro* it was found to have no androgenic activity, but was found to be a potent anti-androgen (as potent as the known anti-androgen flutamide). In vivo studies indicate an anti-androgen-like activity of BBP and its major metabolites.	
Toxicity to birds	
No studies were located on sub-chronic, chronic or developmental toxicity to birds.	
Neurotoxicity to birds	
Monsanto (1992) Cited in ECB (2005) **Neurotoxic NOAEL = 5000 mg/kg bw/day**	Laying hens (strain unspecified) (10/group) received either 0 mg BBP/kg bw/day, 5000 mg BBP/kg bw/day or 500 mg tri-o-tolyl phosphate/kg bw/day (positive control) for 3 consecutive days, which was then repeated 18 days later. No gross symptoms of neurotoxicity were observed in the birds administered BBP, despite symptoms occurring in the positive control group.

The draft RAR identified the NOAEL of 50 mg/kg body weight per day from the rat reproduction toxicity study of Tyl *et al.* (2004) as most suitable for the derivation of the $PNEC_{secpois.biota}$.

3.5.2 PNECs for secondary poisoning of predators

Bioconcentration data

The TGD BCF trigger of 100 in whole fish is exceeded for BBP. Consequently, there is a need to derive PNECs for secondary poisoning. The draft RAR for BBP identifies a number of bioconcentration studies, three with bluegill sunfish (*Lepomis macrochirus*) and one with Eastern oysters (*Crassostrea virginica*) (see Table 3.2).

BCF values for BBP and its metabolites in whole fish range from 188 to 663, indicating a strong potential to bioconcentrate (Table 3.1). However, the BCF values for the parent compound alone are much lower with a whole fish BCF of 12 reported for BBP alone (Carr 1992 cited in ECB 2005). Carr (1992) (cited in ECB 2005) reported that <3% of the accumulated BBP remains as the parent compound with the majority transformed to monobutyl phthalate and monobenzyl phthalate. The metabolites occur within 1-3 days of exposure. Although there are no data on the chronic toxicity of phthalate mono-esters to aquatic organisms, reproductive tests with mammals show that the mono-esters have similar effects and threshold vales to BBP (ECB 2005).

Given the metabolism of BBP and the potential for toxic effects of the mono-esters (albeit in mammalian studies) the draft RAR proposed to use BCF values relating to both, the BBP parent compound and metabolites rather than the parent compound alone. The study by Carr (1992) was deemed of appropriate quality and as the BCF value relates to ^{14}C-labelled compound it also provides a measure of the uptake of the mono-ester metabolites. Consequently, the BCF of 449 reported by Carr (1992) was used for the estimation of secondary poisoning by the RAR.

Table 3.2 Bioconcentration data for BBP

Species	Conc. (mg l^{-1})	Exposure conditions	$BCF_{whole\ fish}$ (parent compound and metabolites)	Comments	Reference
Bluegill sunfish (*Lepomis macrochirus*)	0.00296	17 d 22°C	BCF =188	BCF_{muscle}= 28 $BCF_{viscera}$= 1693 Uptake =143 d^{-1} Depuration $t_{1/2}$= 0.75 d	Heidolph and Gledhill (1979) (Cited in ECB 2005)
Bluegill sunfish (*Lepomis macrochirus*)	0.034	3 d 22°C	BCF = 449 ** (12)*	GLP Total (^{14}C)metabolite BCF_{muscle}=45 (1)* $BCF_{viscera}$=684 (19)*	Carr (1992) (Cited in ECB 2005)
Bluegill sunfish (*Lepomis macrochirus*)	0.00973	21 d 16°C	BCF =663	Depuration $t_{1/2}$= 1-2 d	Barrows *et al.* (1980) (Cited in ECB 2005)
Eastern oyster (*Crassostrea virginica*)	0.012	11 d 19.5°C	BCF =135	GLP Depuration $t_{1/2}$= 1-2 d	Springborn Laboratories (1986) (Cited in ECB 2005)

BCF Bioconcentration factor
* BCF for parent compound (BBP)
** Based on parent compound exposure rather than total radioactivity exposure

PNECs for secondary poisoning

The draft RAR identified a NOAEL of 50 mg/kg body weight per day from a rat reproduction toxicity study (Tyl *et al.* 2004) as most suitable for the derivation of the $PNEC_{secpois.biota}$. The appropriate assessment factors to derive a PNEC based on a chronic $NOAEL_{food}$ from a mammalian study are a conversion factor of 20 and an assessment factor of 30, giving:

$PNEC_{secpois.biota}$ = (NOAEL 50 mg/kg bw x 20)/AF 30 = 33.3 mg/kg in food

Reported BCF values for whole fish range from 188 to 663. However, the draft RAR identified the BCF of 449 reported by Carr (1992) as the most suitable for the estimation of secondary poisoning. Consequently, the concentration in water preventing bioaccumulation in prey to levels >$PNEC_{secpois.biota}$ can be calculated as follows:

$PNEC_{secpois.water}$ = (33.3 mg/kg prey)/BCF (449) = 0.074 mg l^{-1} (74 µg l^{-1})

This concentration is higher than the proposed long-term PNECs for the protection of pelagic communities in both inland and marine water bodies. Therefore, if quality

standards are set on the basis of the freshwater and saltwater PNECs, the protection of predators from secondary poisoning will be covered.

The same conclusion is drawn if the worst-case BCF of 663 from Barows *et al.* (1980) is used in the calculation of the $PNEC_{secpois.water}$.

