Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at Community level of

tris(nonylphenyl) phosphite

ECHA/RAC/CLH-O-0000001402-87-01/A1

EC number: 247-759-6
CAS number: 26523-78-4

Adopted

26 October 2010

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TABLES

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: Tris(nonylphenyl)phosphite

EC Number: 247-759-6

CAS number: 26523-78-4

Registration number (s): -

Purity: 95 – 100% w/w

Impurities:

Nonylphenol (CAS 25154-52-3)

Phenol (CAS 108-95-2)

Di(nonylphenyl)phenylphosphite (CAS 25417-08-7)

TNPP was on the 4th priority list of the Existing Substances Regulation and it is therefore a requirement to harmonise classification for all endpoints justifying classification.

A classification proposal was submitted and discussed at ECB (TC C&L) for health endpoints. Classification R43 (may cause sensitization by skin contact) was concluded by the TC C&L for health. For information, discussions and conclusions of the TC C&L as reported in summary records of the corresponding meetings are presented in Appendix I of the present report. No relevant new data has been identified since TC C&L discussion for health.

The proposal for environmental classification was on hold as additional testing had been requested and was on-going. A summary explanation of the justification for requirement of the new studies according to Commission Regulation (EC) No 466/2008 is presented in Appendix II of the present report.

Further to completion of the required environmental test the whole classification proposal is now submitted to ECHA for all endpoints justifying classification as requested for priority substances under ESR.

In addition, many difficulties has been encountered during the assessment of TNPP as this substance can be considered as difficult to test for the purposes of determining its aquatic toxicity and difficult to classify. In absence of the Classification and Labelling Inventory that is not yet available, it is not possible to know what self-classification is applied by manufacturers and importers and if an appropriate classification for environment is applied. Setting an harmonised classification for environment is therefore justified to ensure the application of an appropriate classification.

In the view of a contingent discussion on the relevance of classification due to impurities, some additional toxicological data is displayed in the present dossier for information.

The only endpoints proposed for harmonisation are however skin sensitisation and environment.

Proposed classification based on Directive 67/548/EEC criteria:

Xi; R43
N; R50/53

Proposed classification based on Regulation (EC) No 1272/2008:

Skin Sens. 1 – H317
Aquatic Acute 1 – H400
Aquatic Chronic 1 – H410
M-factor: none

Proposed labelling based on Directive 67/548/EEC criteria:

R-phrases: R43- 50/53
Symbol(s) : Xi; N
S-phrases: S24–37–60–61

Proposed labelling based on Regulation (EC) No 1272/2008:

Pictograms: GHS07, GHS09

Signal word: Wng

Hazard statement codes: H317, H410

Proposed specific concentration limits (if any):

None

Proposed notes (if any): see below.

Some impurities of TNPP, especially nonylphenol, have a harmonised classification and can be present in TNPP in concentrations that trigger additional classifications of TNPP as discussed in more details in section 1.2.

However, the classification proposed in this dossier as displayed above does not take into account additional classifications based on the impurities as the impurity content can vary depending on the production process and its possible improvements.

According to articles 10 and 11 of Regulation (EC) No 1272/2008 (CLP Regulation), the potential influence of impurities on classification remains of the responsibility of the manufacturer/importer. To inform manufacturer/importer as well as users that it can be necessary to complement the harmonised classification of TNPP based on the impurity content, it was initially proposed that a new note could be created and added to the TNPP proposal. However, the article 11 of the CLP Regulation already mentions that substances are to be classified based on their impurity content and

the article 4(3) states that harmonised classifications need to be complemented when relevant. The CLP Regulation therefore clearly implies that classifications based on the impurity content need to be added if relevant and are not part of the harmonised classification. Thus, an extra note is not necessary.

Available data on skin irritation, eye irritation and reproductive toxicity of TNPP are displayed in the present dossier for information and possible discussions related to potentially present impurities.

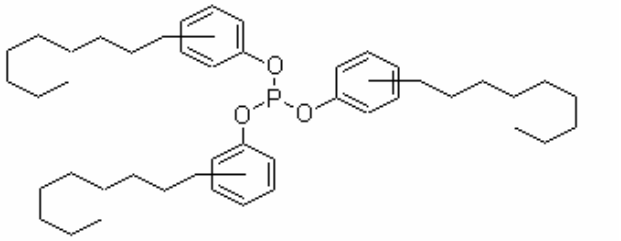
JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

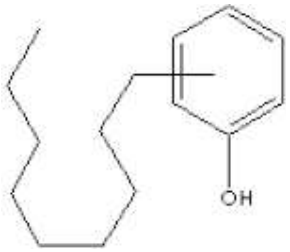
Chemical Name:	Tris(nonylphenyl)phosphite
	Synonyms: Alkanox TNPP, Lowinox TNPP, Irgafos TNPP, Tris(monononylphenyl)phosphite, Tri(nonylphenyl)phosphite, Weston 399, Weston TNPP, Irgastab CH 55, Naugard TNPP, Polygard, Polygard HR, Polygard LC, TNPP, Trisnonylphenylphosphit.
EC Name:	Tris(nonylphenyl)phosphite
CAS Number:	26523-78-4
CAS Name	Phenol, nonyl-,1,1',1''-phosphite
IUPAC Name:	Tris(nonylphenyl)phosphite

1.2 Composition of the substance

Chemical Name:	Tris(nonylphenyl)phosphite (TNPP)
EC Number:	247-759-6
CAS Number:	26523-78-4
IUPAC Name:	Tris(nonylphenyl)phosphite
Molecular Formula:	$C_{45}H_{69}O_3P$
Structural Formula:	
	Alkyl chains may have different degrees of branching which can result in a non-linear structure.
Molecular Weight:	689 g/mol
Typical concentration (% w/w):	-

Concentration range (% w/w):	There are two grades of TNPP that are sold in the marketplace. The purity of the standard TNPP is reported as ca. 95 – 100% w/w. The following impurities may be found in standard TNPP: - Nonylphenol (CAS 25154-52-3) < 5% w/w, - Phenol (CAS 108-95-2) < 0.1% w/w, - Di(nonylphenyl)phenylphosphite (CAS 25417-08-7) 0.05% w/w, A high purity grade of TNPP was introduced into the market in the late 1990s. The impurities found in the high purity TNPP are: - Nonylphenol (CAS 25154-52-3) < 0.1% w/w, - Phenol (CAS 108-95-2) < 0.1% w/w, - Di(nonylphenyl)phenylphosphite (CAS 25417-08-7) 0.05% w/w, TNPP is an unspecific isomeric reaction mass. No information is available on the distribution of the isomers.

Impurities

Chemical Name:	Nonylphenol
EC Number:	246-672-0
CAS Number:	25154-52-3
IUPAC Name:	Nonylphenol
Molecular Formula:	$C_{15}H_{24}O$
Structural Formula:	 Alkyl chain may have different degrees of branching which can result in a non-linear structure.
Molecular Weight:	220.34 g/mol
Typical concentration (% w/w):	< 5% w/w
Concentration range (% w/w):	-

Classification: The following harmonised classification applies to nonylphenol:

According to 67/548/EEC	*According to CLP*
Repr. Cat. 3; R62-63 Xn; 22 C; R34 N; R50/53	Repr. 2 - H361fd Acute Tox. 4 - H302 Skin Corr. 1B - H314 Aquatic Acute 1 - H400 Aquatic Chronic 1 - H410

Chemical Name: Phenol

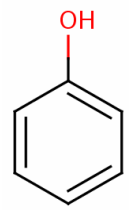
EC Number: 203-632-7

CAS Number: 108-95-2

IUPAC Name: Phenol

Molecular Formula: C_6H_6O

Structural Formula:



Molecular Weight: 94.11 g/mol

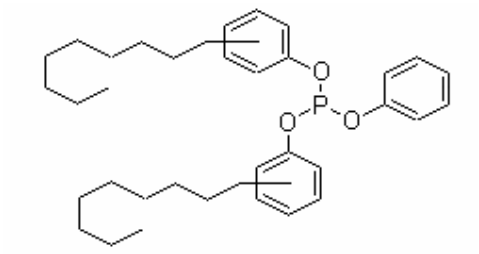
Typical concentration (% w/w): < 0.1% w/w

Concentration range (% w/w): -

Classification: The following harmonised classification applies to phenol:

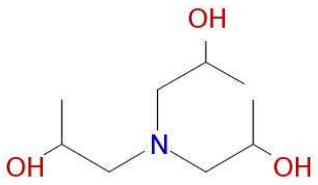
According to 67/548/EEC	*According to Regulation (EC) No 1272/2008*
Muta. Cat.3; R68 T ; R23/24/25 Xn; R48/20/21/22 C; R34 with SCL : T; R23/24/25: C ≥ 10 % Xn; R20/21/22: 3 % ≤ C < 10 % C; R34: C ≥ 3 % Xi; R36/38: 1 % ≤ C < 3 %	Muta. 2; H341 Acute Tox. 3; H301-H311-H331 STOT RE 2; H373 Skin Corr. 1B; H314 with SCL: Skin Corr. 1B: C ≥ 3 % Skin Irrit. 2: 1 % ≤ C < 3 % Eye Irrit. 2: 1 % ≤ C < 3 %

Considering that phenol can be present in TNPP in concentration < 0.1%, no additional classification applies for TNPP due to this impurity.

Chemical Name:	Di(nonylphenyl)phenylphosphite
EC Number:	-
CAS Number:	25417-08-7
IUPAC Name:	-
Molecular Formula:	$C_{36}H_{50}O_3P$
Structural Formula:	 Alkyl chains may have different degrees of branching which can result in a non-linear structure.
Molecular Weight:	561.76 g/mol
Typical concentration (% w/w):	0.05% w/w
Concentration range (% w/w):	-
Classification:	No harmonised classification

Additive

Chemical Name:	Triisopropanolamine (TIPA)
	TIPA is added for hydrolytic stability of TNPP
EC Number:	204-528-4
CAS Number:	122-20-3
IUPAC Name:	1-1',1''-nitrilotripropan-2-ol
Molecular Formula:	$C_9H_{21}NO_3$
Structural Formula:	



Molecular Weight:	191.26 g/mol
Typical concentration (% w/w):	-
Concentration range (% w/w):	0.5 - 1% w/w
Classification:	The following harmonised classification applies:

According to 67/548/EEC	*According to Regulation (EC) No 1272/2008*
Xi; R36 R52/53	Eye Irrit. 2 - H319 Aquatic Chronic 3 - H412

Considering that TIPA can be present in TNPP in concentration 0.5-1%, no additional classification applies for TNPP due to this additive alone.

Only presence of nonylphenol can therefore have an influence on the classification of TNPP.

However, the classification proposed in this dossier as displayed in page 3 does not take into account additional classifications based on impurities as impurity content can vary depending on the production process and its possible improvements.

According to articles 10 and 11 of the CLP Regulation, the potential influence of impurities on classification remains of the responsibility of the manufacturer/importer. To inform manufacturer/importer as well as users that it can be necessary to complement the harmonised classification of TNPP based on the impurity content, it was initially proposed that a new note could be created and added to the TNPP proposal. However, the article 11 of the CLP Regulation already

mentions that substances are to be classified based on their impurity content and the article 4(3) states that harmonised classifications need to be complemented when relevant. The CLP Regulation therefore clearly implies that classifications based on the impurity content need to be added if relevant and are not part of the harmonised classification. Thus, an extra note is not necessary.

Available data on skin irritation, eye irritation and reproductive toxicity of TNPP are displayed in the present dossier for information and possible discussions related to possibly present impurities.

1.3 Physico-chemical properties

Table 1: Summary of physico- chemical properties

REACH ref Annex, §	Property	IUCLID section	Value	[comment/reference]
VII, 7.1	Physical state at 20°C and 101.3 kPa	3.1	Viscous liquid at room temperature	
VII, 7.2	Melting/freezing point	3.2	6°C ± 3°C	Reimer&Associates, 2001b
VII, 7.3	Boiling point	3.3	322°C (degradation)	Reimer&Associates, 2001a
VII, 7.4	Relative density	3.4 density	0.98 g/cm^3 at 20°C	Crompton, 2003
VII, 7.5	Vapour pressure	3.6	0.058 Pa at 25°C	Phoenix_Chemical_Laboratory, 1997
VII, 7.6	Surface tension	3.10	No data	
VII, 7.7	Water solubility*	3.8	Upper value: <0.05 mg.L^{-1} at 20°C Lower value: 3.10^{-16} mg/L	From EC, 2009 : TNO, personal communication Lower value: value obtained using QSAR calculation
VII, 7.8	Partition coefficient n-octanol/water (log value)	3.7 partition coefficient	Experimental > 10 (T° not known)	OECD guidelines 117 HPLC method (Jakupca, 2007)
VII, 7.9	Flash point	3.11	207°C (closed cup)	Pittsburgh_Testing_Laboratory, 1978
VII, 7.10	Flammability	3.13	No data	
VII, 7.11	Explosive properties	3.14	TNPP is not expected to have explosive properties	On the basis of chemical structure
VII, 7.12	Self-ignition temperature		No data	
VII, 7.13	Oxidising properties	3.15	No oxidising properties	EC, 2009
VII, 7.14	Granulometry	3.5	No data	
XI, 7.15	Stability in organic solvents and identity of relevant degradation products	3.17	No data	
XI, 7.16	Dissociation constant	3.21	No data	
XI, 7.17,	Viscosity	3.22	6000 cps at 25°C	Crompton, 2003
	Auto flammability	3.12	440°C	United States Testing Company, 1990
	Reactivity towards container material	3.18	No data	
	Thermal stability	3.19	No data	

* **Explanation of the water solubility value**: A water solubility was estimated using structure activity relationships models developed by the U.S. Environmental Protection Agency and Syracuse Research Corporation (EPIWIN, US EPA and Syracuse Research Corporation, 2001). The water solubility was estimated to 1.3x10^{-15} mg/L (Staples, 2001). Other estimations have been obtained

using a more recent version of EPI suite software (US-EPA and Syracuse Research Corporation, 2004): $3x10^{-16}$ and $6.9x10^{-7}$ mg/L calculated with a water solubility estimate from log Kow (WSKOW v1.41 with a log Kow of 20.05) and a water solubility estimate from fragments, respectively.

