Common Implementation Strategy for the Water Framework Directive

Environmental Quality Standards (EQS)

Substance Data Sheet

Priority Substance No. 24

4-Nonylphenol (branched) and Nonylphenol

CAS-No. 84852-15-3 and 25154-52-3

Final version
Brussels, 31 July 2005

Disclaimer

This data sheet provides background information on the setting of the Environmental Quality Standard in accordance with Article 16 of the Water Framework Directive (2000/60/EC). The information was compiled, evaluated and used as outlined in the Manual [4] and has been discussed in a consultative process with the Expert Advisory Forum on Priority Substances and the Expert Group on Quality Standards. Furthermore, it has been peer-reviewed by the SCTEE [6]. The substance data sheet may, however, not necessarily represent the views of the European Commission.

New upcoming information was considered and included up to the date of finalisation of this data sheet. Information becoming available after finalisation of this document will be evaluated in the review process of priority substances according to Art. 16(4)of the Water Framework Directive. If necessary, the Environmental Quality Standard substance data sheets will then be revised in the light of technical and scientific progress.

1 Identity of substance

Priority Substance No: 24	4-Nonylphenol (branched) and nonylphenol
CAS-Number:	84852-15-3 and 25154-52-3
Classification WFD Priority List *:	PHS

* PS: priority substance; PHS: priority hazardous substance; PSR: priority substance under review; OSC: other substance of concern

2 Proposed quality standards

2.1 Overall quality standards

Ecosystem	**Quality Standard**	**Quality Standard ”rounded value”**	**Comment**
AA-QS all surface waters addressed by the WFD	0.33 µg/l	**0.3 µg/l**	see section 8.1
MAC-QS (ECO)	2.1 µg/l	**2 µg/l**	see section 8.1

2.2 Specific quality standards

Protection Objective	**Quality Standard**	**Comment**
Pelagic community (freshwater & saltwater)	0.33 µ/l	see section 8.1
Benthic community (freshwater & saltwater sediment)	39 µg/kg wet wt 180 µg/kg dry wt	tentative values derived by EP method; see 8.2
Predators (second. poisoning)	10 mg/kg (biota tissue) 3.3 µg/l (corresponding concentration in water)	see 8.3
Food uptake by man	8.7 mg/kg (seafood) 2.9 µg/l (corresponding concentration in water)	see 8.4
Abstraction of water intended for human consumption (AWIHC)	no EU DW abstraction standard set, the derivation of such a standard is not required	see 8.5
Water intended for human consumption (WIHC)	no EU standard set	see 8.5

3 Classification

CAS No.	Substance	R-Phrases and Labelling	Ref.
84852-15-3	nonylphenol 4-nonylphenol, branched	Repr.Cat.3; R62 - Repr.Cat.3; R63 - Xn; R22 - C; R34 - N; R50-53	[7]
25154-52-3	nonylphenol 4-nonylphenol, branched	Repr.Cat.3; R62 - Repr.Cat.3; R63 - Xn; R22 - C; R34 - N; R50-53	[7]

4 Physical and chemical properties

Property	Value	Ref.	Comments
Mol. Weight:	220.34 g/mol	[1]	
Water Solubility	6 mg/l at 20ºC	[1]	[1]: may be pH dependent, discussion in [1]
Vapour Pressure:	circa 0.3 Pa at 25ºC (some evidence that actual value may be lower)	[1]	[1]: extrapolated value
Dissociation constant:	pKa: ca. 10	[1]	[1]: Given that nonylphenol is a weak acid, the pH may have an effect on its adsorptive behaviour. However, the pKa is thought to be around 10, meaning that in most situations encountered in the environment, nonylphenol will be present in the undissociated and hence more hydrophobic form

5 Environmental fate and partitioning

Property	Value	Ref.	Comments
<u>Abiotic degradation</u> Hydrolysis Photolysis	Hydrolysis and photolysis are thought to be negligible removal processes for nonylphenol in the aquatic environment	[1]	
<u>Biodegradation</u>	inherently biodegradable DT50 soil: 300 d DT50 surface water: 150 d	[1]	[1]: The biodegradation depends on several factors, the actual half life in a given environment could belonger or shorter than the estimated values, depending on the prevailing conditions
<u>Partition coefficients</u> Octanol - Water Koc (organic carbon-water) $K_{susp-water}$ (suspended matter-water) $K_{sed-water}$ (sediment-water)	 log Kow 4.48 5360 l/kg 135 m^3/m^3 135 m^3/m^3	[1]	[1]: The partition coefficients for nonylphenol have been calculated using EUSES based on a log Kow of 4.48.
<u>Bioaccumulation</u> Bioconcentration Factor (BCF) Fish Mussel used in the Risk Assessment	 up to 1300 (on fresh weight basis) 2000 – 3000 1280	[1]	[1]: The BCF calculated from the log Kow of 4.48, using the TGD equation is 1280 and agrees well with the measured values. It is therefore be used in the RA

6 Effect data (aquatic environment)

Table 6.1: Summary of the lowest reliable toxicity values of nonylphenol for aquatic species (table 3.14 in [1])