4 Analysis and monitoring

BBP may be analysed by gas chromatography/mass spectrometry and by high-performance liquid chromatography. It is determined in water by an enrichment procedure using sequential reverse osmosis, followed by extraction and analysis by gas chromatography/mass spectrometry (WHO 1999).

Techniques routinely used in modern laboratories using aluminium oxide clean-up and capillary GC coupled with mass spectrometry can achieve limits of detection in the range of 0.01 to 0.02 µg l^{-1} in most environmental waters (Lewis *et al.* 1998). Detection limits of 0.05 µg l^{-1} can be achieved in sediments using fluorisil clean-up coupled with Gas Chromatography with Electron Capture Detector (GC-ECD) (Lewis *et al.* 1998).

Phthalates may be present in laboratory equipment and lead to high procedural blanks. It is recommended that all equipment is cleaned immediately prior to analysis by solvent extraction and muffling. Washing glassware in detergent does not guarantee the removal of all organic matter and muffling is recommended (Lewis *et al.* 1998).

Proposed PNECs derived for BBP range from 0.75 to 50 µg l^{-1} in environmental waters. The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. Using this criterion, it is evident that current analytical methodologies should offer adequate performance to analyse for BBP.

5 Conclusions

5.1 Availability of data

Long-term freshwater toxicity data are available for five taxonomic groups including algae, bacteria, ciliates, crustaceans and fish. Freshwater short-term toxicity data are available for seven taxonomic groups including algae, bacteria, crustaceans, fish, hydroids, insects and molluscs. Long-term saltwater toxicity data are available for three taxonomic groups: algae, crustaceans and fish. Short-term saltwater toxicity data are available for five different taxonomic groups: algae, annelids, crustaceans, fish and molluscs. Based on the available data there appears to be little difference between trophic levels in the sensitivity of organisms to BBP. In addition there are no significant differences in the sensitivity of freshwater or saltwater species of the same taxonomic group.

There were no fresh or saltwater field or mesocosm data available for BBP.

BBP may have both oestrogenic and anti-androgenic effects. However, effects *in vivo* tend to occur at higher concentrations where effects such as mortality may be observed in some species using standard tests. In addition there is still some uncertainty about long-term effects and the issue of parent offspring transfer. Until these issues are resolved it is not possible to incorporate ED effects into the PNEC derivation.

5.2 Derivation of PNECs

The proposed PNECS are described below and summarised in Table 5.1.

5.2.1 Long-term PNEC for freshwaters

Long-term data are available for five taxonomic groups including algae, bacteria, ciliates, crustaceans, fish. The lowest valid long-term freshwater data point was a 30-day NOEC (growth/length) of 0.14 mg l^{-1} for the fathead minnow (*Pimephales promelas*). However, given the similarity in effects in both fresh and saltwater the EU RAR combined the two data sets. In saltwater a 28-day NOEC (reproduction/growth) of 0.075 mg l^{-1} was reported for the mysid shrimp (*Mysidopsis bahia*). This datum was generated in a GLP study under flow through conditions and was regarded by the RAR as fully valid for PNEC derivation.

The long-term freshwater PNEC in the EU RAR for BBP was therefore based on the 28 day NOEC (for reproduction and growth) of 0.075 mg l^{-1} reported for the mysid shrimp (*Mysidopsis bahia*) and an assessment factor of 10 applied, because of the availability of long-term data for three trophic levels and the similarity in sensitivity of trophic levels, resulting in a **$PNEC_{freshwater\_lt}$ of 0.0075 mg l^{-1} (7.5 µg l^{-1}) BBP.**

This value is slightly lower than the existing EQS of 20 µg l^{-1} derived by applying an extrapolation factor of 50 (10 to account for extrapolation from acute effects to acute no effects and a factor of 5 to account for extrapolation to chronic no effects) to the lowest observed acute toxicity values for fish and invertebrates available at the time the EQS

was derived, namely a 96-h LC50 of 0.82 mg l^{-1} for rainbow trout (*Oncorhynchus mykiss*) and a 48-h EC50 of 1.0 mg l^{-1} for *Daphnia magna*.

5.2.2 Short-term PNEC for freshwaters

Freshwater short-term toxicity data are available for seven taxonomic groups including algae, bacteria, crustaceans, fish, insects, hydra and molluscs. The lowest valid short-term freshwater data point was a 72-hour EC50 (growth rate) of 0.64 mg l^{-1} for the alga *Navicula pelliculosa*. However, given the similarity in effects in both fresh and saltwaters the EU RAR combined the two data sets. In saltwater a 96 hour LC50 of 0.51 mg l^{-1} was reported for the shiner perch (*Cymatogaster aggregata*). This datum was generated under flow-through conditions with measured exposure concentrations and was regarded, by the RAR, as fully valid for PNEC derivation.

Although short-term critical data were identified in the RAR, EU RARs do not usually derive intermittent (short-term) PNECs. Consequently, no short-term RAR PNEC was available to be adopted as the EQS. Therefore, a short-term PNEC was derived in this report by applying an assessment factor of 10 to the lowest available datum, because of the availability of reliable short-term data for at least three trophic levels and the similarity in sensitivity of trophic levels resulting in a **$PNEC_{freshwater\_st}$ of 0.051 mg l^{-1} (51 µg l^{-1}) BBP.**

This value is slightly lower than the existing maximum allowable concentration (MAC) of 100 µg l^{-1} proposed to protect freshwater life from episodic exposure to BBP. The MAC was derived by applying an assessment factor of 10 to the lowest acute data (highlighted in the above long-term section) to account for extrapolation from acute effects to acute no effects.

