Committee for Risk Assessment

RAC

Annex 1

Background document (BD)

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

Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate

EC number: 432-770-2

CAS number: 139189-30-3

ECHA/RAC/CLH-O-0000002526-74-03/A1

Adopted

30 November 2012

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Part A.

Part B.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate
EC number:	432-770-2
CAS number:	139189-30-3
Annex VI Index number:	015-192-00-1
Degree of purity:	>= 98.0 — <= 99.0 % (w/w)
Impurities:	Confidential – The substance contains one impurity. This has been taken into consideration and does not additionally contribute to the classification. Further information is provided in the technical dossier.

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	Skin Sens 1: H317 Aquatic chronic 4: H413	Xi R43 R53
Current proposal for consideration by RAC	Removal of Aquatic chronic 4 classification	Removal of R53 classification
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Skin Sens 1: H317	Xi R43

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	None	Not classified	conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	None	Not classified	conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	None	Not classified	conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	None	Not classified	conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	None	Not classified	conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	None	Not classified	conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	None	Not classified	conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	None	Not classified	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	None	Not classified	conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	None	Not classified	conclusive but not sufficient for classification
2.11.	Self-heating substances and	Not classified	None	Not classified	conclusive but not sufficient

	mixtures				for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	None	Not classified	conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	None	Not classified	conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	None	Not classified	conclusive but not sufficient for classification
2.15.	Organic peroxides	Not classified	None	Not classified	conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	None	Not classified	conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Not classified	None	Not classified	conclusive but not sufficient for classification
	Acute toxicity - dermal	Not classified	None	Not classified	conclusive but not sufficient for classification
	Acute toxicity - inhalation	Not classified	None	Not classified	Data lacking
3.2.	Skin corrosion / irritation	Not classified	None	Not classified	conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Not classified	None	Not classified	conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	Not classified	None	Not classified	Data lacking
3.4.	Skin sensitisation	**Skin Sens. 1**	None	**Skin Sens. 1**	Not appropriate
3.5.	Germ cell mutagenicity	Not classified	None	Not classified	conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	None	Not classified	data lacking
3.7.	Reproductive toxicity	Not classified	None	Not classified	data lacking

3.8.	Specific target organ toxicity –single exposure	Not classified	None	Not classified	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	None	Not classified	conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	None	Not classified	data lacking
4.1.	Hazardous to the aquatic environment	Not classified	None	**Aquatic Chronic 4; H413**	conclusive but not sufficient for classification
5.1.	Hazardous to the ozone layer	Not classified	None	Not classified	data lacking

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Warning

Pictogram: GHS 07
Hazard statements: H317
Precautionary statements: P261, P272, P280, P302+352, P333+313, P321, P363, P501

Proposed notes assigned to an entry:

None

Table 4: Proposed classification according to DSD

Hazardous property	**Proposed classification**	**Proposed SCLs**	**Current classification [1)]**	**Reason for no classification [2)]**
Explosiveness	Not classified	None	Not classified	conclusive but not sufficient for classification
Oxidising properties	Not classified	None	Not classified	conclusive but not sufficient for classification
Flammability	Not classified	None	Not classified	conclusive but not sufficient for classification
Acute toxicity	Not classified	None	Not classified	conclusive but not sufficient for classification
Acute toxicity – irreversible damage after single exposure	Not classified	None	Not classified	conclusive but not sufficient for classification
Repeated dose toxicity	Not classified	None	Not classified	conclusive but not sufficient for classification
Irritation / Corrosion	Not classified	None	Not classified	conclusive but not sufficient for classification
Sensitisation	**Xi, R43**	None	**Xi, R43**	Not appropriate
Carcinogenicity	Not classified	None	Not classified	data lacking
Mutagenicity – Genetic toxicity	Not classified	None	Not classified	conclusive but not sufficient for classification
Toxicity to reproduction – fertility	Not classified	None	Not classified	data lacking
Toxicity to reproduction – development	Not classified	None	Not classified	data lacking
Toxicity to reproduction – breastfed babies Effects on or via lactation	Not classified	None	Not classified	data lacking
Environment	conclusive but not sufficient for classification	None	**R53**	conclusive but not sufficient for classification

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Indication of danger:	Xi
R-phrases:	R43
S-phrases:	S2, S24, S37

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

When notified under NONS (00-06-1342), the substance was originally classified as R53 on the basis of the low solubility, lack of biodegradation and partition coefficient. The substance was subsequently classified as Aquatic Chronic 4 when CLP ATP 01 was prepared.

The REACH registration has been claimed for the notified substance and a spontaneous update submitted to ECHA to modify the details of the composition and the data available. The information in this dossier is consistent with that in the registration.