Trophic level	**Species**	**End point**	**Concentration (mg/l)**	**Reference ***	**Validity #**
Freshwater fish	Fathead minnow *Pimephales promelas*	96hr LC_{50}	0.128	Brooke (1993a)	Valid
		33 day $NOEC_{survival}$	0.0074	Ward and Boeri (1991b)	Valid
Saltwater fish	Sheepshead minnow *Cyprinodon variegatus*	96hr LC_{50}	0.31	Ward and Boeri (1990d)	Valid
Freshwater invertebrates	*Ceriodaphnia dubia*	96hr EC_{50}	0.069	England (1995)	Valid
		7 day $NOEC_{reproduction}$	0.0887		
	Daphnia magna	48hr EC_{50}	0.085	Brooke (1993a)	Valid
		21 day $NOEC_{surviving\ offspring}$	0.024	Comber et al (1993)	Valid
	Hyalella azteca	96hr EC_{50}	0.0207	Brooke et al (1993)	Valid
Saltwater invertebrates	*Mysidopsis bahia*	96hr LC_{50}	0.043	Ward and Boeri (1990c)	Valid
		28 day $NOEC_{length}$	0.0039	Ward and Boeri (1991c)	Valid
Fresh water algae	*Selenastrum capricornutum*	96hr $EC_{50(Cell\ growth)}$	0.41	Ward and Boeri (1990b)	Valid
	Scenedesmus subspicatus	72hr $EC_{50\ (Biomass)}$ 72hr $EC_{10\ (Biomass)}$ 72hr $EC_{50\ (Growth\ rate)}$ 72hr $EC_{10\ (Growth\ rate)}$	0.0563 0.0033 0.323 0.0251	Kopf (1997)	Valid
Saltwater algae	*Skeletonema costatum*	96hr $EC_{50(Cell\ growth)}$	0.027	Ward and Boeri (1990a)	Valid
Mesocosm study		20 day NOEC 20 day LOEC	0.005 0.023	Liber et al (1999)	Use with Care

Studies are classed as valid if they fully describe the test material used, the test organism, the test method and conditions and the if endpoint concentration is based upon measured levels.

* Full reference in [1]

Further short term and long term toxicity data evaluated in the risk assessment [1] are presented in Annex 1 to this data sheet.

In July 2005 the Netherlands provided new data of a test with the echinoderm species *Psammechinus miliaris* (Green sea urchin) [5]., The results of this test (48 hr, larval development) are as follows: NOEC 12 µg/l, LOEC 17 µg/l, EC10 5.4 µg/l and EC50 > 28 µg/l. They do however not have an impact on the calculation of the $QS_{saltwater}$ because neither the NOEC nor the assessment factor used as basis fort the QS calculation will change as consequence of the new test result.

6.1 Predicted no effect concentrations (aquatic environment)

Table 6.2: PNECs

Compartment	**Value**	**Reference**
Surface water	0.33 µg/l	[1]
Sediment	0.039 mg/kg wet weight	[1]
$PNEC_{oral}$ (secondary poisoning)	10 mg/kg food	[1]

6.1.1 Calculation of PNEC surface water [1]

The $PNEC_{water}$ was calculated using all the aquatic toxicity data present on nonylphenol (see also appendix 1 to this data sheet). For nonylphenol short term and long term data are available for both freshwater and saltwater species for three trophic levels. The $PNEC_{water}$ was calculated using the assessment factors detailed in the TGD.

The most sensitive species in short term studies appears to be the freshwater invertebrate *Hyalella azteca* with a 96-hour EC_{50} of 0.0207 mg/l. The most sensitive species in long term studies appears to be the freshwater algae *Scenedesmus subspicatus* with a 72-hour $EC_{10(Biomass)}$ of 3.3 µg/l. As long-term NOECs from at least three species representing three trophic levels are available an assessment factor of 10 may be used. Applying this to the long term NOEC for algae gives a $PNEC_{water}$ of 0.33 µg/l.

Data exist indicating toxicity at lower concentrations than the concentrations at which oestrogenic effects are observed. Therefore, the calculated PNEC should be protective for oestrogenic effects in fish as well.

6.1.2 Calculation of PNEC sediment[1]

The TGD states that an equilibrium partitioning method may be used to estimate the $PNEC_{sed}$. In using this method it is assumed that sediment-dwelling organisms and water column organisms are equally sensitive to nonylphenol and that the concentration of nonylphenol in sediment, interstitial water and benthic organisms is at thermodynamic equilibrium. The following formula is used in the risk assessment [1] to derive the $PNEC_{sed}$ from the $PNEC_{water}$

$$PNEC_{sed} = \frac{K_{susp\text{-}water} \times PNEC_{water} \times 1{,}000}{RHO_{susp}} = 0.039 \text{ mg/kg wet wt}$$

$PNEC_{water}$	0.33 µg/l
$K_{susp\text{-}water}$	Partition coefficient suspended matter-water (135 m^3/m^3 EUSES)
RHO_{susp}	Bulk density of suspended matter (1150 kg/m^3)

6.1.3 Calculation of the PNEC for non compartment specific effects relevant for the food chain (secondary poisoning) [1]

Nonylphenol has been shown to bioconcentrate in aquatic species.

No toxicity data are available on avian species; thus a PNEC is derived from laboratory mammal data. From Section 4 (of [1]), a NOAEL of 15 mg/kg body weight was found for reproductive effects.

Using the conversion factor of 20 from Appendix VII of the TGD and a further factor of 3 to allow for the fact that calorific content of a laboratory diet is higher than the diet of fish-eating mammals and birds, this NOAEL is equivalent to a daily dose of 100 mg/kg food. The TGD recommends the use of an assessment factor of 10 on reproductive studies. Therefore the $PNEC_{oral}$ is 10 mg/kg food.

6.2 Summary on the endocrine disrupting potential of nonylphenol

4-Nonylphenol (branched) and nonylphenol: Nonylphenol and nonylphenol ethoxylates do exhibit estrogenic activity. For nonylphenol ethoxylates the activity was found to increase with decreasing chain length, with nonylphenol showing the greatest activity. Most of the tests indicate that oestrogenic effects may start to occur at around 10-20 µg/l	[1]
4-(para)-nonylphenol (CAS 104-40-5): Insufficient data on ED potential (table 4)	[2]

7 Effect data (human health)

Summary on human health data [1]: The hazardous properties of nonylphenol have been evaluated in animals to the extent that the minimum data requirements according to Article 9(2) of Regulation 793/93 have been met. The key health effects of acute toxicity, corrosivity, repeated dose toxicity and reproductive effects have been identified. For acute toxicity, the oral LD_{50} is in the range 1200-2400 mg/kg and the dermal LD_{50} is around 2000 mg/kg. An oral LOAEL for repeated dose toxicity is 15 mg/kg/day. Concerns for mutagenicity and carcinogenicity are low. Regarding the effects on the reproductive system, the observations of oestrogenic activity in *in vitro* and *in vivo* assays, minor perturbations in the repro-ductive system of offspring in a multigeneration study, and testicular changes in gavage studies collectively raise concerns. Overall, the *in vitro* and *in vivo* studies show that nonylphenol has oestrogenic activity of a potency that is between 3 to 6 orders of magnitude less than that of oestradiol. The oral NOAEL for reproductive effects is 15 mg/kg/day.