5.2.3 Long-term PNEC for saltwaters

Long-term saltwater data for BBP were available for the 'base set' of organisms (algae, invertebrates and fish). The long-term saltwater PNEC in the EU RAR for BBP was based on the 28-day NOEC (for reproduction and growth) of 0.075 mg l^{-1} reported for the mysid shrimp (*Mysidopsis bahia*). An assessment factor of 100 was proposed by the EU RAR. A larger assessment factor (than used for the freshwater PNEC) was adopted due to a lack of data for exclusively marine organisms such as echinoderms and molluscs, resulting in a **$PNEC_{saltwater\_lt}$ of 0.00075 mg l^{-1} (0.75 µg l^{-1}) BBP.**

This value is lower than the existing EQS of 20 µg l^{-1}, which was 'read-across' from the freshwater long-term value.

5.2.4 Short-term PNEC for saltwaters

No short-term saltwater PNEC was derived in the EU RAR for BBP. However, the lowest short-term critical data were identified in the RAR. The lowest reliable data point in the combined data set was the 96-hour LC50 of 0.51 mg l^{-1} reported for the shiner perch. An assessment factor of 50 is proposed, because in addition to reliable short-term data for at least three trophic levels, there are short-term data to show that one additional marine taxonomic group (molluscs) is no more sensitive than algae, crustaceans and fish. The use of such an assessment factor with the short-term saltwater data is in line with the guidance within the EU TGD (ECB (2003). This results in a **$PNEC_{saltwater\_st}$ of 0.010 mg l^{-1} (10 µg l^{-1}) BBP.**

This value is lower than the existing maximum allowable concentration (MAC) of 100 µg l^{-1}, which was 'read-across' from the freshwater short-term value.

5.2.5 PNEC for sediments

BBP has a log Kow value of 4.84 which is above the TGD trigger level of 3. As such sediment standards for BBP should be derived. However, it was not possible to locate data on the direct toxicity of BBP to sediment-dwelling organisms. Consequently, it was not possible, at this time, to derive a sediment PNEC.

5.2.6 PNEC for secondary poisoning

The draft RAR identified the NOAEL of 50 mg/kg bw from a rat reproduction toxicity study as most suitable for derivation of $PNEC_{secpois.biota}$. The appropriate assessment factors to derive a PNEC based on a chronic $NOAEL_{food}$ from a mammalian study are a conversion factor of 20 and an assessment factor of 30 resulting in a **$PNEC_{secpois.biota}$ of 33.3 mg/kg BBP in food**

Reported BCF values for whole fish range from 188 to 663. However, the draft RAR identified a BCF of 449 in Bluegill sunfish (*Lepomis macrochirus*) as the most suitable for the estimation of secondary poisoning. Consequently, the concentration in water preventing bioaccumulation in prey to levels >$PNEC_{secpois.biota}$ is a **$PNEC_{secpois.water}$ of 0.074 mg l^{-1} (74 µg l^{-1}) BBP.**

This concentration is higher than the proposed long-term PNECs for the protection of pelagic communities in both inland and marine water bodies. Therefore, if quality standards are set on the basis of the freshwater and saltwater PNECs, the protection of predators from secondary poisoning will be covered.

The same conclusion is drawn if the worst-case BCF of 663 is used in the calculation of the $PNEC_{secpois.water}$.

Table 5.1 Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC (µg l^{-1})	Existing EQS (µg l^{-1})
Freshwater/long-term	7.5	20 (AA)
Freshwater/short-term	51	100 (MAC)
Saltwater/long-term	0.75	20 (AA)
Saltwater/short-term	10	100 (MAC)
Sediment	-	–
Secondary poisoning	74	–

AA = Annual Average
MAC =Maximum Allowable Concentration

5.3 Analysis

BBP may be analysed by gas chromatography/mass spectrometry and by high-performance liquid chromatography.

Proposed PNECs derived for BBP range from 0.75 to 51 µg l^{-1} in environmental waters. The data quality requirements are that, at a third of the EQS total error of measurement should not exceed 50 per cent. Using this criterion, current analytical methodologies should offer adequate performance to analyse for BBP

5.4 Implementation issues

Based on consideration of the information collated within the report and the proposed PNECs the following comments are made re: implementation:-

- The current analytical capability should be adequate for compliance assessment
- The proposed PNECs are consistent with those proposed in the EU Risk Assessment Report.
- The PNECs are suitable for use as EQSs as they are not subject to excessive uncertainty.

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List of abbreviations

AA	Annual Average
AF	Assessment Factor
a.i.	active ingredient
BCF	Bioconcentration factor
BBP	Benzyl Butyl Phthalate
bw	body weight
CAS	Chemical Abstracts Service
DO	Dissolved Oxygen
EC50	Concentration effective against 50 per cent of the organisms or animals tested
ECD	Electron Capture Detection
EHC	Environmental Health Criteria
EQS	Environmental Quality Standard
GC	Gas Chromatography
GLP	Good Laboratory Practice (OECD)
LC50	Concentration lethal to 50 per cent of the organisms or animals tested
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
lt	long-term
MAC	Maximum Allowable Concentration
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
OECD	Organization for Economic Co-operation and Development
PNEC	Predicted No-Effect Concentration
SSD	Species Sensitivity Distribution
RAR	Risk Assessment Report
st	short-term
TGD	Technical Guidance Document
UKTAG	UK Technical Advisory Group
US EPA	US Environmental Protection Agency
WFD	Water Framework Directive

ANNEX 1 Data quality assessment sheets

Identified and ordered by alphabetical order of references.