Experimental water solubility was determined by Reimer & Associates (2001c). The flask method based on OECD Guideline 105 was used. TNPP was not detected in the saturated aqueous test solution. Therefore it is concluded that the water solubility of TNPP is below the detection limit of the substance. This detection limit was estimated to be 0.6 mg/L.

The TNPP Industry commissioned a laboratory to develop a more sensitive analytical method for measuring TNPP so as to better approximate the true water solubility limit. Preliminary efforts were able to establish a new LOQ of 0.05 mg/L. Solubility measurements have been attempted using this new analytical method. Considering the first results of this experiment, it seems that water solubility is still around or below this analytical limit (TNO, personal communication). This value has been used in the TNPP risk assessment (EC, 2009).

The range of water solubility's currently used in the RAR takes into account both the lowest result obtained using QSARs ($3x10^{-16}$ mg/L) and the fact that this substance is expected to have a water solubility below the quantification limit currently available for TNPP (< 0.05 mg/L). The TC NES agreed to go ahead with this range of water solubility.

2 MANUFACTURE AND USES

2.1 Identified uses

Industrial use: stabiliser in the processing of various plastic and rubber products (polyvinylchloride – PVC – film, Polyolefins linear low density polyethylene – LLDPE, High density polyethylene – HDPE rubber).

General public: no identified use

3 CLASSIFICATION AND LABELLING

3.1 Classification in Annex I to Directive 67/548/EEC

No current classification in Annex I to Directive 67/548/EEC or in Annex VI to Regulation (EC) No 1272/2008.

3.2 Self classification(s)

No data.

4 ENVIRONMENTAL FATE PROPERTIES

4.1 Degradation

4.1.1 Stability

Table 2: Degradation of TNPP in air and water

	Value	Reference
Atmospheric degradation (estimated with EPIWIN v3.10)	$kdeg_{air}$ = 3.28 d^{-1} half-life: 0.21 days (5.07 h)	Staples, 2001
Aquatic degradation hydrolysis of TNPP in aqueous media no information on test conditions (pH, temperature) (Test substance: Doverphos HiPure 4-HR (in addition to TNPP, HR grade contains 0.75% of triisopropanol amine, TIPA (added for hydrolytic stability, cf. section 1.2 p. 11!), CAS n°122-20-3): Purity of TNPP: 99.9%, Residual NP: <0.1%).	The TNPP hydrolysis study was performed to determine the extent to which TNPP hydrolyzes to nonylphenol (NP) in aqueous media. Solutions of the TNPP(DP4HPHR) / buffer system (10 mg/L TNPP) were directly injected into a LC-MS, and the amount of nonylphenol was measured. The nonylphenol calibration curve was calculated using the same technique with a branched industry standard nonylphenol. Percent hydrolysis was defined as weight NP * 100/weight TNPP. The level of NP after the first 18.5 hours was assumed equivalent or within experimental error. Thus the hydrolysis of TNPP was less than 0.05%. After 92.5 hours there was a slight increase in the NP level**. Percent hydrolysis was calculated at 0.10%. This level was maintained at 0.1% over 241.5 hours. The formation of NP was approximately 0.1 % after 68 h. The level remained constant the next 6 days (0.15% of NP formed after 241.5 h). Measured concentrations of nonylphenol increased from around 5 µg/L (0, 2, 18.5 h) to 9 µg/L (24 h), 10 µg/L (68 h), and 16 µg/L (92.5 h).**	DAT Laboratories, 2007
Aquatic degradation	A preliminary study on the hydrolysis of (TNPP) was carried out by	TNO, 2004

Preliminary study on the hydrolysis of TNPP (Test substance: Doverphos 4 Hipure)	analyzing the hydrolysis product nonylphenol (NP) as a function of time. The hydrolysis experiments were carried out with TNPP in 10 mM NH4Ac (pH 7.0) at a concentration of 0.1 and 1 µg/L. The solutions were analysed after storage in the dark at 20°C for 1h, 2h, 4h, 16h and 24 h. Under the assumption that the half-life of TNPP was between 13-14 hrs, TNO should have been able to detect NP formed at the two concentrations used (0.1 and 1 µg/L) for the hydrolysis experiment. Also it has been established that NP is likely not adsorbing to the glass containers since TNO was able to find a very good recovery of NP in the calibration solutions prepared similar to the TNPP hydrolysis samples. No NP could be detected in the various hydrolysis solutions above the LOD of 23 ng/L. Moreover, no significant increase in NP could be detected with increasing hydrolysis time up to 24 h. However, to confirm hydrolytic stability in a preliminary test, OECD Test Guideline 111 suggests performing a 5d experiment at 50°C and three pH levels (4, 7, 9). Moreover, according to Appendix II Industry informed that the laboratory had used TNPP with linear NP as reference standard and also the quantification method was developed for linear NP. The commercial TNPP grade tested is supposed to contain largely branched nonylphenyl-chains. So if NP would be formed, it would be the branched NP and that would not have been detected in this study.	
Evidence for TNPP hydrolysis in a test of acute TNPP toxicity to *Daphnia magna* Method: OECD TG 202 (Test substance: Hydrolysed solution of TNPP (TNPP grade according to	Results of this test support the fact that TNPP does produce nonylphenol in water. Indeed, 0.3 mg/L of nonylphenol was formed after leaving TNPP in water for 78 hours at room temperature (detection limit of nonylphenol 0.2 mg/L). Nonylphenol	Hydroqual Laboratories Ltd, 2001a

supplier's characterisation sheet as appended to study report: Doverphos® HiPure 4, CAS 26523-78-4, < 0.1% free nonylphenol).	was only detected in the highest treatment at test initiation (10 mg/L nominal concentration of TNPP).	
Evidence for TNPP hydrolysis in an algal growth inhibition test on TNPP Species : *Pseudokirchneriella subcapitata* Method: OECD TG 201 (Test substance: Hydrolysed solution of TNPP (TNPP grade according to supplier's characterisation sheet as appended to study report: Doverphos® HiPure 4, CAS 26523-78-4, < 0.1% free nonylphenol)	Results of this experiment suggest that hydrolysis was occurring. The test medium contains 0.65 mg/L phosphate. Complete hydrolyses of the test substance (100 mg/L) would yield approximately 12 mg/L of phosphorous acid, and even limited hydrolysis of TNPP as realistically expected would contribute significantly to phosphorous supply of the growth medium. The cell density in the highest test concentration at 72 h was 344 % greater than the controls. This represents approximately 1.5 additional doublings of the cell population exposed to the hydrolysed TNPP solution when compared to the controls. In the control and lowest treatment, maximum cell numbers were already reached after 48h, clearly indicating limitations of growth e.g. due to depletion of an essential nutrient. Continued growth from 48h to 72h in all other treatment levels shows compensation for this limitation. The result indicates that growth stimulation might be caused due to the liberation of phosphorous from TNPP hydrolysis. The samples of the test solutions were analysed for nonylphenol however it was not detected in any of the samples (detection limit of 0.2 mg/L).	Hydroqual Laboratories Ltd, 2001b

Considering all study results together, RAC concludes that despite the very low water solubility of TNPP, the studies do either not confirm full hydrolytic stability or provide evidence for limited hydrolysis leading to nonylphenol concentrations in the range of 5 to 300 µg/L. This conclusion appears also plausible with a view to the ester bonds between the phosphorous- and nonylphenyl-moieties as apparent starting points for hydrolytic action.

4.1.2 Biodegradation

4.1.2.1 Biodegradation estimation

No data.

4.1.2.2 Screening tests

Table 3: Summary of screening tests

Guideline / Test method	Test para-meter	Inoculum		Test substance concentr.	Degradation		Reference
		Type	Adaptation		Incubation period	Degree [%]	
OECD 301D (Test substance: purity of 100% based on a SDS; certificate of analysis not provided)	biological oxygen demand (BOD)	commercial bacterial preparation	No	15.4 mg/L	28 days	< 4% after 28 days	Hydroqual Laboratories Ltd, 2001c
OECD 301B (Purity of TNPP not given)	CO_2 evolution	The inoculum was constituted with activated sludge collected from the sewage treatment plant of Reinach (Switzerland)	The inoculum was pre acclimated to the test medium over night	18.1 mg/L	29 days	1% after 29 days	CIBA-Geigy, 1994

4.1.2.3 Simulation tests

No data.

4.1.3 Summary and discussion of persistence

TNPP is not readily biodegradable in aquatic environments. However, it has been shown that the substance can be hydrolysed into nonylphenol, this hydrolytic product is inherently biodegradable (EC 2002) and classified accordingly (cf. section 1.2, impurities).

Although it cannot be totally ruled out that there might be environmental conditions where significant hydrolysis could occur, hydrolytic degradation of TNPP in the aquatic environment is considered rather limited at 20°C and pH 7 (percent hydrolysis was calculated at 0.1% with 0.15%

of nonylphenol formed after 241 h). This is based on the expected very low water solubility of the substance that would not enable hydrolysis to occur in large amount. Furthermore, the high hydrophobicity of TNPP (high log Kow) will contribute to a large adsorption of the substance on sediment when entering the aquatic compartment thus reducing its availability for hydrolysis. However, resulting low concentrations of nonylphenol are classification relevant due to the high toxicity of this degradation product.

Based on these available studies, we can conclude that TNPP is not rapidly degradable in the environment according to the CLP Regulation.

4.2 Environmental distribution

4.2.1 Adsorption/desorption

The partition coefficients for TNPP have been calculated using EUSES (E.C., 2004) based on a log Kow > 10. These partition coefficients should be interpreted with care, as they are based on a highly uncertain log Kow value, and are presumably outside the domain of the models. They are presented as an example in the following table:

Table 4: Calculated partition coefficients for TNPP with a Log Kow > 10 (actual calculation with Log Kow = 14)

K_{oc}	$2.76x10^{11}$	Partition coefficient organic carbon-water ($L.kg^{-1}$)
Kp_{susp}	$2.76\ x10^{10}$	Partition coefficient solid-water in suspended matter ($L.kg^{-1}$)
Kp_{sed}	$1.38\ x10^{10}$	Partition coefficient solid-water in sediment ($L.kg^{-1}$)
Kp_{soil}	$5.51\ x10^{09}$	Partition coefficient solid-water in soil ($L.kg^{-1}$)
$K_{soil\text{-}water}$	$8.27\ x10^{09}$	Soil-water partition coefficient ($m^3.m^{-3}$)
$K_{susp\text{-}water}$	$6.89\ x10^{09}$	Suspended matter-water partition coefficient ($m^3.m^{-3}$)
$K_{sed\text{-}water}$	$6.89\ x10^{09}$	Sediment-water partition coefficient ($m^3.m^{-3}$)

The high hydrophobicity of TNPP (high log Kow) will contribute to a large adsorption of the substance on sediment when entering the aquatic compartment.

4.2.2 Volatilisation

A Henry's law constant between 799 and $1.33x10^{17}$ $Pa.m^3.mol^{-1}$ was calculated from TGD estimation (eq 21) using a vapour pressure of 0.058 Pa, a molecular weight of 689 $g.mol^{-1}$ and a water solubility of <0.05 $mg.L^{-1}$ (the lowest value obtained using the QSAR result for the water solubility was $3x10^{-16}$ mg/L). These values should be interpreted with care due to the high uncertainty in the water solubility values.

The resulting air-water partition coefficient ($K_{air\text{-}water}$) would then range between 0.337 and $5.62x10^{13}$ $m^3.m^{-3}$ by EUSES v2.1. However, considering the hydrophobicity and the strong adsorption potential of the substance, volatilisation of TNPP from water is not expected to be a major phenomenon.

4.2.3 Distribution modelling

Not relevant for this report.

4.3 Bioaccumulation

4.3.1 Aquatic bioaccumulation

4.3.1.1 Bioaccumulation estimation

A calculated BCF of 3.162 L/kg has been obtained using EpiWin (log Kow used 20.05 (estimated)).