Table 7.1: Oral toxicity data relevant for human health risk assessment [1]

	Effect	**Value**	**Reference**
NOAELoral	reproductive effects (rat)	15 mg/kg/day	[1]
LOAELoral	repeated dose toxicity (rat)	15 mg/kg/day	[1]

8. Calculation of quality standards

8.1 Quality standards for water

Freshwater

The $PNEC_{water}$ as identified in [1] (see table 6.2 of this data sheet) is suggested as the quality standard for inland waters.[1]

$QS_{freshwater}$ = 0.33 µg nonylphenol /l

As the water solubility of nonylphenol is high and the partition coefficient $Kp_{SPM\text{-}water}$ is 536[2] (trigger not met) it is not required to calculate a corresponding $QS_{SPM.water}$ referring to the concentration of nonylphenol in suspended particulate matter (SPM). Nonylphenol is normally analysed in water.

Transitional, coastal and territorial waters

Some effect data for saltwater species (fish, crustaceans, algae, molluscs, echinoderms) are available and (no)-effect concentrations do not obviously differ from those obtained for freshwater species of the same taxonomic groups. Therefore, the $QS_{saltwater}$ can be derived from the same data set as the $QS_{freshwater}$. To this end, the TGD assessment factor method as proposed for the marine effects assessment is used (section 4.3.2.2 of the Manual [4]).

As short-term tests with species representing 2 additional marine taxonomic groups (beside fish, crustaceans, algae) are available and indicate that these are not the most sensitive group an assessment factor of 10 is appropriate to derive the QSsaltwater on the basis of the NOEC/EC10 of the most sensitive species in long term studies (*Scenedesmus subspicatus* with a 72-hour $EC10_{(Biomass)}$ of 3.3 µg/l).

$QS_{saltwater}$ = 0.33 µg nonylphenol /l

Quality standard accounting for transient concentration peaks (MAC-QS)

Acute toxicity data are available for freshwater and marine organisms of different taxonomic groups (beside fish, crustaceans and algae also for annelids, insects, molluscs (clams and snails) and higher plants). The lowest acute toxicity value identified in the risk assessment [1] as fully valid is 0.0207 mg/l for the 96 hr EC_{50} of *Hyalella azteca* (Brooke, 1993a, full ref. in [1]).

The MAC-QS is derived on the basis of the EC50 of Hyallella azteca and the guidance given in the TGD on the effects assessment for intermittent releases (section 4.3.6 of the Manual [4]). As the

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[1] The SCTEE commented on the derivation of quality standards for nonylphenol [6]. The Committee recommended to consider more recent data such as, e.g., included in the risk assessment report of the US-EPA [8] and to re-consider the derived aquatic quality standard in the light of the availability of a mesocosm study. The recommendations of the SCTEE were followed but no new data with relevance for the quality standards derived in this data sheet could be identified and with regard to the mesocosm it is stated in the EU risk assessment report [1] that the study alone is no suitable basis for QS derivation: "… *the field study provides good supporting data for that generated in the laboratory studies, but cannot on its own be used as the basis for deriving a PNEC to protect the aquatic compartment."* Hence, in order to remain consistent with the agreed methodological framework for QS derivation [4], the original proposal for the $QS_{freshwater}$ is maintained.

[2] $Kp_{SPM\text{-}water}$ = Koc * foc (Koc = 5360, see section 5; foc = 0.1, TGD default value of the fraction of organic carbon in suspended solids)

available toxicity data cover a wide range of different taxonomic groups it is suggested to use only a reduced assessment factor of 10 (instead of 100). This suggestion is supported by the values of the long term NOECs, which are higher than the suggested MAC-QS.

MAC-QS = 2.1 µg nonylphenol /l

The derivation of a separate MAC-QS applicable to transient concentration peaks in coastal and territorial waters is not required as it is not probable that significant peaks occur in these waters. For transitional waters the freshwater MAC-QS may be used.

8.2 Quality standard for sediment

Since the partition coefficient $Kp_{SPM\text{-}water}$ is only 536 (trigger value of 1000 not met) the calculation of sediment quality standards is normally not required. However, as a QS referring to sediment was calculated for octylphenol, the respective standard is calculated for nonylphenol as well.

Experimental data with sediment dwelling organisms are not available. Therefore the $QS_{sediment}$ is calculated with the equilibrium partitioning method as follows (see section 4.3.2.3 of the Manual [4]):

$$PNEC_{sed} = \frac{K_{susp\text{-}water}}{RHO_{susp}} \times PNEC_{water} \times 1000 = 0.039 \text{ mg/kg wet wt}$$

$PNEC_{water}$	0.33 µg/l (freshwater & saltwater)
$K_{susp\text{-}water}$	Partition coefficient suspended matter-water (135 m^3/m^3 EUSES)
RHO_{susp}	Bulk density of suspended matter (1150 kg/m^3)

The TGD defines wet SPM as 90% vol/vol water (density 1 kg/l) and 10% vol/vol solids (density 2.5 kg/l), thus giving a wet density of $(0.9 \times 1) + (0.1 \times 2.5) = 1.15$ kg/l. The dry weight of solids is therefore 0.25 kg (per litre wet SPM) and thus the wet:dry ratio is 1.15/0.25 = 4.6.

This results in dry weight based sediment quality standards of:

$QS_{sediment}$ = 39 µg/kg wet wt = 180 µg/kg dry wt

The values derived by the EP-method should only be considered as tentative standards. In order to refine the quality standards for the sediment compartment, long term tests conducted with benthic organisms are required. For the time being no reliable effects based $QS_{sediment}$ can be derived.

8.3 Secondary poisoning of top predators

The relevant $PNEC_{oral}$ identified in the risk assessment [1] is 10 mg/kg food of the predator (section 6.1.3 of this data sheet). The $PNEC_{oral}$ is the quality standard for biota tissue with respect to secondary poisoning of top predators as objective of protection ($\approx QS_{secpois.biota}$).