Data relevant for PNEC derivation were quality assessed in accordance with the so-called Klimisch Criteria (Table A1).

Table A1 Klimisch Criteria*

Code	Category	Description
1	Reliable without restrictions	Refers to studies/data carried out or generated according to internationally accepted testing-guidelines (preferably GLP**) or in which the test parameters documented are based on a specific (national) testing guideline (preferably GLP), or in which all parameters described are closely related/comparable to a guideline method.
2	Reliable with restrictions	Studies or data (mostly not performed according to GLP) in which the test parameters documented do not comply totally with the specific testing guideline, but are sufficient to accept the data or in which investigations are described that cannot be subsumed under a testing guideline, but which are nevertheless well-documented and scientifically acceptable.
3	Not reliable	Studies/data in which there are interferences between the measuring system and the test substance, or in which organisms/test systems were used that are not relevant in relation to exposure, or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for an assessment and which is not convincing for an expert assessment.
4	Not assignable	Studies or data which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature.

* Klimisch H.-J, Andreae M and Tillmann U, 1997 A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*, **25**, 1–5.
** OECD Principles of Good Laboratory Practice (GLP). See: http://www.oecd.org/department/0,2688,en_2649_34381_1_1_1_1_1,00.html

Author	Adams *et al.* 1995

Information on the test species	
Test species used	*Selenastrum capricornutum*
Source of the test organisms	Not stated
Holding conditions prior to test	Acclimated to appropriate temperature before testing
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Method closely followed EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians and complied with US, EEC, OECD guidelines and EPA GLP
Form of the test substance	Colourless liquid, purity ≥95%. Commercially available batch
Source of the test substance	Manufacturer
Type and source of the exposure medium	Injected directly into the test water and homogenised for two minutes before test organisms were added.
Test concentrations used	Not stated
Number of replicates per concentration	2
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static
Measurement of exposure concentrations	Measured concentrations (50% of nominal at end of test)
Measurement of water quality parameters	Diluent water measured at beginning of test. Temperature was measured at intervals of 0, 24, 48, 72 and 96 hours in control only.
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Complied with US EEC ,OECD guidelines and EPA GLP
Overall comment on quality	Conducted according to guidelines, but some study details lacking. In addition the results are based on cell count and the duration of the experiment (6 days) mean that the data are not comparable with OECD methods. The EU RAR has stated that studies of such long durations are not valid.

Reliability of study	**Unreliable**
Relevance of study	**Relevant**
Klimisch Code	**3 (Not Reliable)**

Author	Adams *et al.* 1995

Information on the test species	
Test species used	*Daphnia magna* *Paratanytarsus parthenogenetica* *Mysidopsis bahia*
Source of the test organisms	Not stated
Holding conditions prior to test	Acclimated to appropriate temperature before testing
Life stage of the test species used	<24 hours old Second to third instar <24 hours old

Information on the test design	
Methodology used	Method closely followed EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians and complied with US, EEC, OECD guidelines and EPA GLP
Form of the test substance	Colourless liquid, purity ≥95%. Commercially available batch
Source of the test substance	Manufacturer
Type and source of the exposure medium	Injected directly into the test water and homogenised for two minutes before test organisms were added.
Test concentrations used	5 and a control
Number of replicates per concentration	2
Number of organisms per replicate	10
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static
Measurement of exposure concentrations	Measured concentrations (50% of nominal at end of test)
Measurement of water quality parameters	Hardness, alkalinity, DO and pH measured at beginning of test. Temperature and DO were measured at intervals of 0, 24, 48 hours
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Complied with US EEC ,OECD guidelines and EPA GLP
Overall comment on quality	Conducted according to guidelines and with reasonable description of methods and results. Based on available data the study appears to be of high quality

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1 (Reliable without restriction)**

Author	Adams *et al.* 1995
Information on the test species	
Test species used	*Lepomis macrochirus*
Source of the test organisms	Not stated
Holding conditions prior to test	Acclimated to appropriate temperature prior to testing. Fish were observed for 14 days before testing.
Life stage of the test species used	Juvenile, 29-40 mm in length
Information on the test design	
Methodology used	Method closely followed EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians and complied with US, EEC, OECD guideline 203 and EPA GLP
Form of the test substance	Colourless liquid, purity ≥95%. Commercially available batch
Source of the test substance	Manufacturer
Type and source of the exposure medium	Diluted in solvent. Solvent concentration did not exceed 0.1 ml/l.
Test concentrations used	5 and a control
Number of replicates per concentration	2
Number of organisms per replicate	10
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static
Measurement of exposure concentrations	Measured concentration (50% of nominal at end of test)
Measurement of water quality parameters	Diluent water measured at beginning of test. Dissolved oxygen and pH was measured at intervals of 0, 24, 48, 72 and 96 hours in all tests. Temperature was measured at the same intervals in the control samples only.
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Yes GLP
Overall comment on quality	Generally good quality and valid for use. RAR also reported study results as valid for PNEC derivation.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR Valid)**

Author	Adams *et al.* 1995

Information on the test species	
Test species used	*Oncorhynchus mykiss*
Source of the test organisms	Not stated
Holding conditions prior to test	Acclimated to appropriate temperature prior to testing. Fish were observed for 14 days before testing.
Life stage of the test species used	Juvenile, 39-62 mm in length