Using EUSES v2.1 calculation, a bioconcentration factor of 479 L/kg could be calculated for fish taking into account a log Kow >10 (the worst case for BCF obtained when using the parabolic equation giving the BCF for fish based on the K_{ow}, (E.C., 2003)).

4.3.1.2 Measured bioaccumulation data

Measured data on bioaccumulation of TNPP are not available.

4.3.2 Terrestrial bioaccumulation

Not relevant for this report.

4.3.3 Summary and discussion of bioaccumulation

The bioaccumulation factors calculated for TNPP based on log Kow of 8 and >10 as a worst case indicate a high bioaccumulation potential. Nevertheless, the bioaccumulation potential of TNPP based on these calculations should be considered with precaution for the following reasons:

- molar weight is near 700 g/mol (689 g/mol) and certain classes of substances with molecular mass greater than this threshold are not readily taken up by fish and are unlikely to bioaccumulate significantly.
- Information on the molecular size of TNPP is also available (personal communication, Kazumi Kawahara, CERI, 20th October 2005). Based on this study, it seems that, taking into account the calculated molecular size of TNPP, the bioaccumulation potential is negligible. The calculation of the mean diameter for six different three dimension structures of TNPP has led to a lowest value of 13.9 Å. This conclusion has been reached based on a cut-off value for the ability of a chemical to pass through fish gill membrane has been established at 9.5 Å (Opperhuizen *et al.*, 1985). However, it should also be considered that the current cut-off value proposed by the REACH Guidance is a mean diameter higher than 17 angstroms.

- A worst case value has been taken into account for the calculation of BCFs for TNPP. However, there are some indications that the Kow of TNPP could be much higher than this value (HPLC method estimated log Kow > 10).

- The molecular dimensions (D_{max}[1] and D_{eff}[2]) of two representative isomers of commercial TNPP were estimated with a demonstration version of Molecular Operating Environment software (version 2006.08) (Schocken, 2007). The TNPP isomers, comprised of nonylphenol ligands that are "slightly or highly branched" were each sorted into their lowest potential energy state conformations in aqueous solution and the lowest-energy conformations averaged to obtain the requisite molecular dimensions. The approach taken was to use two different programs of MOE, namely, conformational import and dynamics simulation. Results showed that D_{max} average, currently considered the most important molecular dimension and defined as the average diameter of the smallest spheres circumscribing the low-energy conformations for a given TNPP isomer, ranged from 23.7 Å for the slightly branched TNPP isomer to 22.8 Å for the highly branched TNPP isomer using the conformational import approach and from 24.3 Å to 21.2 Å for the slightly branched and highly branched TNPP isomer using the dynamics simulation method, respectively. These values all exceed the 17.4-Å cutoff currently used to preclude absorption of organic chemicals via fish gills. Coupled with TNPP's high experimentally determined log Kow >10 and its high molecular weight (689 grams/mole), it is unlikely that this chemical would be bioaccumulative in the aquatic environment.

- Mammalian toxicity of TNPP is described in section 5 of this report. In animals, TNPP has a very low acute toxicity by the oral route, with a LD50 value of about 19.5 +/- 3.3 g/kg bw for the rat. Two-year studies provide a profile of limited repeated dose toxicity for TNPP. In these 2-year studies, 3300 ppm of TNPP in the diet (corresponding to 167 mg/kg/d in rats), was derived as a NOAEL, both for rat and dog.

The low mammalian toxicity of TNPP could be linked to a limited absorption potential. However in the absence of specific toxicocinetic study, only quantitative information was derived from the physico-chemical properties of the substance.

According to Commission Regulation (EC) No 466/2008 of 28 May 2008, information on the structure of TNPP is required. The TC NES confirmed the need for modelling the TNPP structure using OASIS. This information remains not available to date. Consequently, it is not possible to conclude on the bioaccumulation based on a weight of evidence approach. However, the cut-off value of log Kow ≥ 4 (as the experimental BCF measure is not available) set out in the CLP Regulation is exceeded.

4.4 Secondary poisoning

Not relevant for this report.

[1] Defined as the diameter of the smallest sphere into which the molecule may be placed.

[2] Defined as the diameter of the smallest cylinder into which the molecule may be placed.

5 HUMAN HEALTH HAZARD ASSESSMENT

All studies included in this section were previously reviewed under the Existing Substances Regulation. No relevant new data has been identified and included here.

When considered useful in the view of a discussion on the relevance of classification due to impurities, some additional toxicological data is displayed in the present dossier for information.

The only health endpoint proposed for harmonisation is however skin sensitisation.

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

No specific toxicocinetic study was conducted with trisnonylphenyl phosphite.

5.2 Acute toxicity

5.2.1 Acute toxicity: oral

Table 5: Acute toxicity by oral route

Species	LD_{50} (mg/kg)	Observations and Remarks	Ref.
Rat	19.5 +/- 3.3 gram/kg bw (TNPP purity not givzn)	Gross pathological findings included hemorrhagic lesions in the gastric mucosa and/or duodenum in a few rats that died, and hemorrhagic lungs.	Food and Drug Research Laboratories, 1957.
Rat	> 10.0 ml/kg bw (eq. to 9.8 g/kg bw) (TNPP purity: not known; considered to be 100%)	No mortality occurred during the study	Hill Top Research, 1965

5.2.2 Acute toxicity: inhalation

No data

5.2.3 Acute toxicity: dermal

Table 6: Acute toxicity by dermal route

Species	LD_{50} (mg/kg)	Observations and Remarks	Ref.
Rat	> 2000 mg/kg bw (TNPP purity not given)	No mortality occurred during the study	Tay, 2001a
Rat	> 2000 mg/kg bw (TNPP purity > 94%)	No mortality occurred during the study	Ciba-Geigy, 1992

5.2.4 Acute toxicity: intraperitoneal routes

Table 7: Acute toxicity by IP route

Species	LD_{50} (mg/kg)	Observations and Remarks	Ref.
Rat	> 1000 mg/kg bw (TNPP purity not given)	No mortality occurred during the study	Ciba-Geigy, 1983

5.2.5 Summary and discussion of acute toxicity

➔ According to the criteria of the Directive 67/548/EEC and of the CLP Regulation, this chemical doesn't need to be classified on the basis of its acute toxicity (LD_{50} > 2000 mg/kg by oral and dermal route).
Information for this endpoint is given for information only.

5.3 Irritation

5.3.1 Skin

Table 8: Skin irritation

Species	N° of animals	Exposure time (h/day)	conc. (wt/wt)	Dressing : Occlusive semi-occlusive open	Observations and remarks (specify the experimental conditions, score and evaluation method)	Ref.
Rabbit	3	4 hours	A dose of 0.5 ml liquid test substance (TNPP purity: 99.3%)	Semi-occlusive	Very slight erythema was observed in three out of three rabbits following a 4-hour exposure. By the 24-hour observation point, the irritation was reversed. OECD 405 Reactions graded according to the Draize scoring scale.	Tay, 2001b
Rabbit	6	24 hours	A dose of 0.5 ml liquid test substance (TNPP purity not given)	Occlusive	In 3/6 animals, the application sites showed necrosis. In 5/6 animals the erythemas extended beyond the treated areas. Erythema and edema of intact skin were reversed within 7 days. Reactions graded according to the Draize scoring scale.	Ciba-Geigy, 1981

5.3.2 Eye

Table 9: Eye irritation

Species	N° of animals	Exposure time (h/day)	conc. (wt/wt)	Observations and remarks (specify the experimental conditions, score and evaluation method)	Ref.
Rabbit	4	Single instillation, unrinsed (TNPP purity: 99.3%)	0.1 ml of the undiluted test substance	Slight conjunctival redness and chemosis were observed at the 1-hour observation point and were resolved within 24 to 48 hours. OECD 404	Tay, 2001c

Rabbit	6	Single instillation rinsed within 30 seconds in half on the animals (TNPP purity not given)	0.1 ml of the undiluted test substance	Reversible slight redness of conjunctiva and chemosis were observed. Mean scores for conjunctive redness were 1, 0.3 and 0.7 at 24, 48 and 72h, respectively in non rinsed eyes (mean 0.7). Mean scores for chemosis were 1, 0.3 and 0.3 at 24, 48 and 72h, respectively in non rinsed eyes (mean 0.6).	Ciba-Geigy, 1981

5.3.3 Respiratory tract

No data

5.3.4 Summary and discussion of irritation

➔ According to the criteria of the Directive 67/548/EEC and of the CLP Regulation, this chemical doesn't need to be classified as an irritant to the skin nor to the eye.

Indeed, for skin irritation, the conclusion is based on the guideline study with semi-occlusive application that shows mean 24-48-72h scores of 0 for both erythema and edema (reversibility of erythema already observed at 24h).

For eye irritation, reversible effects were observed on conjunctiva with mean 24-48-72h scores below 2 in both studies.

Information for this endpoint is given for information only.

5.4 Sensitisation

5.4.1 Skin

Table 10: Skin sensitisation

Species	Type o f test	N^{o} of animals (c, t)	Incidence of reactions observed (c, t)	Ref.
Guinea pig	Maximisation Test OECD 406 (TNPP purity > 94%) Induction with 5% TNPP intradermal and 10% topical. Challenge with 1% TNPP.	c : 10 t : 20	There were 12/20 (60%) and 15/20 (75%) positive animals respectively 24h and 48h after occlusive epidermal application (showing erythema scores of 1 to 2) and none in the negative control group.	Ciba-Geigy, 1992d
Guinea pig	Buehler Sensitisation Test OECD 406 Challenge and induction with neat substance. (TNPP purity: 99.3%)	c: 15 t : 20	All animals showed no sign of erythema or oedema at the 24 and 48-hour observation points for the challenge phase.	Tay, 2001d

c : control group ; t : test group

5.4.2 Respiratory system

No data

5.4.3 Summary and discussion of sensitisation

➔ According to the guidance on the application of the CLP criteria (CLP guidance), a substance may be classified as skin sensitiser on the basis of a positive test result in one of the animal tests 1) mouse local lymph node assay (LLNA), 2) guinea pig maximisation test (GPMT) and 3) Buehler occluded patch test. The positive result in the maximisation test (more than 30% of animals with a positive reaction in an adjuvant type guinea pig test method) warrants classification with R43 (Skin Sens. 1 – H317 according to the CLP Regulation). The observed dose-response relationship, i.e. 5% intradermal induction and 75% incidence of sensitised guinea pigs, corresponds to a “moderate” potency according to the CLP guidance, which is covered by the generic concentration limit of 1%. Regarding the purity of the tested TNPP grade (> 94%), up to < 5% nonylphenol (NP) might contribute to the test results. However NP is not classified as skin sensitiser and the GPMT with technical TNPP is thus considered sufficiently valid for classification.

5.5 Repeated dose toxicity

5.5.1 Repeated dose toxicity: oral

Table 11: Repeated toxicity by oral route

Species	Dose mg/kg/body weight mg/kg diet	Duration of treatment	Observations and Remarks	Ref.
Rat	NOAEL = 1% TNPP in the diet (about 1000 mg/kg bw)	90 days	Pathological changes were observed in the lung and the kidney.	Food and Drug Research Laboratories, 1957
Rat	NOAEL = 3300 ppm in the diet (about 167 mg/kg bw)	2 years	Limited observed effects (slight retardation of growth in males and elevation of the liver weight in F0 females at the highest dose level).	Food and Drug Research Laboratories, 1961
Dog	NOAEL = 3300 ppm in the diet	2 years	Limited observed effects (chronic inflammation in renal pelvis in one male dog at the highest dose level, slight to moderate degree of hyperplasia of the thyroid (with focal collections of lymphocytes) in two female dogs at the highest dose level) group	Food and Drug Research Laboratories, 1961
Rat	NOAEL = 200 mg/kg bw for males NOAEL > 1000 mg/kg bw for females	4 weeks for F0 males 10 weeks for F0 females 85 days for F1 generation	Renal lesions observed in F0 and F1 males.	Tyl et al., 2002

5.5.2 Repeated dose toxicity: inhalation

No data

5.5.3 Repeated dose toxicity: dermal

No data

5.5.4 Summary and discussion of repeated dose toxicity:

➔ According to the criteria of the Directive 67/548/EEC and of the CLP Regulation, this chemical doesn't need to be classified on the basis of its repeated dose toxicity (absence of significant and/or severe effects at doses relevant for classification).

Information for this endpoint is given for information only.

5.6 Mutagenicity

Not evaluated in this dossier

5.7 Carcinogenicity

Not evaluated in this dossier

5.8 Toxicity for reproduction

RAC did not further scrutinise the information on reproductive toxicity. The dossier submitter had incorporated selected information just to supply a contingent discussion on the role of nonylphenol (NP) impurities. However, during further processing of the CLH proposal it has been clarified that NP impurities have to be dealt with according to articles 10, 11 of the CLP Regulation, but are not to be covered by the proposal for harmonised classification of TNPP. A potential classification for reproductive toxicity of TNPP was previously discussed at TC C&L (see summary records in Appendix I). TC C&L finally concluded no classification justified. Since then, neither the dossier submitter nor the public consultation revealed new information on reproductive toxicity of TNPP to be considered and prepared for the present CLH proposal.