Based on this quality standard referring to biota tissue, a corresponding quality standard referring to the nonylphenol concentration in water is calculated with the bioconcentration factors (BCF) used in the risk assessment [1] for fish (1248) and the BCF for mussels (2000-3000, see section 5 of this data sheet).

$QS_{secpois.biota}$ = 10 mg nonylphenol /kg food (wet weight)

$QS_{secpois.water}$ = 10 [mg/kg] * 3000^{-1} [kg/l] = 3.3 µg nonylphenol /l

Even with the highest BCF of mussels the concentration in water not to be exceeded in order to prevent secondary poisoning of predators is by far higher than the quality standard required to protect the freshwater and saltwater pelagic communities.

8.4 Quality standard referring to food uptake by humans

The lowest relevant $NOAEL_{oral}$ identified in the risk assessment [1] is 15 mg/kg bw d^{-1} for effects on reproduction in rats. If the usual assessment factor of 100 is applied to extrapolate from animal to man the $NOAEL_{oral.human}$ is 0.15 mg/kg bw d^{-1} (10.5 mg d^{-1} for a person with 70 kg body weight as relevant threshold level).

In the Manual (section 4.3.2.6) [4] it is suggested that the relevant threshold level may not be exhausted for more than 10% by consumption of fishery products (i.e. 1 mg d^{-1}).

The average fish consumption of an EU citizen is 115 g d-1 (TGD [3]). Thus, 115 g fishery product must not contain more than 1 mg nonylphenol.

$$\mathbf{QS_{hh.food}} = \frac{\text{1 mg nonylphenol}}{\text{115 g fishery product}} * 1000\text{ g} = \textbf{8.7 mg nonylphenol / kg fishery product}$$

Given the BCF_{fish} used in the risk assessment [1] (1248 l/kg), a tissue concentration of 8.7 mg nonyl-phenol per kg fish results in a water concentration of:

$$\mathbf{QS_{hh.food.water}} = \frac{\text{8.7 mg/kg}}{\text{1248 l/kg}} * 1000 = \textbf{7 µg nonylphenol /l}$$

The BCF of mussels appears to be higher than the BCF_{fish}. A maximum of 3000 was identified in the risk assessment [1]. With a tissue concentration of 8.7 mg/kg the BCF_{mussel} results in a water concentration of 2.9 µg/l nonylphenol.

The QS_{water} is by far lower than the standard derived for the protection of human health against adverse effects due to ingestion of fishery products. It is therefore not required to establish a quality standard referring to ingestion of fishery products by humans.

8.5 Quality standard for drinking water abstraction

No "guide values" or quality standards have been set in the context of Council Directives 75/440/EEC or 98/83/EC. Therefore, a provisional drinking water quality standard is calculated based on the recommendations given in the TGD[3].

The lowest relevant $NOAEL_{oral}$ identified in the risk assessment [1] is 15 mg/kg bw d^{-1}. If the usual assessment factor of 100 is applied to extrapolate from animal to man the $NOAEL_{oral.human}$ is 0.15 mg/kg bw d^{-1} (threshold level for human health).

The provisional quality standard for drinking water is calculated with the provision that uptake by drinking water should in any case not exceed 10% of the threshold level for human health [3].

$$QS_{DW.provisional} = \frac{0.1 * TL_{HH} * BW}{Uptake_{DW}} = 0.525\text{ mg nonylphenol /l}$$

with:

$QS_{DW.provisional}$	provisional quality standard for drinking water (mg/l)
TL_{HH}	threshold level for human health (0.15 mg nonylphenol /kg body weight per day)
BW	body weight (70 kg)
$Uptake_{DW}$	uptake drinking water (2 l per day)

The provisional drinking water quality standard is by far higher than the AA- and MAC- quality standards required to protect the aquatic community. It appears therefore not necessary to derive a quality standard referring to drinking water abstraction as objective of protection.

8.6 Overall quality standard

The specific quality standard derived for the protection of aquatic life in both inland and marine waters is the lowest and therefore proposed as overall annual quality standard for all surface waters addressed by the WFD.

9 References

[1] European Union Risk Assessment Report: 4-nonyl-phenol (branched) and nonylphenol, CAS No: 84852-15-3, 25154-52-3 EINECS No: 284-325-5, 246-672-0. Series: 2nd Priority List Volume: 10. European Commission – Joint Research Centre, Institute for Health and Consumer Protection, European Chemicals Bureau (ECB). © European Communities, 2002, EUR 20387 EN. The final report is available at the internet site of the European Chemicals Bureau: http://ecb.jrc.it/existing-chemicals/ ⇒ tick ESIS button, then enter CAS or EINECS number of substance.

[2] COM(2001)262 final: Communication from the Commission to the Council and the European Parliament on the implementation of the Community Strategy for Endocrine Disrupters – a range of substances suspected of interfering with the hormone system of humans and wildlife

[3] Technical Guidance Document on Risk Assessment in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) No 1488/94 on Risk Assessment for Existing Substances and Directive 98/8/EC of the European Parliament and the Council Concerning the placing of biocidal products on the market. Part II. European Commission Joint Research Centre, EUR 20418 EN/2, © European Communities 2003. Available at the internet-site of the European Chemicals Bureau: http://ecb.jrc.it/existing-chemicals/

[4] Manual of the Methodological Framework Used to Derive Environmental Quality Standards for Priority Substances of the Water Framework Directive. Peter Lepper, Fraunhofer-Institute Molecular Biology and Applied Ecology, 15 November 2004. Available at the internet-site of the European Commission: http://europa.eu.int/comm/environment/water/water-dangersub/pri_substances.htm

[5] AquaSense (2005). Toxicity tests with priority substances in the Water Framework Directive. Sponsor: Institute for Inland Water Management and Waste Water Treatment (RIZA). Report number: 2034

[6] Opinion of the Scientific Committee on Toxicity, Ecotoxicity and the Environment (SCTEE) on "The Setting of Environmental Quality Standards for the Priority Substances included in Annex X of Directive 2000/60/EC in Accordance with Article 16 thereof", adopted by the CSTEE during the 43rd plenary meeting of 28 May 2004, European Commission Health & Consumer Protection Directorate General, Brussels. http://europa.eu.int/comm/health/ph_risk/committees/sct/documents/out230_en.pdf

[7] ESIS: European Chemicals Bureau – ESIS (European Substances Information System), July 2005. http://ecb.jrc.it/existing-chemicals/ ⇒ tick ESIS button, then enter CAS or EINECS number of substance.