Information on the test design	
Methodology used	Method closely followed EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians and complied with US, EEC, OECD guidelines and EPA GLP
Form of the test substance	Colourless liquid, purity ≥95%. Commercially available batch
Source of the test substance	Manufacturer
Type and source of the exposure medium	The diluter system was modified to incorporate a mixing chamber with a sonicator and magnetic stirrer into which the test chemical was directly injected. Stock solutions were carefully prepared to ensure no surface film was transferred to the test solution.
Test concentrations used	5 and a control
Number of replicates per concentration	2
Number of organisms per replicate	10
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Measured concentration (Concs. remained constant)
Measurement of water quality parameters	Diluent water measured at beginning of test. Dissolved oxygen and pH was measured at intervals of 0, 24, 48, 72 and 96 hours in all tests. Temperature was measured at the same intervals in the control samples only.
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Yes GLP
Overall comment on quality	Generally good quality and valid for use. RAR also reported study results as valid for PNEC derivation.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR Valid)**

Author	Adams *et al.* 1995

Information on the test species	
Test species used	*Pimephales promelas*
Source of the test organisms	Not stated
Holding conditions prior to test	Acclimated to appropriate temperature prior to testing. Fish were observed for 14 days before testing.
Life stage of the test species used	Juvenile, 29-44 mm in length

Information on the test design	
Methodology used	Method closely followed EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians and complied with US, EEC, OECD guidelines and EPA GLP
Form of the test substance	Colourless liquid, purity ≥95%. Commercially available batch
Source of the test substance	Manufacturer
Type and source of the exposure medium	The diluter system was modified to incorporate a mixing chamber with a sonicator and magnetic stirrer into which the test chemical was directly injected. Stock solutions were carefully prepared to ensure no surface film was transferred to the test solution.
Test concentrations used	5 and a control
Number of replicates per concentration	2
Number of organisms per replicate	10
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static
Measurement of exposure concentrations	Measured concentration (Concs. remained constant)
Measurement of water quality parameters	Diluent water measured at beginning of test. Dissolved oxygen and pH was measured at intervals of 0, 24, 48, 72 and 96 hours in all tests. Temperature was measured at the same intervals in the control samples only.
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Yes GLP
Overall comment on quality	Generally good quality and valid for use. RAR also reported study results as valid for PNEC derivation.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR Valid)**

Author	Adams *et al.* 1995

Information on the test species	
Test species used	*Cyprinodon variegatus*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Juvenile, 6-17 mm.

Information on the test design	
Methodology used	Method closely followed EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians and complied with US, EEC, OECD guidelines and EPA GLP
Form of the test substance	Colourless liquid, purity ≥95%. Commercially available batch
Source of the test substance	Manufacturer
Type and source of the exposure medium	Injected directly into the test water and homogenised for two minutes before test organisms were added.
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static
Measurement of exposure concentrations	Measured concentration.
Measurement of water quality parameters	Dilutent water measured at beginning of test. Dissolved oxygen and pH was measured at intervals of 0, 24, 48, 72 and 96 hours in all tests. Temperature was measured at the same intervals in the control samples only.
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Yes GLP
Overall comment on quality	Generally good quality and valid for use.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1 (Reliable without restriction)**

Author	Barera and Adams 1981

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	Obtained from laboratory cultures of parthenogenetic females
Holding conditions prior to test	Culturing technique involved placing 10 *Daphnia* in 1l battery jars with 500 ml well water, 20 mg l^{-1} Purina trout chow (PR-11) and water mixture and approximately 0.5 mg l^{-1} *Selenastrum capricornutum*. Daphnids were exposed to a 8:16 hour light:dark cycle using fluorescent lights. The cultures wed fed daily and water was renewed three times/week.
Life stage of the test species used	<24 hours

Information on the test design	
Methodology used	ASTM Standard E 729-80 with minor modifications
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	3
Number of organisms per replicate	10
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static
Measurement of exposure concentrations	Nominal concentrations
Measurement of water quality parameters	Temperature. Dissolved oxygen, alkalinity, hardness, conductivity and pH were monitored
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Recommended dilution water standards (ASTM Standard E729-80) was met
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Some details lacking, but conforms to guideline testing procedures. Although results are based on nominal concentrations the EU RAR states that the study is valid for PNEC derivation

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR valid)**

Author	Carolina Ecotox 1995

Information on the test species	
Test species used	*Navicula pelliculosa*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	OECD 201
Form of the test substance	Not stated, but expected to follow OECD method
Source of the test substance	Not stated, but expected to follow OECD method
Type and source of the exposure medium	Not stated, but expected to follow OECD method
Test concentrations used	0.075-2.4 mg l^{-1}
Number of replicates per concentration	Not stated, but expected to follow OECD method
Number of organisms per replicate	Not stated, but expected to follow OECD method
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Not stated, but expected to follow OECD method
Measurement of exposure concentrations	Measured concentrations (34-69% of nominal after 72 hours)
Measurement of water quality parameters	Not stated, but expected to follow OECD method
Test validity criteria satisfied	Not stated, but expected to follow OECD method
Water quality criteria satisfied	Not stated, but expected to follow OECD method
Study conducted to Good Laboratory Practice	Yes GLP
Overall comment on quality	The results of this study were reported to be fully valid by the EU RAR