5.8.1 Effects on fertility

Table 12: Effects on fertility

Species	Route	Dose	Number of generations exposed	Observations and Remarks	Ref.
Rat	Oral (diet)	50, 167 and 500 mg/kg/d NOAEL for reproduction : 167 mg/kg/day) (TNPP purity not given)	3 (F0 to F3)	Growth was normal at all dosage levels in F0, F1 and F2 females. At the dose level of 500 mg/kg/d, there was a slight but statistically significant retardation in growth of the F2 (p=0,001) and F3 (p=0,05) males and of the F3 females (p=0,001), along with a decrease in the efficiency of food utilisation for F2 males (p=0.05) at the highest dose and F3 females at the 2 highest doses used (p=0.001). In F3 females, the decrease of food utilisation efficiency was dose related. There was no indication of adverse effect in the F0 generation at any dose level. Diminution in the number of pups born per litter in the F1 and F2 high dose groups, and a small decrease in the fertility and viability indexes in F2 at this same high dose level exposure were observed (see table 12a below).	Food and Drug Research Laboratories, 1961
Rat	Oral (gavage)	50, 200 and 1000 mg/kg/d NOAEL for maternal and offspring toxicity = 200 mg/kg/day (TNPP	1 (F0 to F1) Modified OECD 421*	Effects were only observed in the highest dose group and were the following : - Three of ten pregnant F0 females at 1000 mg/kg/day died in late pregnancy (gestation day 22). These deaths may have been related to dystocia, since the dams appeared to be unable to deliver their normal appearing pups. Two F0	Tyl et al., 2002

		purity: 99.98%)		females respectively exposed to 50 mg/kg/day (at GD20) and 1000 mg/kg/day (at lactation day 15) were also found dead. But these deaths were attributed to dosing errors and were not considered treatment related. - Ovary weights (absolute and relative to terminal body and brain weights- see table 12b for details) were significantly decreased at 1000 mg/kg/day in F0 but not F1 adult females. These findings were not related to microscopic findings - There was a reduction of the litter size on pnd0 observed at 1000 mg/kg/d and significant on pnd4 (see table 12c below). - In F1 males, paired epididymides weight, relative to terminal body weights, were significantly decreased at 1000 mg/kg/day (see table 12d below). Mating, fertility, pregnancy and gestational indices were equivalent across groups ; gestational length was equivalent across all groups. Andrology parameters, time of vaginal opening, preputial separation, normality and length of oestrous cycles were also checked and did not reveal any changes compared to control.	

* The modified OECD TG 421 exceeds the OECD TG 421 study design as follows: enhanced evaluation of toxicity in the F0 generation, including the evaluation of a recovery group of males; evaluation of developmental landmarks in the F1 generation (time of vaginal opening or preputial separation, normality and length of oestrous cycle) ; and following the F1 offspring to adulthood, with continued exposure and assessment of reproductive structures and functions including potential effect on sperm.

Table 12a : Comparison of first two matings in three generations of rats (FDRL, 1961)

Dose	**Generation**	**Total No. of mating**	**No. litters born alive**	**Pups born alive**	**Pups per litter born**	**No. litters weaned**	**Average weight of pups at weaning[1]**	**F.I.[2]**	**G.I.[3]**	**V.I.[4]**	**L.I.[5]**
Mg/kg							**Gm**				
None	**F0**	49	41	328	8.0	34	40.0	98.0	82.9	87.2	96.2
	F1	20	19	216	11.3	19	36.3	95.0	100.0	87.0	89.5
	F2	20	17	151	8.9	16	42.7	90.0	94.5	93.2	87.5
50	**F0**	49	40	354	8.8	36	36.5	91.8	90.0	91.8	88.0
	F1	20	20	213	10.7	20	41.6	100.0	100.0	96.0	90.0
	F2	20	19	159	8.4	16	40.0	95.0	94.5	87.6	81.1
167	**F0**	50	45	415	9.2	41	37.9	94.0	95.7	95.7	87.7
	F1	20	20	212	10.6	20	40.1	100.0	100.0	95.5	94.5
	F2	20	19	151	8.0	12	42.6	95.0	100.0	94.5	71.0
500	**F0**	48	40	337	8.4	37	36.0	100.0	83.3	93.8	87.3
	F1	17	16	113	7.0	13	36.0	100.0	100.0	93.5	96.0
	F2	20	17	122	7.3	13	43.8	85.0	100.0	79.7	89.7

[1] At 21 days
[2] Fertility index = (No. pregnancies / No. matings) X 100
[3] Gestation index = (No. litters born alive / pregnancies) X 100
[4] Viability index = (No. pups at 1d. / No. pups born alive) X 100
[5] Lactation index = (No. pups at 21d. / No. pups at 1d.) X 100

Table 12b : Summary and Statistical analysis of the F0 female paired ovary weight (absolute and relative) (Tyl et al., 2002)

	Trisnonylphenyl Phosphite (mg/kg/day)			
	0	50	200	1000
Paired ovary weight (g)	0.1488 ± 0.0041 N = 10	0.1426 ± 0.0062 N = 9	0.1512 ± 0.0077 N = 10	0.1137 ± 0.0010 ** N = 5[a]
Relative Paired ovary weight (% sacrifice weight)	0.0456 ± 0.0016 N = 10	0.0458 ± 0.0028 N = 9	0.0466 ± 0.0023 N = 10	0.0355 ± 0.0009 N = 5[a]

** p< 0.01 ; Dunett's test for pairwise comparisons to control
* p< 0.05 ; Dunett's test for pairwise comparisons to control
[a] Decrease in N is due to one paired ovary weight being a statistical outlier and therefore it was excluded.

Table 12c: Summary of F1 offspring toxicity (Tyl et al., 2002)

	Trisnonylphenyl Phosphite (mg/kg/day)			
	0	50	200	1000
N° of live litters Postnatal Day 0	10	8	10	7
N° of live litters Postnatal Day 4	10	7[a]	10	7
Average number of live pups per litter (pnd 0)	14.9 ± 0.5	12.8 ± 1.6	15.9 ± 0.6	12.0 ± 1.4
Average number of live pups per litter (pnd 4, precull)**	14.8 ± 0.5	14.3 ± 0.6	15.6 ± 0.5	12.0 ± 1.4*

[a] The entire litter for female 30 was missing and presumed dead on postnatal day 4.
* p< 0.05 ; Dunett's test
** p<0.01; Test for linear trend

Table 12d: Summary and Statistical analysis of the F1 male paired epididymides weight (absolute and relative) (Tyl et al., 2002)

	Trisnonylphenyl Phosphite (mg/kg/day)			
	0	50	200	1000
Paired epididymes weight (g)	1.1557 ± 0.0209 N = 10	1.1229 ± 0.0233 N = 10	1.1663 ± 0.0162 N = 10	1.1189 ± 0.0215 N = 10
Relative Paired epididymes weight (% sacrifice wt)**	0.2335 ± 0.0070 N = 7	0.2226 ± 0.0074 N = 10	0.2321 ± 0.0057 N = 10	0.2168 ± 0.0027* N = 10

* p< 0.05 ; Individual t-test for pairwise comparisons to control in robust regression model
** p< 0.05 ; Linear trend test in robust regression model

5.8.2 Developmental toxicity

Table 13: Developmental toxicity

Species	Route	*dose mg/kg/day ppm **Conc. (mg/L)	Exposure period : - number of generations or - number of days during pregnancy	Observations and remarks	Ref.
Rat	Oral (gavage)	50, 200 and 1000 mg/kg/d NOAEL terato > 1000 mg/kg/day (TNPP purity: 99.98%)	Exposure during the whole pregnancy Modified OECD 421*	No OECD TG 414 test was provided. Information on developmental toxicity was derived from a screening test according to a modified OECD TG 421*. In this study, no developmental effect was observed, up to the dose level of 1000 mg/kg/day, whether on pnd4 or 21.	Tyl et al., 2002

* The modified study design used in this study provides, for continuation of the F1 offspring, with continuing exposure until sexual maturity. Thus, to provide data on the pnd 4 pups, the pups culled to standardise litters on pnd 4 were euthanised and subjected to complete gross necropsy, but this was done on a very reduced number of pups, since F1 litters were culled on pnd 4 to yield, as nearly as possible, five males and five females per litter. This leads to nearly 2 animals in the highest dose group and 4 in the other groups. The other pups were subjected to a complete gross necropsy at weaning (pnd 21), except for at least one male and one female per litter that were selected to continue treatment for seven more weeks.

5.8.3 Human data

No data

5.8.4 Summary and discussion of reproductive toxicity

➔ According to the criteria of the Directive 67/548/EEC and of the CLP Regulation, this chemical doesn't need to be classified as toxic to reproduction based on the following rationale:

– The effect on reproductive organ weight seen at a high dose in the screening one-generation study (OECD 421) is not considered sufficient to provide evidence of a toxicity to fertility in absence of histological damages or direct effects on fertility in this study and considering the absence of effects related to fertility in the 3-generation study. Phenomenon of dystocia observed in dams at the highest dose in the study of Tyl (2002) is viewed as

maternal toxicity, due from the adjustments of dosing volume on gd 14 and especially on GD 20, resulting in over dosing the dams in late gestation.

– Absence of observation of significant developmental effects.

Information for this endpoint is given for information only, see also introduction to section 5.8.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this dossier

7 ENVIRONMENTAL HAZARD ASSESSMENT

Due to analytical method limits (the water solubility of TNPP is below the quantification limit of the substance) all the test results for TNPP are based on nominal concentrations.

All the studies set out in this report on the environmental aspects (except the long-term Daphnia study) have been reviewed under the Existing Substances Regulation (ESR).

The key study used for a harmonised environmental classification for TNPP is the short-term Daphnia magna study performed by Hydroqual Laboratories Ltd (2001a).

Additional ecotoxicological data is displayed in the present report for information.

7.1 Aquatic compartment (including sediment)

7.1.1 Toxicity test results

7.1.1.1 Fish

Acute toxicity to fish

The following table shows a summary of the acute toxicity tests that were performed with fish species. The toxicity limits reported are above the upper limit of the estimated water solubility (solubility < 50 µg/L).

Table 14: Acute toxicity to fish

Test #	Species	References	Comment	Validity*
1	Species: *Oncorhynchus mykiss* LC_{50} (96 hours) : No effect up to the limit of water solubility Test condition: Static Method: OECD TG 203 (TNPP purity: 99.8%) NP limit of detection 0.2 mg/L	Guterson, 2001	The acute toxicity of an hydrolysed solution of TNPP (purity 99.8%) has been tested on *Oncorhynchus mykiss* according to the OECD guideline 203. The fish were held 33 days before initiating the test on TNPP. Mortality in the stock culture was less than 0.1 % the week prior to test initiation. The fish were fed a daily ration of trout chow equal to 5 % of their body weight but were not fed 24 h prior to test initiation or during the test. The dilution water was dechlorinated City of Calgary tap water (charcoal filtered and aerated) and had a hardness of 198 mg CaCO3/L, alkalinity of 140 mg CaCO3/L, pH of 7.6, and a conductance of 446 ms/cm. **The test solutions were prepared from a stock solution initially containing 100.0 mg/L of TNPP.** The solutions were gently aerated for 78 h at room temperature (20 ± 2 °C). The supernatants containing the hydrolysis products of TNPP were then decanted for preparation of the test solutions. The stock solutions and 200 L of dilution water were cooled to the test temperature overnight in a controlled environment chamber (15 °C with aeration). At test initiation, dissolved oxygen, temperature, and pH ranged from 8.7 to 9.2 mg/L (98% to 100% saturation), 14 to 16 °C, and 7.7 to 8.0 units, respectively. At test termination, the temperature and pH of the test solutions were 15 °C and 7.8, respectively. Dissolved oxygen levels ranged from 6.2 to 6.8 mg/L (69 to 75 % saturation). The test solutions were only analysed for nonylphenol (detection limit of 0.2 mg/L) but nonylphenol was not detected in any of the test solutions collected at test initiation and termination. There were no signs of stress or unusual behaviour exhibited by the fish in any of the treatment concentrations. No fish died at any concentration at any time point**.** The highest non-lethal concentration tested was set as greater than or equal to the 100 mg/L of TNPP hydrolysis products. LC50 was > 100 mg/L after 24, 48, 72 and 96h. This study should be considered as valid with restrictions. Indeed, tested concentrations were far above the water solubility of TNPP. Nonylphenol has been measured but not detected in any sample. The way test solutions were prepared should have enable the observation of effects triggered off by metabolites (nonylphenol). The result from this test can be used to support the fact that no toxicity of TNPP is expected above its water solubility (< 50 µg/L).	2