[8] EPA Ambient Aquatic Life Water Quality Criteria for Nonylphenol – Draft. United States Environmental Protection Agency, Office of Water 4304T. EPA 822-R-03-029, December 2003

Annex 1:

Further short term and long term toxicity data evaluated in the European Union Risk Assessment Report [1]

Criteria for validation of toxicity test results [1]

Studies are classed as valid if they fully describe the test material used, the test organism, the test method and conditions and the if endpoint concentration is based upon measured levels. Where only some of these criteria are described the tests may be used with care or considered not valid. Studies marked ‘use with’ care can be used to support valid studies. For some studies a ‘lack of data’ marking is given. In these cases the original paper has not been received but only a citation. However the results from these non-validated studies are higher than those from the studies already checked so validating such references will not change the outcome of the PNEC derivation.

A1.1 Fish

Table A.1 summarises the toxicity test results for fish exposed to nonylphenol.

Acute toxicity:

The lowest 96-hour LC_{50} reported from a fully valid study is 0.128 mg/l for the freshwater species, the fathead minnow (*Pimephales promelas*) (Brooke, 1993a). It has not been possible to validate the data from some of the remaining studies on freshwater species.

The lowest 96-hour LC_{50} reported for seawater species is 0.017 mg/l for the winter flounder (*Pleuronectes americanus*) (Lussier et al). This study is given a validity marking of ‘use with care’ because only a summary report is available. The lowest value from a valid study is a 96-hour LC_{50} of 0.31 mg/l for sheepshead minnow (*Cyprinodon variegatus*) (Ward and Boeri, 1990d).

Long-term toxicity:

A long-term study on fathead minnow (*Pimephales promelas*) embryos (Ward and Boeri, 1991b) gives a 33-day $LOEL_{survival}$ of 0.014 mg/l and a 33-day $NOEL_{survival}$ of 0.0074 mg/l (LOEL and NOEL are taken to be equivalent to LOEC and NOEC respectively). Brooke (1993b) reports the results from a 28-day study on fathead minnow (*Pimephales promelas*); a 28-day $NOEC_{mortality}$ of 0.0775 mg/l and a 28-day $LOEC_{mortality}$ of 0.193 mg/l are reported. Both of these studies are valid.

A.1.2 Aquatic invertebrates

Table A.2 summarises the toxicity of nonylphenol to aquatic invertebrates.

Short term toxicity:

From the data presented, the lowest acute toxicity value from a fully valid study for freshwater aquatic invertebrates is a 96-hour EC_{50} of 0.0207 mg/l for the amphipod *Hyalella azteca* (Brooke et al, 1993a). The lowest acute toxicity value for *Daphnia magna* from a fully valid study is a 48-hour $EC_{50(Immobilisation)}$ of 0.085 mg/l (Brooke, 1993a). For marine invertebrates the lowest value from a validated study is a 96-hour LC_{50} of 0.043 mg/l for the mysid *Mysidopsis bahia* (Ward and Boeri, 1990c).

Long-term toxicity:

Long-term toxicity data are reported for marine and freshwater invertebrates. The lowest value from a fully valid study on freshwater organisms is a 21-day $NOEC_{surviving\ offspring}$ of 24 µg/l for *Daphnia magna* (Comber et al, 1993). For seawater species a 21-day $NOEC_{length}$ of 3.9 µg/l is reported for the mysid *Mysidopsis bahia* (Ward and Boeri, 1991c). This test is considered valid for use in the risk assessment.

A1.3 Aquatic algae and plants

Table A.3 summarises the toxicity of nonylphenol to aquatic algae.

Short term toxicity:

From Table A.3 the lowest 72-hour EC_{50} value for freshwater species is 0.0563 mg/l for the alga *Scenedesmus subspicatus* based upon change in biomass (Kopf, 1997). The lowest 96-hour EC_{50} value for saltwater species is 0.027 mg/l for the alga *Skeletonema costatum*, based upon biomass (Ward and Boeri, 1990a). Both these values are from valid studies and are taken as short term test results.

Long term toxicity:

The Technical Guidance Document states that for long term studies an EC_{10} may be taken as a long term NOEC if no long term NOEC is available. A 72 hour EC_{10} value of 0.0033 mg/l based upon biomass is reported for the freshwater alga *Scenedesmus subspicatus* (Kopf, 1997). This value will be taken as equivalent to a long-term NOEC.

A1.4 Amphibians

Ward and Boeri (1992) studied the toxicity of nonylphenol to the tadpole *Rana catesbiana*. In the test, tadpoles were exposed for up to 30 days to nonylphenol in a sediment/water system. Nonylphenol was added to the sediment in the test vessels and dilution water added on a flow through basis. The 30 day LC_{50} was 260 mg/kg dry weight and the 30 day EC_{50} was 220 mg/kg dry weight. At 10, 20 and 30 days the lowest observed effect level (LOEL) was 390 mg/kg dry weight and the no observed effect level (NOEL) was 155 mg/kg dry weight. The authors noted that the levels of nonylphenol in the water were high enough to cause the observed toxicity and it is not possible to attribute the toxic effect to either water or sediment exposure.