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR valid)**

Author	Carolina Ecotox 1995

Information on the test species	
Test species used	*Scenedesmus subspicatus*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	OECD 201
Form of the test substance	Not stated, but expected to follow OECD method
Source of the test substance	Not stated, but expected to follow OECD method
Type and source of the exposure medium	Not stated, but expected to follow OECD method
Test concentrations used	0.075-2.4 mg l^{-1}
Number of replicates per concentration	Not stated, but expected to follow OECD method
Number of organisms per replicate	Not stated, but expected to follow OECD method
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Not stated, but expected to follow OECD method
Measurement of exposure concentrations	Measured concentrations
Measurement of water quality parameters	Not stated, but expected to follow OECD method
Test validity criteria satisfied	Not stated, but expected to follow OECD method
Water quality criteria satisfied	Not stated, but expected to follow OECD method
Study conducted to Good Laboratory Practice	Yes GLP
Overall comment on quality	The results of this study were reported to be fully valid by the EU RAR

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR valid)**

Author	EG&G Bionomics 1978

Information on the test species	
Test species used	*Selenastrum capricornutum, Mycrocystis aeruginosa, Navicula pelliculosa, Skeletonema costatum and Dunaliella tertiolecta*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	US EPA 1971 guidelines for testing with freshwater algae
Form of the test substance	Commercial grade BBP
Source of the test substance	Monsanto company
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static
Measurement of exposure concentrations	Nominal
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Few study details with which to assess study. The EU RAR states that due to the exposure time (96 hours) and the fact that results are based on cell count the study should be treated with caution

Reliability of study	**Questionable reliability**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR 'use with care')**

Author	EG&G Bionomics 1981

Information on the test species	
Test species used	*Pimephales promelas*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not stated
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	0.02, 0.03, 0.07, 0.14 and 0.36 mg l^{-1}
Number of replicates per concentration	Not stated
Number of organisms per replicate	120 embryos at each concentration
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Measured
Measurement of water quality parameters	Yes temperature
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Only limited data were available with which to assess the study, but the EU RAR stated that there was no factor that made the results unsuitable for PNEC derivation

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR valid)**

Author	Gledhill *et al.* 1980

Information on the test species	
Test species used	*Mysidopisis bahia*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	US EPA 1975
Form of the test substance	Commercial grade BBP. Pesticide grade organic solvents were used for all extractions and dilutions
Source of the test substance	Monsanto
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or Flow-through, duration, feeding)	Not stated
Measurement of exposure concentrations	Measured concentration
Measurement of water quality parameters	Temperature and salinity of water are reported, but frequency of testing is not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Moderate. Many study details lacking, but the study was run using a standard method.

Reliability of study	**Moderate reliability**
Relevance of study	**Relevant**
Klimisch Code	**2 (reliable with restriction)**

Author	HLS (Huntingdon Life Sciences) 2000

Information on the test species	
Test species used	*Navicula pelliculosa*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	OECD 201
Form of the test substance	Not stated, but expected to follow OECD method
Source of the test substance	Not stated, but expected to follow OECD method
Type and source of the exposure medium	Not stated, but expected to follow OECD method
Test concentrations used	Six (0.046, 0.1, 0.22, 0.46, 1 and 2.2 mg l^{-1})
Number of replicates per concentration	3
Number of organisms per replicate	10^4 cells ml^{-1}
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static
Measurement of exposure concentrations	Measured concentrations
Measurement of water quality parameters	Not stated, but expected to follow OECD method
Test validity criteria satisfied	Not stated, but expected to follow OECD method
Water quality criteria satisfied	Not stated, but expected to follow OECD method
Study conducted to Good Laboratory Practice	Yes GLP
Overall comment on quality	These studies were first conducted with non-standard cell numbers (too high). They were then re-run at the request of the RAR with the correct cell densities. The results were regarded as fully valid by the RAR.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR valid)**

Author	HLS (Huntingdon Life Sciences) 2000

Information on the test species	
Test species used	*Scenedesmus subspicatus*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	OECD 201
Form of the test substance	Not stated, but expected to follow OECD method
Source of the test substance	Not stated, but expected to follow OECD method
Type and source of the exposure medium	Not stated, but expected to follow OECD method
Test concentrations used	Six (0.1, 0.22, 0.46, 2.2 and 4.6 mg l^{-1})
Number of replicates per concentration	3
Number of organisms per replicate	10^4 cells ml^{-1}
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static
Measurement of exposure concentrations	Measured concentrations
Measurement of water quality parameters	Not stated, but expected to follow OECD method
Test validity criteria satisfied	Not stated, but expected to follow OECD method
Water quality criteria satisfied	Not stated, but expected to follow OECD method
Study conducted to Good Laboratory Practice	Yes GLP
Overall comment on quality	These studies were first conducted with non-standard cell numbers (too high). They were then re-run at the request of the RAR with the correct cell densities. The results were regarded as fully valid by the RAR.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR valid)**

Author	Monsanto (1986)

Information on the test species	
Test species used	*Hexagenia limbata*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not stated
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Measured
Measurement of water quality parameters	Measured
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	This study was conducted in-house by Monsanto. There are very few details with which to assess the quality of the study

Reliability of study	**Unknown**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR not assignable)**

Author	Monsanto 1984

Information on the test species	
Test species used	*Daphnia magna* *Chironomus tentans*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	ASTM guidelines
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static
Measurement of exposure concentrations	Nominal
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	There are few details with which to assess this study. However, the EU RAR classified the results as valid for PNEC derivation

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR Valid)**

Author	Monsanto 1982

Information on the test species	
Test species used	*Chironomus tentans*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	US EPA 1975 guidelines
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static
Measurement of exposure concentrations	Nominal
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Yes GLP
Overall comment on quality	There are few details with which to assess this study. However, the EU RAR classified the results as valid for PNEC derivation