Test #	Species	References	Comment	Validity*
2	Species: *Brachydanio rerio* LC_{50} (96 hours) < 10 mg/L LC_{50} (48 hours) = 16 mg/L Test condition: Static Method: Dir. 84/449/EEC C.1 (TNPP purity > 94%) No analytical measurements of TNPP or NP in treatments	CIBA-Geigy, 1992a	The acute toxicity of TNPP (purity >94%) has been tested on *Brachydanio rerio* according to Directive 84/449/EEC, C.1. **Five concentrations plus one control were tested (10, 18, 32, 58 and 100 mg/l).** The control was performed in the test medium, i.e. dechlorinated tap water with an hardness of 171 mg CaCO3/L. Other test parameters were as follow: pH between 7.3 and 7.9, temperature = 22 +/- 1°C. During the test, 10 fish were disposed per aquarium. They were acclimated 125 days prior the test and adapted to test medium 24 hours prior testing and no food was delivered 24 hours prior to exposure. A gentle aeration was started after 48 hours exposure. The test was conducted under a fluorescent light, 16 hours daily. The stock solution contained a mixture of 4 g. test substance and 160 mg Alkylphenol-Polyglycol-Ether (ARKOPAL) completed to 2 L with water. During the test, the oxygen saturation ranged from 89-97% at 24 hours, 68-83% at 48 hours, and 60-76% at 72 hours. In the preliminary test, 10 mg/L TNPP had no effect to the fish after 96 hours of exposure. In the main test, 10 mg/L showed no effect to the fish after 48 hours. However, the oxygen concentration in the water was determined to be low at 48 hours and a gentle aeration was started at this time. After 72 hours of exposure with the test substance, all fish were dead. It is also important to notice that undissolved test substance was observed at the surface of the test vessels. No LC50 could be estimated after 96h but some results were calculated at intermediate times: LC50(48h)=16 mg/L (95% CL 12-19 mg/L) ; LC50(24h)=29 mg/L (95% CL 23-36 mg/L). No mortality occurred in blank and in the vehicle controls. This study has to be considered as invalid for the following reasons: - The tested concentrations were probably very far above the actual water solubility of the substance. - No analytical follow-up of the test concentrations was performed. As there was no equilibration time to allow dissolution of the substance during the preparation of the test concentration, it is not even clear that the maximum solubility in the test medium was achieved. The report mentions that undissolved substance was observed at all test concentrations. - The tested TNPP grade does not exclude that observed effects have to be attributed to nonylphenol present as impurity (5% nonylphenol could result in 0.5 to 5 mg/L NP in the treatment levels and all of these concentrations are well in the range of NP fish toxicity reported in the EU RAR for NP).	3

Test #	Species	References	Comment	Validity*
3	Species: *Leuciscus idus* LC_{50} (48 hours, estimated) = 7.1 mg/L Test condition: Static Method: DIN 38412-L15 (Purity of TNPP: commercial grade; no further information available.) No analytical measurements of TNPP or NP in treatments	CIBA-Geigy, 1988a	A static test was performed with *Leuciscus idus*. Test organisms were acclimated 22 days with no food distribution three days prior to testing and for the test, mean fish size and weight were respectively 44 mm (35-50 mm) and 0.59 g. (0.29-0.85 g.). This led to a loading of 0.39 g/L in the test aquariums (test volume = 15 L.). 10 fish were disposed per concentration and control and dechlorinated tap water was used as dilution water. A hardness of 254 mg CaCO3/L (Ca/Mg = 4/1) was measured. During the test, dissolved oxygen, pH and temperature were measured at 0, 24 and 48 hours: [O2] > 91% saturation, pH = 7.9-8.2 and T = 20 +/- 1°C. The test medium was gently aerated during the test and a fluorescent light was used 16 hours a day. The stock solution of TNPP was prepared using a vehicle solvent, DMF. 5 g. of TNPP were dissolved in made up to 50 mL with DMF. This resulted in a concentration of DMF of 950 mg/L for the highest TNPP concentration tested. **Fish were exposed during 48 hours to six TNPP concentrations (5.8, 10, 18, 32, 58 and 100 mg/L)** plus a blank and a control with the vehicle solvent used. Different symptoms were observed at the different test concentrations: moderate effects on swimming behaviour were observed after 24 and 48 hours at the concentration of 5.8 mg/L. Slight effects on the respiratory function has been observed after 48 hours, at 5.8 mg/L (one fish died at this concentration). All fish died at concentrations down to 10 mg/L. A LC50 of 7.1 mg/L was calculated. This study has to be considered as invalid for the following reasons: - The tested concentrations were probably very far above the actual water solubility of the substance. - No analytical follow-up of the test concentrations was performed. As there was no equilibration time to allow dissolution of the substance during the preparation of the test concentration, it is not even clear that the maximum solubility in the test medium was achieved. - Due to lack of information about the tested TNPP grade it is not excluded that observed effects have to be attributed to nonylphenol present as impurity (e.g. 5% nonylphenol could result in 0.29 to 5 mg/L NP in the treatment levels and all of these concentrations are well in the range of NP fish toxicity reported in the EU RAR for NP).	3

* 1 = valid; 2 = valid with restrictions; 3 = invalid; 4 = not assignable

Chronic toxicity to fish

No chronic toxicity test with fish is available.

7.1.1.2 Aquatic invertebrates

Acute toxicity to aquatic invertebrates

The following table shows a summary of the acute toxicity tests that were performed with aquatic invertebrate species.

Table 15: Acute toxicity to aquatic invertebrates

Test #	Species	References	Comment	Validity
1	Species: *Daphnia magna* TNPP (nominal) EC_{50} (48 hours) = 0.3 mg/L NP (estimated) EC_{50} (48 hours) = 0.009 mg/L Test condition: Static Method: OECD TG 202 (Test substance: Hydrolysed solution of TNPP – TNPP grade according to supplier's characterisation sheet as appended to study report: Doverphos® HiPure 4, CAS 26523-78-4, < 0.1% free nonylphenol) NP limit of detection 0.2 mg/L	Hydroqual Laboratories Ltd, 2001a	The test was initiated with young daphnids less than 24 h old from in-house cultures. Mortality in the stock culture was less than 1% in the week prior to test initiation. Dilution water was dechlorinated City of Calgary tap water (charcoal filtered and aerated). The dilution water had a hardness of 188 mg CaCO3/L, alkalinity of 100 mg CaCO3/L, pH of 8.1, and conductivity of 421 ms/cm. The ratios of calcium-to-magnesium and sodium-to-potassium on a weight-to-weight basis were 3.4 and 4.0 respectively. The concentration of dissolved oxygen was 8.2 mg/L (100 % saturation at the test temperature 20 +/- 1°C). The test solutions were prepared from a stock solution initially containing 100 mg/L of TNPP. The mass of TNPP selected for the test was based on initial attempts to get enough of the hydrolysis products in solution to be acutely toxic to *Daphnia magna*. The method detailed below provided a stock solution that was acutely lethal to *Daphnia magna*. TNPP (100 mg) was weighed onto a glass Petri dish. The dish and test substance were placed into a two-litre, glass Erlenmeyer flask containing 1 L of dilution water. A magnetic stir bar was added and the mouth of the flask sealed with Parafilm®. The test substance was gently stirred for 78 h at room temperature (20 ± 2 °C). The supernatant containing the hydrolysis products of TNPP was then decanted for preparation of the test solutions. **A stock was prepared from the hydrolysed TNPP solution by diluting 100 mL of the supernatant with 900 mL of dilution water (10 mg/L nominal, highest test concentration). This solution was then serially diluted with laboratory dilution water to obtain the other eight test concentrations (5.00, 2.50, 1.25, 0.63, 0.31, 0.16, 0.08, and 0.04 mg/L). The concentrations were nominal values based on the total mass of TNPP initially added to the flask and hydrolysed for 78 h (100.0 mg/L).** The organisms were then added to the test vessels in a random fashion (final loading density of one organism per 10 mL of test solution). There were four replicates for each test concentration containing 5 daphnids. The daphnids were not fed during the test. Beakers were placed on a tray and covered with a glass sheet. The test was conducted at conditions similar to the culture conditions. The test vessels were examined at 24 and 48 h, and the number of immobilised organisms recorded along with any observations of unusual behaviour. The samples of the test solutions were analysed for the major hydrolysis product of TNPP, nonylphenol. Nonylphenol was only detected in the highest treatment (10 mg/L TNPP) at test initiation (0.3 mg/L NP based on the results of duplicate analyses;	2

Test #	Species	References	Comment	Validity
…1 cont	Species: *Daphnia magna* TNPP (nominal) EC_{50} (48 hours) = 0.3 mg/L NP (estimated) EC_{50} (48 hours) = 0.009 mg/L Test condition: Static Method: OECD TG 202 (Test substance: Hydrolysed solution of TNPP (TNPP grade according to supplier's characterisation sheet as appended to study report: Doverphos® HiPure 4, CAS 26523-78-4, < 0.1% free nonylphenol) NP limit of detection 0.2 mg/L	Hydroqual Laboratories Ltd, 2001a	detection limit of 0.2 mg/L). Toxicity values were derived based on this measured concentration of nonylphenol. The test concentrations for toxicity values were derived from the single measured value available for nonylphenol (starting value that was serially diluted by a factor of 2 to obtain the numerical values for the test concentrations, all of which were below the detection limit of 0.2 mg/L for nonylphenol). At test initiation the concentration of dissolved oxygen, temperature, and pH ranged from 8.2 to 8.3 mg/L (99% saturation), 19°C, and 8.1 to 8.3 units, respectively. At test termination, the concentration of dissolved oxygen, temperature, and pH ranged from 7.6 to 7.8 mg/L (96 to 100% saturation), 21°C, and 8.2 to 8.3 units, respectively. Immobilised organisms were considered dead. Toxicity values were derived based on the mass of TNPP hydrolysis products initially present in the test solutions based on nominal concentrations. These nominal values were likely higher than actual concentrations because of the sparingly soluble nature of the test substance and hydrolysis products. The concentrations and 95 % confidence limits of the nominal TNPP concentrations resulting in immobilisation of 50 % of the daphnids at 24 and 48 h were 2.2 mg/L (1.7 to 3.0 mg/L) and 0.3 mg/L (0.2 to 0.4 mg/L), respectively. This would correspond to a 24-h LC50 of 66 µg/L and a 48-h LC 50 of 9 µg/L expressed as estimated concentrations of the major hydrolysis product NP. The toxic response and presence of detectable levels of the hydrolysis product in solution confirmed that the TNPP had undergone hydrolysis during preparation of the stock solution. No explanation can be found to explain the low effect concentrations observed during this short-term toxicity testing with daphnids. Indeed, the toxicity observed could not be attributed solely to nonylphenol. The test result expressed in terms of nonylphenol concentrations was one order of magnitude lower than the acute Daphnia test reported in the nonylphenol RAR (EC_{50} = 0.085 mg/L, (EC, 2002)). However, most nonylphenol concentrations are extrapolated from analytical results for only the highest treatment level, lower arthropod effect concentrations are also reported in the EU RAR for nonylphenol, and physical effects of undissolved TNPP cannot be excluded although there was no reported identification of undissolved material during this test. This test shows definitely the presence of toxic effects to daphnia, if TNPP is used as parent compound. This toxicity is also confirmed by the results obtained on *Lumbriculus variegatus* (Picard, 2008). In addition, this test confirmed the formation of nonylphenol in water (0.3 mg/L after leaving TNPP in water for 78 hours) which is classified as hazardous to the aquatic environment (nonylphenol is classified: N; R50-53).	2

Test #	Species	References	Comment	Validity
2	Species: *Daphnia magna* EC_{50} (48 hours, estimated) = 0.42 mg/L Test condition: Static Method: Dir. 84/449/EEC C.2 (Purity of TNPP > 94%) No analytical measurements of TNPP or NP in treatments	CIBA-Geigy, 1992b	Calculated amounts of the test material to produce the desired concentrations were added to the water and were homogeneously distributed. Values are based on the **nominal concentrations (0.058, 0.1, 0.18, 0.32, 0.58 and 1.0 mg/L).** Parts of the test substance were visible on the surface of the water at concentrations of 0.1-1.0 mg/L. One day before the start of exposure, reproductive *Daphnia* are separated from the young (0-24 hours old) by sieving all individuals through an 800 µm sieve. This procedure is repeated immediately prior to exposure and the young are retained for the test. The Daphnia (4 replicates of 5 Daphnia each) were then transferred into the beakers. Cultures of Daphnia were maintained in glass vessels containing approximately 2.5 litres of reconstituted water and maintained at 20 +/- 1°C. The oxygen content ranged from 97 to 103%, the pH ranged from 7.8 to 8.0, and the water temperature was maintained at 21-24°C throughout the experiment. The EC-50 values were calculated according to the maximum likelihood method, probit model. EC-values were graphically determined on gausso-logarithmic probability paper. The EC_{50} values at 24 and 48 h were 2.6 and 0.42 mg/L, respectively. Although the test result is comparable with the results of test #1, this study has to be considered as invalid for the following reasons: - The tested concentrations were probably very far above the actual water solubility of the substance. - No analytical follow-up of the test concentrations was performed neither for TNPP nor for its degradation product (nonylphenol). As there was no equilibration time to allow dissolution of the substance during the preparation of the test concentration, it is not even clear that the maximum solubility in the test medium was achieved. The report mentions that undissolved substance was observed at all test concentrations. - Due to the low purity of the tested TNPP grade, the effects might be attributed rather to nonylphenol as impurity rather than hydrolysis product.	3

* 1 = valid; 2 = valid with restrictions; 3 = invalid; 4 = not assignable

Chronic toxicity to aquatic invertebrates

The following table shows a summary of the chronic toxicity test that was performed with aquatic invertebrate species.