Table A.1: Toxicity of nonylphenol to fish

Species	Chemical tested	Age/Size	Stat/ Flow	Temp (°C)	Dissolved oxygen (mg/l)	Hardness (mg $CaCO_3$ /l)	pH	Endpoint	Concentration (mg/l)	Reference *	Validity#
Freshwater species											
Fathead minnow *Pimephales promelas*	nonylphenol (91% 4-nonylphenol; 4% 2-nonylphenol; 5% dinonylphenol)	31-35 days 220 mg	flow	24.6 ± 1.4	Mean 7.4 Range 4.6-8.8	Mean 44.9 Range 42.2-46.6	6.9-7.7	96hr LC_{50} 96hr $LOEC_{lethargy}$ 96hr $LOEC_{loss\ of\ equilibrium}$	0.135 (m) 0.187 (m) 0.098 (m)	Holcombe et al (1984)	Valid
	nonylphenol, 4- branched CAS No. 84852-15-3	embryos < 24 hrs old	flow	25 ± 1.5	7.1 - 8.2	160-180 mg/l	7.4-8.1	33 day $LOEC_{survival}$ 33 day $NOEC_{survival}$	0.014 (m) 0.0074 (m)	Ward and Boeri (1991b)	Valid
	nonylphenol	25-35 days	flow					96hr LC_{50} 96hr EC_{50}	**0.128 (m)** 0.096 (m)	Brooke (1993a)	Valid
	nonylphenol							96hr LC_{50}	0.51	Waldock and Thain (1991)	Lack of data
	nonylphenol	30 day	flow					96hr $NOEC_{mortality}$ 96hr $LOEC_{mortality}$ 28 day $NOEC_{mort}$ 28 day $LOEC_{mort}$	0.0831 (m) 0.23 (m) 0.0775 (m) 0.193 (m)	Brooke (1993b)	Valid
Bluegill *Lepomis macrochirus*	nonylphenol	< 1 year	flow					96hr LC_{50} 96hr EC_{50}	0.209 (m) 0.203 (m)	Brooke (1993a)	Valid
	nonylphenol	10-12 weeks	flow					96hr $NOEC_{mortality}$ 96hr $LOEC_{mortality}$ 28 day $NOEC_{mort}$ 28 day $LOEC_{mort}$	0.0865 (m) 0.211 (m) 0.0595 (m) 0.126 (m)	Brooke (1993b)	
Killifish *Oryzias latipes*	4-nonylphenol	0.2 g	stat			25	7.0	96hr LC_{50}	0.4	Yoshimura (1986)	Lack of data
Stickleback *Gasterostrus aculeatus*	nonylphenol							48hr LC_{50}	1.4	Granmo (1991)	Lack of data
Brook trout *Salvelinus fontinalis*	nonylphenol							96hr LC_{50}	0.145	Armstrong and Kingsbury (1979)	Lack of data
Rainbow trout *Oncorhynchus Mykiss*	nonylphenol	fingerling						96hr LC_{50}	0.23	Armstrong and Kingsbury (1979)	Lack of data
	nonylphenol	45 days post hatch	flow					96hr LC_{50} 96hr EC_{50}	0.221 0.109	Brooke (1993a)	Valid
	nonylphenol	embryo juvenile						24hr LC_{50}	0.48	Ernst et al (1980)	Lack of data
Golden orfe *Leuciscus idus melanotus*	nonylphenol	6 ± 2 cm	stat	20 ± 2	> 5 mg/l		7.2-7.3	48hr LC_{50}	0.56 (n)	Hüls (1996f)	Use with care

Freshwater species											
Species	**Chemical tested**	**Age/Size**	**Stat/ Flow**	**Temp (°C)**	**Dissolved oxygen (mg/l)**	**Hardness (mg $CaCO_3$ /l)**	**pH**	**Endpoint**	**Concentration (mg/l)**	**Reference ***	**Validity#**
Atlantic salmon *Salmo salar*	nonylphenol	juvenile	flow					96hr LC_{50}	0.13-0.16 mg/l	McLeese et al (1981)	Use with care
		juvenile	stat					96hr LC_{50}	0.19 mg/l	McLeese et al (1981)	Use with care

Saltwater species											
Species	**Chemical tested**	**Age/Size**	**Stat/ Flow**	**Temp (°C)**	**Dissolved oxygen (mg/l)**	**Salinity °/oo**	**pH**	**Endpoint**	**Concentration (mg/l)**	**Reference**	**Validity**
Cod *Gadus morhua*	nonylphenol			17				96hr LC_{50} 15 day LC_{50}	3 0.1	Swedmark et al (1971)	Lack of data
Guppy *Poecilia reticulata*	4-nonylphenol	3 weeks old	stat	25		28	8	96hr LC_{50} 96hr NOEC	0.44 (n) 0.18 (n)	Personal communication	Use with care
Hook nose *Agonus cataphractus*	nonylphenol							96hr LC_{50}	0.3	Etnier (1985)	Lack of data
Sheepshead minnow *Cyprinodon variegatus*	nonylphenol, 4- branched CAS No. 84852-15-3	juvenile	flow	22 ± 2	7.0 - 8.8	15-17	7.4-8.1	96hr LC_{50} 96hr NOEC	0.31 (m) 0.24 (m)	Ward and Boeri (1990d)	Valid
	4-nonylphenol CAS No. 84852-15-3		flow					96hr LC_{50}	0.142 (m)	Lussier et al	Use with care
Winter flounder *Pleuronectes americanus*	4-nonylphenol CAS No. 84852-15-3		stat					96hr LC_{50}	0.017 (n)	Lussier et al	Use with care
Inland silversides *Menidia beryllina*	4-nonylphenol CAS No. 84852-15-3		flow					96hr LC_{50}	0.069 (m)	Lussier et al	Use with care

stat – static system flow – flow through system n – nominal concentration m-measured concentration

Studies are classed as valid if they fully describe the test material used, the test organism, the test method and conditions and the if endpoint concentration is based upon measured levels.