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR Valid)**

Author	Monsanto (EG&G Bionomics 1979)

Information on the test species	
Test species used	*Lepomis macrochirus*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	US EPA 1975 guidelines
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static
Measurement of exposure concentrations	Nominal
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Yes GLP
Overall comment on quality	Very few details with which to assess study. EU RAR states that tests solutions were cloudy or had a film or both and as such the tests should be used with caution

Reliability of study	**Questionable reliability**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR 'use with care')**

Author	Monsanto 1985

Information on the test species	
Test species used	*Selenastrum capricornutum*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not stated
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Not stated
Measurement of exposure concentrations	Nominal
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Very few details presented with which to assess this study. The EU RAR states that due to the study duration (14 days) the study is not valid

Reliability of study	**Unreliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR not valid)**

Author	Monsanto 1983

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Performed according to an in-house method – Environmental Science Method ES-80-M-42
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Range form 0.12-2.0 mg l^{-1}
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Semi-static
Measurement of exposure concentrations	Measured concentration (new stock solutions were 70-100% of nominal, but concs. reduced to <0.06 mg l^{-1} by the end of the test)
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Yes GLP
Overall comment on quality	The EU RAR states that this study is fully valid

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR Valid)**

Author	Monsanto 1985

Information on the test species	
Test species used	*Selenastrum capricornutum*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not stated
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Not stated
Measurement of exposure concentrations	Nominal
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Very few details presented with which to assess this study. The EU RAR states that due to the study duration (96 hours) the study should be used with care.

Reliability of study	**Questionable Reliability**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR 'use with care')**

Author	Monsanto 1986

Information on the test species	
Test species used	*Mysidopisis bahia*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	ASTM 1985
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	0.031-0.5 mg l^{-1}
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Measured (63-150% of nominal)
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Yes GLP
Overall comment on quality	Although few experimental details are presented in the RAR this study is classified as fully valid for PNEC derivation

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR Valid)**

Author	Ozretich *et al.* 1983

Information on the test species	
Test species used	*Cymastogaster aggregata*
Source of the test organisms	Field collected (Yaquina Bay, Oregon, USA)
Holding conditions prior to test	Quarantined for one week. Healthy fish were transferred to holding tanks for four weeks followed by a transfer to an acclimation room for at least one week prior to experimentation. Raw seawater was used during quarantine and holding, while sand-filtered and UV-sterilised water was used during acclimation. Dissolved oxygen concentrations were maintained at greater than 80% saturation by aeration. The quarantine/holding area was sunlit, while the acclimation room was on a 16:8 hour light:dark cycle with no transition. The salinity of the water was between 30-34%.
Life stage of the test species used	Juveniles

Information on the test design	
Methodology used	ASTM Standard Practices
Form of the test substance	98% purity
Source of the test substance	Pflatz and Bauer, Stamford CN, catalogue #B27750
Type and source of the exposure medium	Solutions of benzyl butyl phthalate in 95% ethanol were pumped into water streams at nominal flow rates of 0.05 ml/min.
Test concentrations used	Five concentrations plus a sixth tank containing seawater with the ethanol solvent carrier solvent serving as a control.
Number of replicates per concentration	Not stated
Number of organisms per replicate	20
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Measured concentration (97% recovery)
Measurement of water quality parameters	Yes, at a frequency recommended by ASTM
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Good documentation of holding conditions and assay procedures. With measured exposure concs. The test appears valid for PNEC derivation. The EU RAR also reported these results to be valid

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR Valid)**

Author	Randall *et al.* 1983

Information on the test species	
Test species used	*Paraphrys vetulus*
Source of the test organisms	Field collected (Yaquina Bay Estuary)
Holding conditions prior to test	Held in aerated flowing seawater (25-32%) holding tanks. After 1 week, fish were treated with 0.25% formalin solution for 1 hour to remove Gyrodactylus parasites and moved to 270 l glass aquaria with aerated seawater flow rates of at least three turnovers/day. Fish were maintained on an artificial diet of 25% squid, 22.5% spinach, 25% beef liver, 2% cod liver oil, 25% wheat germ and 0.5% Nopstress vitamin mix for a minimum of three months prior to the test.
Life stage of the test species used	Juvenile

Information on the test design	
Methodology used	Followed ASTM procedure, except for the absence of bottom substrate.
Form of the test substance	Dissolved in 95% EtOH and added directly to the tank via a circulation pump intake. Alcohol concentrations in the test solution and solvent control never exceeded 0.5 ml/l.
Source of the test substance	Pfaltz & Bauer, Inc., Stamford, CT. Product code B-27750
Type and source of the exposure medium	Sterilised filtered seawater
Test concentrations used	Nominal concentrations of 0, 0.13, 0.216, 0.36, 0.60 and 1 mg l^{-1}.
Number of replicates per concentration	Not stated
Number of organisms per replicate	20
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Flow-through and static
Measurement of exposure concentrations	Measured concentration. (Mean recovery of BBP was 98 +/- 7.1%) (but results reported as nominal)
Measurement of water quality parameters	Water quality is stated, but frequency of testing is not stated.
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	Generally well documented study, but a few details are lacking. The EU RAR States that the results should be used with caution
Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR 'use with care')**

Author	Rhodes *et al.* 1995

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	Springborn Laboratories
Holding conditions prior to test	Acclimated to appropriate temperature before test.
Life stage of the test species used	<24 hours