Table 16: Chronic toxicity to aquatic invertebrates

Test #	Species	References	Comment	Validity*
1	Species: *Daphnia magna* NOEC (21 days) : No effect up to the limit of water solubility Test condition: Static-renewal conditions Method: OECD TG 211, OECD Series on Testing and Assessment Number 23 (OECD, 2000) (Test substance: Doverphos 4 High Pure (DP4HP, with less than 0.1% NP remaining as impurity)) No analytical measurements of TNPP or NP in treatments	Sayers, 2009	A full life-cycle toxicity test was conducted with Daphnia magna, Following OECD Guideline 211 (Springborn Smithers Laboratories, 2009). **The exposure was performed at a single nominal concentration (0.1 mg/L)** under static-renewal conditions for a period of 21 days. During the 21-day toxicity test, the test concentration was prepared at test initiation and at each renewal interval (i.e., every 24 hours) based on a 1.0 mg/mL primary stock solution, prepared weekly by adding, for example, 0.0102 g of TNPP to 10 mL of acetone. The resulting stock solution was observed to be clear and colorless and contained no visible undissolved test substance. A 0.10 mg/L water accommodated fraction (WAF) was prepared by adding 0.40 mL of the 1.0 mg/mL primary stock solution to 4.0 L of dilution water. This stock solution was mixed overnight (20 to 26 hours) under slow stir conditions to prevent possible emulsification of the test substance. The water soluble fraction was removed by siphoning into another vessel. Following siphoning, the solution was observed to be clear and colorless with no visible undissolved test substance. The culture water had a total hardness and total alkalinity as calcium carbonate (CaCO3) of 170 mg/L and 100 mg/L, respectively, a pH range of 8.0 to 8.2, a temperature range of 19 to 21 ºC, a dissolved oxygen concentration range of 7.8 to 9.5 mg/L, and a specific conductivity of 550 micromhos per centimeter (µmhos/cm). Test organisms were < 24 hours old at the initiation of the test. The toxicity test was conducted in 100-mL clear glass beakers, each containing 80 mL of test solution. Thirty replicate test vessels were established for the nominal concentration (0.1 mg/L) and the controls. Test vessels were placed in a temperature-controlled (20 ± 1 ºC) water bath. The photoperiod during testing was the same as that provided in the culture area (16 hours of light, 8 hours of darkness). Light at an intensity range of 8.6 to 13 µE·m-2·s-1 at the surface of the exposure solutions was provided by fluorescent bulbs. The nominal concentration for the definitive toxicity test represents a saturated solution at the limit of solubility for this test substance under testing conditions. The test was conducted as a limit test at a single nominal concentration of 100% WAF of a 0.10 mg/L stock solution.	3

Test #	Species	References	Comment	Validity*
…1 cont	Species: *Daphnia magna* NOEC (21 days): No effect up to the limit of water solubility Test condition: Static-renewal conditions Method: OECD TG 211, OECD Series on Testing and Assessment Number 23 (OECD, 2000) (Test substance: Doverphos 4 High Pure (DP4HP, with less than 0.1% NP remaining as impurity)) No analytical measurements of TNPP or NP in treatments	Sayers, 2009	The dissolved oxygen concentrations in the treatment and control solutions ranged from 6.2 to 9.7 mg/L. Continuous temperature monitoring of the water bath established a minimum/maximum temperature range of 18 to 22 ºC during the exposure period. Measurements of pH ranged from 7.9 to 8.5 in the treatment and control solutions. Total hardness, total alkalinity and specific conductance measured in the treatment level and the dilution water control ranged from 170 to 180 mg/L CaCO3, 100 to 110 mg/L CaCO3 and 600 to 680 µmhos/cm, respectively. These results established that the water quality conditions established for the test remained within acceptable ranges for the survival, reproduction and growth of Daphnia magna. The exposure system provided conditions that were appropriate for promoting acceptable survival and reproduction of the test species and met the minimum standard criteria established by OECD guidelines (i.e., ≥ 80% survival and ≥ 60 offspring per female). Based on survival, reproduction and growth, the 21-day NOEC for TNPP was determined to be ≥ 100% WAF of a 0.10 mg/L stock solution. New comments: After an in depth assessment of the long term *Daphnia magna* study and following relevant comments of other Member States we consider this study invalid for the following reasons: - No analytical follow-up of the test concentrations was performed. Consequently, we have no proof of the presence of TNPP or nonylphenol in water. - Due to renewal every 24h with freshly prepared test solutions, the test design insufficiently covers the key issue of possible nonylphenol formation from TNPP hydrolysis - In order to limit adsorption to negatively charged biological marterial, such as algal cells, the feeding of daphnids should have been done a few hours before test medium renewal (it was not the case in this experiment because daphnids were fed after test medium renewal).	3

* 1 = valid; 2 = valid with restrictions; 3 = invalid; 4 = not assignable

7.1.1.3 Algae and aquatic plants

The following table shows a summary of the toxicity tests that were performed with algae species.

Table 17: Algae and aquatic plants toxicity

Test #	Species	References	Comment	Validity*
1	Species: *Pseudokirchneriella subcapitata*. NOEC (72 hours, growth) : No effect up to the limit of water solubility Test condition: Static Method: OECD TG 201 (Test substance: Hydrolysed solution of TNPP (TNPP grade according to supplier's characterisation sheet as appended to study report: Doverphos® HiPure 4, CAS 26523-78-4, < 0.1% free nonylphenol) NP limit of detection 0.2 mg/L	Hydroqual Laboratories Ltd, 2001b	The test was initiated with exponentially growing cells from in-house cultures maintained at 23 ± 2°C under continuous light (3,770 lux). The cultures were grown under axenic conditions in 2-L flasks containing 1 L of artificial media, aerated with filtered sterile air. Cell numbers were obtained from optical density measurements at 430 nm calibrated against particle and cell counts at test termination. The dilution water was dechlorinated City of Calgary tap water (charcoal filtered and aerated) spiked with nutrients. The dilution water had a hardness of 198 mg CaCO3/L, alkalinity of 146 mg CaCO3/L, pH of 7.6, and conductance of 446 ms/cm. The test solutions were prepared from a stock solution initially containing 100 mg of TNPP in 1 L of dilution water. The substance was weighed on a glass Petri dish (100 mg) and the dish placed into a 2 L glass, Erlenmeyer flask containing 1 L of dilution water. A magnetic stir bar was added and the mouth of the flask sealed with Parafilm®. The test substance was stirred gently for 78 hours at room temperature (21 ± 2 °C). The test solutions were then prepared from the stock solution of TNPP hydrolysis products as recommended by the OECD for the testing of difficult substances. A 100 mL volume of the hydrolysed stock solution was poured into a 250 mL plastic container for **the highest test concentration (100 mg/L nominal test concentration).** A second 100 mL volume of the stock solution was poured into another 250-mL container and serially diluted with 100-mL volumes of dilution water to obtain the remaining **test concentrations (50.0, 25.0, 12.5, 6.3, 3.1, and 1.6 mg/L nominal test concentrations).** The solutions were spiked with 1 mL of a concentrated nutrient solution and then inoculated (1 mL) to give an initial cell density of 9,664 + 154 cells/mL. The	2

Test #	Species	References	Comment	Validity*
…1 cont	Species: *Pseudokirchneriella subcapitata*. NOEC (72 hours, growth) : No effect up to the limit of water solubility Test condition: Static Method: OECD TG 201 (Test substance: Hydrolysed solution of TNPP (TNPP grade according to supplier's characterisation sheet as appended to study report: Doverphos® HiPure 4, CAS 26523-78-4, < 0.1% free nonylphenol) NP limit of detection 0.2 mg/L	Hydroqual Laboratories Ltd, 2001b	inoculum was taken from an exponentially growing culture, washed twice with a sodium bicarbonate solution, and the cell number adjusted to give the desired initial cell density in the 100-mL test volume. The test was conducted in a controlled environment chamber at 23 + 2°C under continuous light with intensity at the plate surface of 4,370 lux provided by cool white fluorescent lights. Two sets of samples were collected for chemical analysis. The first set consisted of samples of the test solutions and control at test initiation. The second set consisted of samples of the test solutions and control incubated under the test conditions for 72 h. The samples were not analysed for TNPP because it is insoluble in water. The samples of the test solutions were analysed for nonylphenol however, in contrast to similar study Hydroqual 2001a, it was not detected in any of the samples (detection limit of 0.2 mg/L). The pH at test initiation and termination in the controls and 100.0 mg/L test solution ranged from 7.0 to 8.0. The initial and final control cell densities were 9,664 cells/mL and 404,000 cells/mL, respectively. This was a 42-fold increase in cell density over the 72-h test period. A 16-fold increase was required for a valid test. The test medium contains 0.65 mg/L phosphate. Complete hydrolysis of the test substance (100 mg/L) would yield approximately 12 mg/L of phosphorous acid, and even limited hydrolysis of TNPP as realistically expected would contribute significantly to phosphorous supply of the growth medium. The cell density in the highest test concentration at 72 h was 344 % greater than the controls. This represents approximately 1.5 additional doublings of the cell population exposed to the hydrolysed TNPP solution when compared to the controls. In the control and lowest treatment, maximum cell numbers were already reached after 48h, clearly indicating limitations of growth e.g. due to depletion of an essential nutrient. Continued growth from 48h to 72h in all other treatment levels shows compensation for this limitation. Only in the two highest treatment levels some attenuation of stimulation has been recorded, possibly due to incipient	2

Test #	Species	References	Comment	Validity*
			nonylphenol toxicity. The result indicates that growth stimulation might be caused due to the liberation of phosphorous from TNPP hydrolysis. The LOEC as well as the 24, 48 and 72 h EC50 values were >100 mg/l. The NOEC was the highest concentration tested of 100 mg/l. The level of nonylphenol present in the test solutions under the conditions in which the stock solution was prepared, diluted, and tested was not toxic to unicellular green alga.	
2	Species: *Scenedesmus subspicatus* NOEC (72 hours, biomass) : No effect up to the limit of water solubility Test condition: Static Method: Dir. 87/302/EEC, part C., p. 89 (Purity of TNPP > 94%) No analytical measurements of TNPP or NP in treatments	CIBA-Geigy, 1992c	**Nominal test concentrations of 0, 1.23, 3.7, 11, 33 and 100 mg/L** were used (three replicates for the test concentrations and 6 replicates for the blank). The stock solution was prepared by mixing 200 mg of the test substance with 80 mL water and 1 mL of a 0.8% alkylphenol-polyglycol ether and made up to 100 mL with water. This 100 mL solution was then made up to 1 liter with water. Calculated amounts of the stock solution to produce the desired test concentrations were added to the water. The algae were then transferred into the flasks (100 mL Erlenmeyer flasks, stoppered with aluminium caps, on Lab-Shaker). The cell densities were measured at 24, 48, and 72 hour. The temperature was continuously measured and maintained at 23 +/- 1°C. The pH was measured at 0 and 72 hours and ranged from 7.8 to 8.1. The test was conducted under continuous illumination, cold white fluorescent light, 118 µE/m² sec +/- 20% (approx. 8000 lux.). No significant effects upon biomass were observed at any test concentration. However, no analytical measurements of TNPP or nonylphenol in the treatments were reported and the presence or actual concentrations are unknown.	3

* 1 = valid; 2 = valid with restrictions; 3 = invalid; 4 = not assignable

7.1.1.4 Sediment organisms

The following table shows a summary of the toxicity test that was performed with sediment species.