* Full reference in [1]

Table A.2: Toxicity of nonylphenol to aquatic invertebrates

Freshwater											
Species	**Chemical tested**	**Age/Size**	**Stat/ Flow**	**Temp (°C)**	**Dissolved oxygen (mg/l)**	**Hardness (mg $CaCO_3$/l)**	**pH**	**Endpoint**	**Concentration (mg/l)**	**Referenc *e**	**Validity#**
Water flea *Daphnia magna*	4-nonylphenol	24 hrs old	stat	20			8 ± 0.2	24hr EC_{50} 24hr EC_0 24hr EC_{100}	0.18 (n) 0.09 (n) 0.34 (n)	Bringmann and Kühn (1982)	Use with care
	nonylphenol							48hr EC_{50}	0.44	Monsanto (1985)	Lack of data
	nonylphenol (91.8% nonylphenol, 86.1% 4-nonylphenol)	<24 hrs old	stat	20 ± 1		180 ± 20	8.25 ± 0.25	24hr LC_{50} 48hr LC_{50} 7 day LC_{50} 14 day LC_{50} 21 day LC_{50} 21 day $NOEC_{surviving\ offspring}$ 21 day $NOEC_{length}$	0.30 (m)[c] 0.19 (m)[c] 0.12 (m)[c] 0.12 (m)[c] 0.10 (m)[c] 0.024 (m)[c] 0.039 (m)[c]	Comber et al (1993)[c]	Valid
	nonylphenol	<24 hrs old	stat					48hr EC_{50}	0.085	Brooke (1993a)	Valid
	nonylphenol 25154-52-3	<24 hrs old	stat	20 ± 1		294	7.5	24hr EC_{50} (immobilisation) 48hr EC_{50} (immobilisation)	0.218 (n) 0.14 (n)	Hüls (1992c)	Valid
	nonylphenol CAS No. 25154-52-3	<24 hrs old	semi-stat	20 ± 1				21 day $NOEC_{reproduction}$	≥ 0.1 (n)	Hüls (1992a)	Valid
	nonylphenol CAS No. 25154-52-3	<24 hrs old	semi-static	20 ± 1				21 day $NOEC_{reproduction}$ $LOEC_{reproduction}$	0.1 (n) 0.14 (n)	Hüls (1992b)	Valid
	nonylphenol		static					21 day $NOEC_{reproduction}$	0.001 (n)	Kopf (1997)	Use with care
Water flea *Daphnia pulex*	nonylphenol							48hr EC_{50}	0.14-0.19	Ernst et al (1980)	Lack of data
Water flea *Ceriodaphnia dubia*	nonylphenol			25				48hr EC_{50}	Mean 0.47 (n)	Ankley et al (1990)	Use with care
Water flea *Ceriodaphnia dubia*	nonylphenol CAS No. 84852-15-3 (>95% 4-nonylphenol)	1st instar < 24 hrs old	stat	24-25	6.4-7.9	144-172	8.3-8.6	96hr LC_{50} 96hr EC_{50} 7 day LC_{50} 7 day EC_{50} 7 day $NOEC_{survivial}$ 7 day $LOEC_{survivial}$ 7 day $NOEC_{reproduction}$ 7 day $LOEC_{reproduction}$	0.276 (m) 0.069 (m) 0.258 (m) 0.0992 (m) 0.202 (m) 0.377 (m) 0.0887 (m) 0.202 (m)	England (1995)	Valid

Freshwater											
Species	**Chemical tested**	**Age/Size**	**Stat/ Flow**	**Temp (°C)**	**Dissolved oxygen (mg/l)**	**Hardness (mg $CaCO_3$/l)**	**pH**	**Endpoint**	**Concentration (mg/l)**	**Referenc *e**	**Validity#**
Clam *Anodonta cataractae*	nonylphenol	Adult 15 g	stat	10				6 day LC_{50}	5.0 (n) 1.7 (m)	McLeese et al (1980b)	Use with care
Annelid *Lumbriculus variegatus*	nonylphenol CAS No. 25154-52-3	adult 0.005 g	flow					96hr LC_{50} 96hr EC_{50} (inacitivity)	0.342 (m) 0.268 (m)	Brooke et al (1993a)	Valid
Snail *Physella virgata*	nonylphenol CAS No. 25154-52-3	adult 476±218 mg	flow					96hr LC_{50} 96hr EC_{50} (inacitivity	0.774 (m) 0.378 (m)	Brooke et al (1993a)	Valid
Dragonfly *Ophiogomphus sp.*	nonylphenol CAS No. 25154-52-3		flow					96hr EC_{50} (loss of equilibrium)	0.596 (m)	Brooke et al (1993a)	Valid
Damselfly *Ischnura elegans*	Nonylphenol		Static					96hr EC_{50} 96hr LC_{50}	0.057 (m) 0.108 (m)	Sims et al (1997)	Use with care
Freshwater shrimp *Gammarus pulex*	Nonylphenol		Static					96hr EC_{50} 96hr LC_{50}	0.0127 (m) 0.0246 (m)	Sims et al (1997)	Use with care
Painted shrimp *Hyalella azteca*	nonylphenol CAS No. 25154-52-3		flow					96 hr EC_{50} (loss of mobility)	0.0207 (m)	Brooke et al (1993a)	Valid
Painted shrimp *Hyalella azteca*	nonylphenol CAS No. 84852-15-3 (>95% 4-nonylphenol)	Juvenile 2-3 mm	flow	21	1.4-8.0	152-158	7.9-8.7	96hr LC_{50} 96hr EC_{50}	0.17 (m) 0.15 (m)	England and Bussard (1994)	Valid

Seawater											
Species	**Chemical tested**	**Age/Size**	**Stat/ Flow**	**Temp (°C)**	**Dissolved oxygen (mg/l)**	**Salinity (°/₀₀)**	**pH**	**Endpoint**	**Concentration (mg/l)**	**Reference**	**Validity**
Clam *Mya arenaria*	nonylphenol	adult 20 g	stat	10				15 day LC_{50}	> 1 (n) > 0.7 (m)	McLeese et al (1980b)	Use with care
Coot Clam *Mulinia lateralis*	4-nonylphenol CAS No. 84852-15-3		stat					96hr LC_{50}	0.038 (n)	Lussier et al	Use with care
Mussel *Mytilus edulis*	nonylphenol CAS No. 25154-52-3	adult 40-50 mm	semi-stat	17 ± 1		32		96hr LC_{50} 15 day LC_{50} 35 day LC_{50}	3 (n) 0.5 (n) 0.14 (n)	Granmo et al (1989)	Use with care
Crustacean *Nitocra spinipes*	nonylphenol							96hr LC_{50}	0.118 0.139	Wahlberg et al (1990)[d]	Not valid
Brown shrimp *Crangon crangon*	4-nonylphenol							96hr LC_{50}	0.6	Granmo (1991)	Lack of data
	nonylphenol							96hr LC_{50}	0.42	Waldock and Thain	Lack of data