Information on the test design	
Methodology used	No method specified, but protocol well documented.
Form of the test substance	Colourless liquid, purity ≥95%
Source of the test substance	Chemical Manufacturers Association (CMA) Phthalate Ester Panel
Type and source of the exposure medium	Not stated
Test concentrations used	0.073 (0.03), 0.23 (0.09), 0.28 (0.23), 1.4 (0.3) and 2.4 (0.4) mg l^{-1} (measured concentrations, SD in brackets)
Number of replicates per concentration	4
Number of organisms per replicate	20
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Measured concentrations
Measurement of water quality parameters	Dissolved oxygen and temperature were measured every weekday within one replicate from each treatment level and the control. Total hardness, alkalinity, specific conductivity and pH were monitored weekly within one replicate from each treatment level and the control.
Test validity criteria satisfied	Methods of analysis were within acceptable limits. Exposure levels were constant and within acceptable limits
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Yes GLP
Overall comment on quality	Well documented methodology and results. Measured concentrations. EU RAR reported study as fully valid

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR Valid)**

Author	Rhodes *et al.* 1995

Information on the test species	
Test species used	*Oncorhynchus mykiss*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Eyed embryos

Information on the test design	
Methodology used	Closely followed those described in US EPA – Toxic Substances Control Act (EPA-TSCA) 40 CRF, Part 797.1600 as modified in Testing Consent Agreement 40 CRF, Part 799 and ASTM Standard Guideline for Conducting Early Life-Stage Toxicity Tests with Fishes
Form of the test substance	Colourless liquid, purity ≥95%
Source of the test substance	Chemical Manufacturers Association (CMA) Phthalate Ester Panel
Type and source of the exposure medium	Not stated
Test concentrations used	0.012 (0.0046), 0.021 (0.0058), 0.044 (0.0091), 0.095 (0.017) and 0.020 (0.036) mg l^{-1} (mean measured concentrations, SD in brackets) and vehicle blank receiving solvent.
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Measured concentrations
Measurement of water quality parameters	Measurements of water quality were made, but frequency at which they were checked is not stated.
Test validity criteria satisfied	
Water quality criteria satisfied	On a few occasions, minor temporary excursions were noted in temperature
Study conducted to Good Laboratory Practice	Yes
Overall comment on quality	Conducted in agreement with EPA methodology and to GLP. No-effects were reported at any concentration so NOEC >0.02 mg l^{-1}. Study reported as valid in EU RAR.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR Valid)**

Author	Springborn Bionomics 1987

Information on the test species	
Test species used	*Mysidopsis bahia*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	US EPA/OTS 1985
Form of the test substance	BBP dissolved in acetone
Source of the test substance	Not stated
Type and source of the exposure medium	Filtered sea water
Test concentrations used	0.13-2 mg l^{-1}
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Measured (<40% of nominal)
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Yes GLP
Overall comment on quality	EU RAR reported that the results of this study are valid for PNEC derivation

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR Valid)**

Author	Springborn Bionomics 1986

Information on the test species	
Test species used	*Paleomonetes vulgaris*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	ASTM 1985
Form of the test substance	BBP dissolved in acetone
Source of the test substance	Not stated
Type and source of the exposure medium	Filtered sea water
Test concentrations used	0.52-2.9 mg l^{-1}
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Measured (63-93% of nominal)
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Yes GLP
Overall comment on quality	EU RAR reported that the results of this study are valid for PNEC derivation

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR Valid)**

Author	Springborn Bionomics 1986

Information on the test species	
Test species used	*Crassostrea virginica*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	US EPA 1985
Form of the test substance	BBP dissolved in acetone
Source of the test substance	Not stated
Type and source of the exposure medium	Filtered sea water
Test concentrations used	0.28-2.9 mg l^{-1}
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Measured (42-68% of nominal)
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Yes GLP
Overall comment on quality	EU RAR reported that the results of this study are valid for PNEC derivation

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR Valid)**

Author	Springborn Bionomics 1986

Information on the test species	
Test species used	*Nereis virens*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	ASTM 1985
Form of the test substance	BBP dissolved in acetone
Source of the test substance	Not stated
Type and source of the exposure medium	Filtered sea water
Test concentrations used	0.31-3 mg l^{-1}
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Measured (60-100% of nominal)
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Yes GLP
Overall comment on quality	EU RAR reported that the results of this study are valid for PNEC derivation

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR Valid)**

Author	Suggatt and Foote 1981

Information on the test species	
Test species used	*Selenastrum capricornutum* *Skeletonema costatum*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not stated
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Not stated
Measurement of exposure concentrations	Nominal
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	There are very few details with which to assess the study. The study duration is non-standard and the EU RAR states that algal studies presented in the paper are primitive. Consequently the EU RAR stated that the results are not of suitable quality for PNEC derivation

Reliability of study	**Unreliable**
Relevance of study	**Relevant**
Klimisch Code	**(EU RAR not valid)**

Author	U.S. Environmental Protection Agency 1978

Information on the test species	
Test species used	*Pseudokirchneriella subcapitata*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not stated
Form of the test substance	Formulation
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	0.02-0.25 mg l^{-1}
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Not stated
Measurement of exposure concentrations	Nominal
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to Good Laboratory Practice	Not stated
Overall comment on quality	There is a lack of data with which to assess this study. There was no chemical analysis and the study duration was non-standard (i.e. 96 h and not 72 h as in OECD 201)

Reliability of study	**Unknown**
Relevance of study	**Relevant**
Klimisch Code	**4 (Not assignable)**