Table 18: Toxicity to sediment organisms

Test #	Species	References	Comment	Validity
1	Species: *Lumbriculus variegatus* Reproduction and biomass: LOEC(28 days) = 63 mg a.i./kg dw NOEC (28 days) < 63 mg a.i./kg dw Estimated NOECs: $EC_{10(reproduction)}$ = 44 mg a.i./kg dw $EC_{10(biomass)}$ = 25 mg a.i./kg dw Test condition: Static Organic carbon content: 1.8% Method: OECD TG 225 (Doverphos 4 Hi Pure (DP4HP, CAS No.: 26523-78-4): Purity: 99.9% as tris (nonylphenyl) phosphate with less than 0.1% NP remaining as impurity) No analytical measurements of TNPP or NP in treatments	Picard, 2008	A 28-day sediment-water toxicity test using spiked sediment was conducted with *Lumbriculus variegatus*, following OECD guideline 225. Artificial sediment was prepared (6.0% sphagnum peat, 20% kaolin clay, 37% fine sand and 37% of coarse sand) and characterized (**organic carbon content 1.8%,** pH of 6.3, and a percent moisture 11.8%). **TNPP was applied as appropriate amount of stock solution (100 mg/L in acetone) to 50 g fine silica sand, which after acetone evaporation was mixed with 1.0 kg sediment, resulting in nominal test concentrations of 63, 130, 250, 500 and 1000 mg a.i./kg dw[3].** Prior to test termination, no observations of mortality or abnormal behavior were evident during this study. However, turbidity of the overlying water caused by oligochaete burrowing activity made accurate observations of the test organisms difficult. At test termination (day 28), the number of living oligochaetes recovered within the 63, 130, 250, 500 and 1000 mg a.i./kg dw treatment levels was 21, 19, 14, 15 and 12, respectively. A statistically significant difference in number of total oligochaetes recovered in all treatment levels tested compared to the pooled control organisms was established. Mean biomass in the 63, 130, 250, 500 and 1000 mg a.i./kg dw treatment levels was 20, 21, 15, 15 and 8.7 mg, respectively. A statistically significant difference in mean biomass in all treatment levels tested compared to the pooled control organisms was established. Since all concentrations of TNPP caused a statistically significant reduction of both oligochaete reproduction and biomass, the NOEC value for these endpoints was empirically estimated to be < 63 mg a.i./kg dw. The LOEC for this exposure was determined to be 63 mg a.i./kg dw. Based on linear regression, an EC10 value was calculated as an estimate of the NOEC for reproduction and biomass. The NOEC values for reproduction and biomass were estimated to be 44 and 25 mg a.i./kg dw, respectively. One deviation from the OECD guideline 225 was observed in the report, the total ammonia content was analysed only in Solvent control and in the highest dose. The guideline indicates the analysis at least in one replicate of the controls and in one test vessel of each concentration level at the start of the exposure period, and subsequently 3 x per week.	2

* 1 = valid; 2 = valid with restrictions; 3 = invalid; 4 = not assignable

[3] No analytical verification of TNPP or nonylphenol concentrations in any treatment

7.1.1.5 Other aquatic organisms

No data available.

7.1.2 Calculation of Predicted No Effect Concentration (PNEC)

Not relevant for this type of dossier.

7.2 Terrestrial compartment

7.2.1 Toxicity test results

No data available.

7.2.1.1 Toxicity to soil macro organisms

No data available.

7.2.1.2 Toxicity to terrestrial plants

No data available.

7.2.1.3 Toxicity to soil micro-organisms

No data available.

7.2.1.4 Toxicity to other terrestrial organisms

Toxicity to birds

No data available.

Toxicity to other above ground organisms

No data available.

7.2.2 Calculation of Predicted No Effect Concentration (PNEC_soil)

Not relevant for this type of dossier.

7.3 Atmospheric compartment

No data available.

7.4 Microbiological activity in sewage treatment systems

7.4.1 Toxicity to aquatic micro-organisms

The following table shows a summary of the toxicity tests that were performed with micro-organisms.

Table 19: Toxicity to aquatic micro-organisms

Test #	Species	References	Comment	Validity*
1	STP activated sludge 1.6-1.7 g/L IC_{50} = 16 mg/L for the reference substance (3,5-dichlorophenol) NOEC: n.d. Method: OECD TG 209 (Purity of TNPP: commercial grade; further information not available)	CIBA-Geigy, 1988b	Instead of a centrifuged sludge, a settled sludge was used. Due to the very low solubility and the expected low toxicity of the substance, only one concentration (100 mg/L) was tested in duplicates during three hours. The test substance was directly added to the test vessel. In one replicate no inhibition was recorded, in the other an inhibition of 24% was observed. This test must be considered invalid because of the large difference between both replicates.	3
2	STP activated sludge NOEC = 18.1 mg/L Method: OECD TG 301B (Purity of TNPP not given)	CIBA-Geigy, 1994	After 7 days and 20 days, the biodegradation of the reference substance (Sodium benzoate) reaches respectively 71 and 86%. The controls of reference and reference together with the test substance meet the specification for ready biodegradability. Therefore, it can be concluded that the test substance has no inhibitory effect on the bacteria at the concentration tested (18.1 mg/L) which is above the solubility limit of TNPP.	2

* 1 = valid; 2 = valid with restrictions; 3 = invalid; 4 = not assignable

7.4.2 PNEC for sewage treatment plant

Not relevant for this type of dossier.

7.5 Calculation of Predicted No Effect Concentration for secondary poisoning (PNEC_oral)

Not relevant for this type of dossier.

7.6 Conclusion on the environmental classification and labelling

Based on the available studies, taking into account the specific physicochemical properties of TNPP, and in response to comments during the public consultation, the dossier submitter concluded with the classification rational provided in Appendix III. It is the opinion of RAC that the justification for the proposed classification has to be further strengthened for clarification of the underlying key considerations:

Available data and information show that TNPP is not rapidly degradable according to the CLP Regulation. Experimental key information is lacking to conclude on its bioaccumulation. However, the cut-off value of log Kow ≥ 4 set out in the CLP Regulation is exceeded. Due to specific physicochemical properties of TNPP, in particular its very low water solubility and a high octanol-water partitioning quotient, both yet estimated with considerable uncertainties, experimental studies testing TNPP have to be scrutinised very carefully. No ecotoxicological study provides evidence for effects directly exerted by TNPP. The key issue for an adequate classification decision relates to the role of nonylphenol (NP, [Aquatic Acute 1; H400, Aquatic Chronic 1; H410] in Annex VI to the CLP Regulation) as degradation product resulting from TNPP hydrolysis. At the same time, a range of from < 0.1% to < 5 % of NP can occur as impurity in various TNPP grades on the market – as impurity, NP has to be considered according to articles 10 and 11 of the CLP Regulation, but not for the harmonised classification proposed in the present document.

Two relevant studies on hydrolysis of TNPP are available, both basically analysing the formation of NP due to hydrolytic cleavage of the ester-bonds between the phosphorous and NP-moieties of TNPP. In a preliminary study roughly following OECD Test Guideline (TG) 111 at 20°C and pH 7 for 24h, no NP could be detected beyond the limit of detection (LOD) of 23 ng/L. However, the analytical method has apparently not been optimised for the tested TNPP grade with branched NP-chains, and moreover OECD TG 111 suggests conducting a preliminary study at 50°C and three pH levels (4, 7, 9) for 120h to confirm hydrolytic stability. In a second study not referring to a standard method and with no pH and temperature details reported, the tested TNPP grade included 0.75% TIPA which is added as stabiliser e.g. against hydrolysis. Limited formation of NP resulted in increasing NP concentrations during the first 4 days, then remaining on a constant level of ca. 15 µg/L NP (accounting for ca. 0.1 % hydrolysis) until test termination after 10 days.

In an acute toxicity test with the water flea *Dapnia magna* the test solutions were prepared from a high purity grade (< 0.1% free NP) TNPP stock solution, 78 hours subjected to conditions for potential hydrolysis prior to the test. In the highest treatment level (nominal 10.0 mg/L TNPP) 0.3 mg/L NP were detected, of which a maximum of 0.01 mg/L might be attributed to impurities. No NP was detected in lower treatment levels due to the relatively high LOD of 0.2 mg/L NP. The observed effects follow a concentration-response curve with an effect concentration EC50 of 0.3 mg/L TNPP (nominal). This value corresponds to an estimated NP-related EC50 = 0.009 mg/L, which is even lower than results from corresponding *Daphnia* tests with NP (lowest 48h EC 50 = 0.085 mg/L), reported in the EU Risk Assessment of NP as part of a comprehensive description of NP aquatic ecotoxicity (EC, 2002 – see e.g. page 117, table 3.15).

In an algae study with the same 78 hours preparatory period for potential hydrolysis in the stock solution, the most pronounced effect was growth stimulation, which might be caused due to the liberation of phosphorous from TNPP hydrolysis.

As supportive evidence, a sediment-water *Lumbriculus variegatus* toxicity test using spiked sediment following OECD TG 225 is available. Although the presence and concentrations of TNPP and NP are not verified by analytical measurements, the test-design provides appropriate conditions for NP formation from hydrolytic TNPP degradation, and clear concentration-dependent effects on reproduction and growth could be plausibly attributed to NP.

All other studies are not useful for classification considerations due to severe limitations as:

- no analytical verifications of TNPP or NP concentrations;
- high limits of detection foreclosing analytical verification of low NP concentrations, yet expected to affect tested organisms;
- either high or unspecified impurity grades of tested TNPP, which foreclose to discern NP as impurity and NP from TNPP hydrolysis;
- insufficient test conditions for hydrolysis (e.g. daily renewal in 21d daphnid study).

Overall, despite the low water solubility and high log Kow of TNPP, formation of NP due to limited hydrolysis has to be expected. Due to low degradation rates, TNPP remains in the environment, mainly adsorbed e.g. to sediment, from where hydrolytic release of NP can also be expected. The key study with water fleas provides sufficient evidence that under environmental conditions the transformation products resulting from applied nominal TNPP concentrations cause adverse, classification relevant ecotoxic effects.

The available data do not allow an adequate description of the apparent bottleneck between undissolved TNPP on one side, and dissolved amounts of TNPP and its major hydrolysis product NP, not to mention other potential transformation products, on the other side. With a view to this lack of information, RAC dismissed the option to classify TNPP in analogy to its major transformation product NP. RAC concludes that nevertheless TNPP loadings below the corresponding classification criterion of 1 mg/L might be sufficient to result in concentrations of NP and possibly other transformation products, altogether causing classification relevant effects. Thus, combined with persistency, the classification R50/53 (DSD) / Aquatic Acute 1, Aquatic Chronic 1 (CLP) is justified.

RAC has thoroughly discussed several M-Factor options. Based on the available information and its considerable uncertainties, RAC concludes to recommend no harmonised M-Factor (and no SCL). The scientific uncertainty could be significantly reduced by adequate experimental data, carefully taking into account the specific TNPP properties and regulatory needs. As an adequate framework for developing and agreeing appropriate test protocols, the REACH substance evaluation process appears supposable.

Proposed classification based on Directive 67/548/EEC:

N; R50/53

Proposed specific concentration limits (if any):

none

Proposed classification based on Regulation (EC) No 1272/2008:

Aquatic Acute 1 – H400

Aquatic Chronic 1 – H410

M-factor: none

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

TNPP was on the 4th priority list of the Existing Substances Regulation and it is therefore a requirement to harmonise classification for all endpoints justifying classification.

A classification proposal was submitted and discussed at ECB (TC C&L) for health endpoints. Classification R43 was concluded by TC C&L for health. For information, discussions and conclusions as reported in summary records of the corresponding meetings are presented in Appendix I of the present report. No relevant new data has been identified since TC C&L discussion for health.

The proposal for environmental classification was on hold as additional testing had been requested and was on-going. A summary explanation of the justification for requirement of the new studies according to Commission Regulation (EC) No 466/2008 is presented in Appendix II of the present report.

Further to completion of the required environmental test the whole classification proposal is now submitted to ECHA for all endpoints justifying classification as requested for priority substances under ESR.

In addition, many difficulties has been encountered during the assessment of TNPP as this substance can be considered as difficult to test for the purposes of determining its aquatic toxicity and difficult to classify. In absence of the Classification and Labelling Inventory that is not yet available, it is not possible to know what self-classification is applied by manufacturers and importers and if an appropriate classification for environment is applied. Setting a harmonised classification for environment is therefore justified to ensure the application of an appropriate classification.

In the view of a contingent discussion on the relevance of classification due to impurities, some additional toxicological data is displayed in the present dossier for information.

The only endpoints proposed for harmonisation are however skin sensitisation and environment.

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APPENDIX I

Collection of discussions on TNPP classification at ECB

TNPP classification was first discussed in written procedure of TC NES III 04. For health effects, it was then discussed at the Technical Committee of Classification and Labelling (TC C&L) in March 2005 and in November 2005. Health classification was concluded at the TC C&L in November 2005. Environmental effects were not further discussed.

Extract from document ECBI/141/04 Rev. 4 - Final Summary table of the written procedure for Substances from TCNES III 04

Substance (Rapporteur)	Index No	Current Classification-S-Phrases	Proposed Classification-S-Phrases	Comments to proposal (HH: human health, ENV: environment)	**Revised Classification S-Phrases**	Comments to revised proposal
TNPP, Tris (nonylphenyl) phosphite (France)	Not listed CAS 26523-78-4		HH: R43 ENV: N: R50-53 S: (2-)46-24-37-60-61	**BE**: HH: agrees. **EL**:HH: agrees. **IRL**: HH: agrees but add Xi (substance is a skin sensitiser) and revise S Phrases to: S24-37-60/61. **NL**: HH: agrees with R43 but R62 should also be discussed. Provide additional information on old skin/eye irritation test. **UK**: HH: agrees. **S**: HH: agrees. **DK**: HH: suggests also application of Repr. Cat. 3 R62. **DE**: HH: Revise chapter on reproductive toxicity according to comments. **UK:** HH: agrees. **S**: ENV: agrees, but give a better rational for classification	----------	

Extract from document ECBI/55/05 - Draft Summary Record - Meeting of the Technical Committee C&L on the Classification and Labelling of Dangerous Substances - Arona, 15-18 March 2005

TNPP, Tris (nonylphenyl) phosphate (F) Not in Annex I CAS 26523-78-4 Proposal: R43 ENV: N: R50-53 S: (2-) 46-24-37-60-61

Documents:

ECBI/141/04 Rev. 4 ECB, Final Summary of proposals and comments distributed instead of substance sheets

ECBI/127/04 FR, C&L proposal

F presented their proposal. They proposed no classification for skin irritation and for skin sensitisation based on results of guinea pig maximisation test. Concerning reprotoxic effects there were for fertility two oral studies on rats. A decrease in the number of pups born was seen at 500 mg and a small decrease in fertility. In the other studying the absence of systemic toxicity deaths were seen at the higher dose (1000 mg/kg) – probably due to distoxia – that was discussed at TCNES and considered as a reprotoxic effect. **The Group** provisionally agreed for R43. IND could react in the follow-up period. Based on the possible reaction, MS might comment and reconsider their decision. **NL** had no concerns for R62.