Seawater											
Species	**Chemical tested**	**Age/Size**	**Stat/ Flow**	**Temp (°C)**	**Dissolved oxygen (mg/l)**	**Salinity (°/₀₀)**	**pH**	**Endpoint**	**Concentration (mg/l)**	**Reference**	**Validity**
										(1991)	
Sand shrimp *Crangon septemspinosa*	nonylphenol	adult 1.3 g	stat	10				96hr LC_{50}	0.4 (n) 0.3 (m)	McLeese et al (1980b)	Use with care
			stat					96hr LC_{50}	0.3 (m)	McLeese et al (1981)	Use with care
Grass shrimp *Palaemonetes pugio*	4-nonylphenol CAS No. 84852-15-3		flow					96hr LC_{50}	0.059	Lussier et al	Use with care
Lobster *Homarus americanus*	nonylphenol	20 g	stat	10				96hr LC_{50}	0.2 (n) 0.17 (m)[a]	McLeese et al (1980b)	Use with care
	4-nonylphenol CAS No. 84852-15-3		stat					96hr LC_{50}	0.071 (n)	Lussier et al	Use with care
Mysid *Mysidopsis bahia*	nonylphenol CAS No. 84852-15-3 (4-nonylphenol, branched)	< 24 hrs old	flow	23.8-25.3	6.5-7.8	20	7.3-8.2	96hr LC_{50} 96hr NOEC	0.043 (m) 0.018 (m)	Ward and Boeri (1990c)	Valid
			stat	23.3-26.4	5-8.5	20-21	7.5-8.2	28 day $LOEC_{length}$ 28 day $NOEC_{length}$	0.0067 (m) 0.0039 (m)	Ward and Boeri (1991c)	Valid
	4-nonylphenol CAS No. 84852-15-3		flow					96hr LC_{50}	0.06 (m)	Lussier et al	Use with care
Mud crab *Dyspanopeus sayi*	4-Nonylphenol CAS No. 84852-15-3		flow					96hr LC_{50}	0.2 (m)	Lussier et al	Use with care
Amphipod *Leptocheirus plumulosus*	4-nonylphenol CAS No. 84852-15-3		flow					96hr LC_{50}	0.062 (m)	Lussier et al	Use with care

Benthic (Sediment dwelling) organisms											
Species	**Chemical tested**	**Age/Size**	**Stat/ Flow**	**Temp (°C)**	**Dissolved oxygen (mg/l)**	**Salinity (°/₀₀)**	**pH**	**Endpoint**	**Concentration (mg/l)**	**Reference**	**Validity**
Midge *Chironomus tentans*	nonylphenol	larvae	flow	20 ± 1		138-158	7.7-8.3	14 day LC_{50}	0.119	England and Bussard (1993)	Valid

stat – static system flow – flow through system n – nominal concentration m-measured concentration

Studies are classed as valid if they fully describe the test material used, the test organism, the test method and conditions and the if endpoint concentration is based upon measured levels.

* Full reference in [1]

Table A.3: Toxicity of nonylphenol to aquatic algae and plants

Species	Chemical	Experimental conditions	Endpoint/Effect	Concentration (mg/l)	Reference *	Validity#
Freshwater species						
Duckweed *Lemna minor*	nonylphenol CAS No. 25154-52-3		96hr NOEC 96hr LOEC(Frond production)	0.901 (m) 2.08 (m)	Brooke et al (1993a)	Valid
Green alga *Chlorella pyrenoidosa*	nonylphenol		Growth reduction 24hr LC_{50} 24hr LC_{100}	0.025-7.5 1.5 25	Weinberger and Rea (1981)	Lack of data
Green alga *Scenedesmus subspicatus*	nonylphenol	UBA GLP	72hr EC_{50} (Cell growth) 72hr EC_{10} (Cell growth)	1.3 0.5	Hüls (1996d)	Valid
	nonylphenol CAS No. 2515-52-3	EN 28692/ISO 8692 DIN 38412 9	72hr EC_{50} (Biomass) 72hr EC_{10} (Biomass) 72hr EC_{50} (Growth rate) 72 hr EC_{10} (Growth rate)	0.0563 0.0033 0.323 0.0251	Kopf (1997)	Valid
Flagellate *Chlamydomonas reinhardii*	nonylphenol	Ultrastructure examined under electron microscope	Cell membrane disorganisation; distorted flagellae	0.5-0.7	Weinberger and Rea (1981)	Lack of data
	nonylphenol		Inhibition of photosynthesis 55% 100%	 0.5 0.75	Moody and Weinberger (1983)	Lack of data
Alga *Selenastrum capricornutum*	nonylphenol CAS No. 25154-52-3		96hr NOEC 96hr LOEC (Cell production)	0.694 (m) 1.480 (m)	Brooke et al (1993a)	Valid
	nonylphenol CAS No. 84852-15-3 (95% 4-nonylphenol)	Temp 23.2-23.7 °C pH 7.4-7.5 to 8.2-8.9	96hr EC_{50} (Cell growth)	0.41 (m)	Ward and Boeri (1990b)	Valid
Saltwater species						
Marine alga *Skeletonema costatum*	nonylphenol CAS No. 84852-15-3 (95% 4-nonylphenol)	Temp 21-22 °C pH 7.9-8.1 to 8.3-9.6 Salinity 30 ‰	96hr EC_{50} (Cell growth)	0.027 (m)	Ward and Boeri (1990a)	Valid

m-measured concentration

Studies are classed as valid if they fully describe the test material used, the test organism, the test method and conditions and the if endpoint concentration is based upon measured levels.

* Full reference in [1]