Repr. Cat 3 – R62

D could not find anything in the dossier on quantitative data. They wanted to have quantitative data. They thought that it was a case between R62 and no classification. **F** said that they would revise the proposal upon further discussions of Industry. **NL** and **DK** agreed with that.

ECB said that TNPP will end up now in the regular follow-up as the written procedure was over and the substance is in the pipeline for the normal procedure. Reprotoxicity was postponed to the next meeting.

7.6.1.1 Conclusion: The TC C&L agreed to classify the substance as R43. The Repr. Cat. 3; R62 classification will be discussed at the next meeting.

Extract from document ECBI/60/05 Rev. 3 - Draft Summary Record of the Meeting of the Technical Committee on the Health Effects of New Substances, Pesticides, Biocides, Existing Chemicals, and on General Issues –

Ispra, 14 - 17 November 2005

TNPP, Tris(nonylphenyl)phosphite (F042)

(CAS number 26523-78-4, EC number 247-759-6)

Not in Annex 1

Proposal: R43 - N; R50-53

ECBI/127/04 Classification proposal and Rev. 1

In **March 2005** R43 was provisionally agreed. Some member states were concerned over the reproductive toxicity and France submitted a revised document (Rev 1) in the follow-up period.

France introduced their paper (Rev 1) in which, following earlier requests, a detailed review of fertility data had been undertaken. The paper concluded that there was no case for classification for fertility.

With the exception of Denmark, who expressed a strong reservation, the Group agreed that no classification for fertility was required.

APPENDIX II

Summary explanation why the new studies have been required by Commission Regulation (EC) No 466/2008 :

Decisions taken to perform tests on TNPP result from the last TC NES meeting (TC NES I ’08).

- **Acute toxicity test with *Daphnia magna* and long term *Daphnia* test (depending on outcome of acute *Daphnia* test):** The Rapporteur had identified some uncertainties in the acute toxicity test with *Daphnia* and the test had been redone with measurements of both TNPP and NP. The test would provide more information on the formation of NP during the toxicity tests. The Rapporteur expected that NP would not be formed in the toxicity tests. It was discussed during the last TC NES meeting that for poorly soluble substances it might take time before effects could be observed and that might be a reason for not seeing effects in short-term tests, whereas effects might be shown in long term tests. Moreover, the results of the acute test could eventually be used to determine the test concentration range of the long term *Daphnia* study. Consequently, a long term *Daphnia* test has been required in order to know the effects of TNPP on aquatic organisms. Indeed, due to the low solubility of the substance uptake by filtering organisms such as *Daphnia* occurred not only via water but also via suspended matter, for example through adhesion to algae.

- **Information on structure of TNPP:** Information on structure of TNPP has been required for the evaluation of the bioaccumulation potential of the substance. Indeed, the molecular dimensions Dmax and Deff had been estimated with Molecular Operating Environment (MOE) software. In the discussions of the PBT WG it had been noticed that the MOE software seemed not to calculate the parameter Dmax. UK recommended during the TC NES meeting to double check what exactly had been calculated and if possible to compare with OASIS prediction. ECB concluded that the Rapporteur was asked to have further look into the calculations used for the molecular diameter and to base the conclusion on bioaccumulation potential on an evaluation of all weight of evidence including the (lack of) toxic effects. *(This information is not available at this time).*

- **Information on solubility:** The improvement of the analytical method led to perform a new water solubility measurement. This need was confirmed by the QSARs estimate which showed that TNPP is sparingly soluble in water.

- **Log Kow determination:** ECB noted that in this section the basis was laid for the sensitivity analysis using a range of log Kow values from 7 – 22. Industry recommended the use of a higher value of log Kow in the sensitivity analysis. Industry believed that a log Kow of 7 was not reliable. Industry emphasised that it was not likely that TNPP does bioaccumulate in fish, a higher value was also supported by QSAR estimates. Industry concluded that the higher value of log Kow was more realistic. The Rapporteur explained that the upper value of the log Kow range had been chosen because it was the highest value obtained with QSARs. The Rapporteur reminded though that the QSARs could not be considered valid above log Kow of 10, as indicated in the TGD. Therefore the Rapporteur was reluctant to use the upper value in the risk assessment. The Rapporteur noted that the value of 7 might be too low, but the true hydrophobicity of TNPP was unknown.

Consequently, the TC NES supported the request to do a Kow measurement using HPLC method based on OECD guideline 117. We noted that log Kow was used for the evaluation of the bioaccumulation potential of the substance.

- **Hydrolysis test:** ECB noted that the hydrolysis study was a crucial test and the conclusions from the test affected the rest of the risk assessment.
  The first hydrolysis study (2001) suffered from serious problems. It was not known what was happening in that study. There was a lack of material balance. The LOQ was high and that was assumed to be the water solubility. Therefore the study was interpreted improperly. Then industry had asked TNO to provide a new hydrolysis study according to the OECD guidelines. TNO had established a much lower LOQ and they used an indirect way to show whether TNPP did hydrolyse or not because it is sparingly soluble in water. They did this both by measuring in the calibration solutions which showed that the amount of nonylphenol was the same. Then they used the water samples where TNPP was added to show if nonylphenol was formed. TNO was not able to detect more nonylphenol then was present initially in the samples as impurity, thereby showing that no further nonylphenol was formed in the 24h study that they conducted.

  Industry informed the meeting on recent developments on the hydrolysis study of 2004. The testing laboratory had used TNPP with linear NP as reference standard. Also a sensitive method was developed for the detection and quantification of linear NP. Commercial TNPP contained largely branched NP-chains. So if NP would be formed, it would be the branched NP and that would not have been detected in the 2004 hydrolysis study. Therefore the relevance of the 2004 study was questionable. Industry was looking further into the possibilities of doing a new test. ECB noted that this particular hydrolysis study was very relevant for the whole risk assessment as the meeting had come to the conclusion that hydrolysis of TNPP did not take place under environmental conditions on the basis of this study. Therefore if this study was questioned, large parts of the risk assessment would have to be revisited on the basis of these comments. ECB noted that a repeat study might be necessary. Industry agreed that a new study was urgently needed to measure hydrolysis. ECB asked the Rapporteur to evaluate the results of the new study and to consider the results in a revised risk assessment.

- **Sediment test with *Lumbriculus variegatus*:** it appeared that the target environmental compartment was sediment due to the low water solubility and high log Kow of TNPP. The Rapporteur pointed out that conclusion (i) was proposed for sediment for all stages of the life cycle of TNPP because of the absence of data on toxicity of TNPP towards benthic organisms. Considering the low solubility in water and the suspected high adsorption potential of TNPP toxicity to sediment dwelling organisms should be studied.
  UK agreed that further sediment testing could be useful given all difficulties to test surface water exposure with *Daphnia*. Perhaps a single limit test with *Lumbriculus* could be done just to test out that there is no observed chronic toxicity. Maybe that could be combined with some bioaccumulation testing to get some idea on this at the same time. *Lumbriculus* was the preferred species because the ingestion route was addressed. If the test showed no long term toxicity, this would mean that the substance was not bioavailable. The TC NES agreed to ask for a *Lumbriculus* test.

- **Monitoring data for sites with PEC/PNEC>1:** Monitoring data have been required in order to refine the PEC value and then recalculate the Risk Characterisation Ratio when it was higher than 1. *(This information is not available at this time).*

APPENDIX III

Classification rational provided by dossier submitter in CLH report version as re-submitted in June 2010 after public consultation:

"Before concluding on the environmental classification and labelling of TNPP, we would like to point out the difficulties encountered during the assessment of TNPP as this substance can be considered as difficult to test for the purposes of determining its aquatic toxicity. Indeed, there were still uncertainties regarding some physico-chemical properties of TNPP (cf. LOQ, log Kow, Koc) and in addition, the formation of its degradation product: nonylphenol in the aquatic environment raises issues for interpretation of test results. In fact, the analytical determination of the water solubility was not done with accuracy (we obtained a range of water solubility based on QSAR and on the LOQ value) as the substance seems to be sparingly soluble. The lack of consistent analytical methods has an impact on results of toxicity tests performed on aquatic organisms. Indeed, toxicity test results are based on nominal concentrations instead of measured concentrations advisable for TNPP. Nominal concentrations seem to be inappropriate for this kind of substance due to its intrinsic properties (particularly its potential of adsorption and its low solubility); the follow-up of the substance is really important in this case to guarantee a good interpretation of toxicity data. Because of the inability to measure TNPP at very low levels, the formation of its degradation product (nonylphenol) had been measured. However, this degradation product raises issues for interpretation of test results due its intrinsic toxicity to aquatic organisms (nonylphenol is classified: N; R50-53). In addition to analytical difficulties and its degradation product, the log Kow of TNPP estimated by the HPLC method may be interpreted with care as the results obtained are outside the applicability domain of the method. Consequently, the Koc value based on this log Kow value also has to be interpreted carefully.

On account of all uncertainties regarding analytical methods, physico-chemicals properties values of TNPP and formation of nonylphenol which is classified as hazardous to the aquatic environment, we propose, below, a conservative and protective environmental classification and labelling.

Short-term toxicity tests available performed with fish and algae and that were considered as valid (validity of 1 or 2) did not conclude on a toxic effect up to the limit of water solubility; consequently they do not justify a classification. Thus we focused on toxicity tests performed with invertebrates. After an in-depth assessment of the long-term *Daphnia magna* study (Sayers, 2009), we propose to invalidate this study (validity of 3) owing to the lack of analytical follow-up of the test concentrations (neither for TNPP nor for its degradation product (nonylphenol)). The short-term toxicity test performed by Ciba-Geigy (1992b) has to be considered as invalid too (validity of 3) for the same reasons (no analytical follow-up) but it is used as supportive data. Consequently and after an in-depth assessment of the short-term *Daphnia magna* study performed by Hydroqual Laboratories Ltd (2001a), we propose to consider this latter as valid (validity of 2) and as the key study to base the harmonised classification of TNPP. Indeed this study definitely shows the presence of toxic effects to daphnids, if TNPP is used as parent compound. This toxicity is confirmed by the results obtained on *Lumbriculus variegatus* (Picard, 2008). In addition the daphnid test confirmed the formation of nonylphenol in water (0.3 mg/L after leaving TNPP in water for 78 hours) which is classified as hazardous to the aquatic environment (nonylphenol is classified: N; R50-53). Despite the fact that EC_{50} nominal value on TNPP (EC_{50} (48 h) = 0.3 mg/L) was confirmed by the short-term test performed by CIBA-Geigy (1992b) (EC_{50} (48 h) = 0.42

mg/L), this value seems to be higher than what may have been measured concentrations. This is due to the sparingly soluble nature of the test substance and its potential of adsorption to test vessels. In addition, these nominal concentrations are widely above the actual water solubility of the substance (WS < 0.05 mg/L). As a consequence and according to the CLP Regulation, due to its potential acute effect on invertebrates at a concentration probably inferior to 0.3 mg/L and due to its low degradability and its log Kow > 4, TNPP should be classified as R50-53 (Aquatic Acute 1 – Aquatic Chronic 1).

Concerning the proposition of an M-factor value for TNPP, we suggested the most conservative and protective value based on the short-term *Daphnia magna* study performed by Hydroqual Laboratories Ltd (2001a). This test was performed without analytical monitoring of TNPP. In addition due to its intrinsic properties, a very low water solubility on the one hand and a high potential of adsorption on the other hand, toxicity value obtained probably under-estimate the real toxicity of TNPP. Therefore applying an M-factor value of 1 for TNPP (corresponding to the EC_{50} (48 h) of 0.3 mg/L) is not protective enough for the aquatic environment. In addition this value (EC_{50} (48 h) of 0.3 mg/L) seems much above the range of water solubility (between 3.10^{-16} and 0.05 mg/L). If we considered that the acute toxicity of TNPP is probably roughly close to its water solubility, then the M-factor applied will be > 10 (corresponding to a water solubility < 0.05 mg/L). This result is consistent with the acute toxicity value observed in the key study (Hydroqual Laboratories Ltd 2001a) (for its degradation product: nonylphenol (EC_{50} (48 h) = 0.009 mg/L) for which an M-factor value of 100 will be applied. On account of the uncertainties of the quantitative result for TNPP obtained with the short-term test with *Daphnia magna* and due to its low water solubility value and the high toxicity of its degradation product, we proposed to apply a safety M-factor of 100 for TNPP.

The same approach was applied to determine specific concentration limits according to Directive 67/548/EEC."