2.2 Short summary of the scientific justification for the CLH proposal

This proposal has been prepared by CS Regulatory Ltd in accordance with Article 37(6) of CLP, and submitted by the UKCA. The justification for the CLH proposal to remove the environmental classification of Chronic Category 4 (under CLP) and R53 (under DSD) is based upon relevant study data and QSAR estimates available for the substance itself and a closely structurally-related aryl phosphate. The data are summarised as:

- Bioaccumulation
  - Test data (Sewell, I.G. & Bartlett, A.J. (1995))
  - QSAR data (Green, S. (2011a) and Green, S. (2011b))
- Chronic toxicity to Daphnia (Makiko Anai (2010) and Makiko Anai (2011))
- long-term effects on sediment organisms (Goodband T. / Mullee D.M. (2011a) and Goodband T. / Mullee D.M. (2011a))
- Acute Toxicity to Earthworm (Goodband T. (2011))
- Toxicity to terrestrial plants (Goodband T. / Mullee D.M. (2011))
- Effects on soil micro-organisms (nitrogen transformation) (Clarke, N. (2011))

The bioaccumulation test achieved a BCF of < 0.02 but was conducted using a dispersing agent that is considered to potentially affect the uptake to the test species. In support of this result and to add weight of evidence the results of two QSAR assessments according to EPIWIN and CAESAR achieved BCFs of 8.99 and 6 respectively. The data do not provide a conclusive result but the weight of evidence is considered adequate to determine that the BCF of the substance is below the qualifying criteria for BCFs (>100 for DSD and >500 for CLP) and therefore that the substance does not show the potential to bioaccumulate in the aquatic environment. Both the chronic Daphnia studies show an absence of chronic effects at the solubility limits determined in the studies. Furthermore, all of the data endpoints available sediment and soil species and terrestrial plants showed no toxic or inhibitory effects up to the maximum dose volume required by the test guidelines.
For full details on the justification for the removal of the classification please see the results section of this dossier and Section 5.6: Conclusions on classification and labelling for environmental hazards.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Skin Sens 1 H317

Aquatic Chronic 4 H413

Signal Word: Warning

Pictogram: GHS 07

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

Xi; R43

R53

S 2-24-37-61

2.4 Current self-classification **and labelling**

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

The current classification in Annex VI is used but the proposed classification and labelling is;

Skin Sens 1: H317

Signal Word: Warning

Pictogram: GHS 07

2.4.2 Current self-classification and labelling based on DSD criteria

The current classification in Annex VI is used but the proposed classification and labelling is;

Xi; R43

S 2-24-37

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

This dossier has been prepared by CS Regulatory Ltd 1L-2 in accordance with Article 37(6) of CLP. The substance is currently listed on Annex VI of CLP and is classified with Aquatic Chronic 4 (R53 in accordance with Dir 67/548/EEC). Data are available to demonstrate that this classification is incorrect and therefore a proposal to amend the classification is justified.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

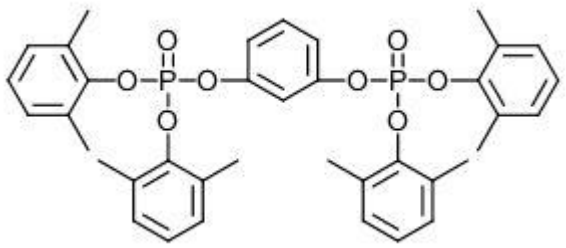
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	432-770-2
EC name:	Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate
CAS number (EC inventory):	139189-30-3
CAS number:	139189-30-3
CAS name:	Phosphoric acid, P,P'-1,3-phenylene P,P,P',P'-tetrakis(2,6-dimethylphenyl) ester
IUPAC name:	Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate
CLP Annex VI Index number:	015-192-00-1
Molecular formula:	C38 H40 O8 P2
Molecular weight range:	687

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate	>80% w/w	Confidential	Concentration range is claimed as confidential and is not provided in this public document. The value is provided in the accompanying IUCLID dossier. The confidential information does not affect the classification proposal.

Current Annex VI entry:

Skin Sens 1: H317

Aquatic Chronic 4: H413

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
confidential			

Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate contains 1 process impurity which is considered to not contribute to the classification and labeling. Further detail is provided in the technical dossier.

Current Annex VI entry: Not Classified

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

Current Annex VI entry:

Not Applicable

1.2.1 Composition of test material

All study data were developed on technical grade material with purity (98.4 %w/w) and impurity profile meeting the composition stated in the registration dossier.

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	The substance is a white powder at room temperature	IUCLID 4.1 study report, PX-200: Determination of General Physico-Chemical Properties, 519/005, 1999, Hogg, A.S. Method: Visual assessment Purity: 98.4%	Visual assessment
Melting/freezing point	368 K	IUCLID 4.2 Reference: study report, PX-200: Determination of General Physico-Chemical Properties, 519/005, 1999, Hogg, A.S. Method: EU Method A.1 (Melting / Freezing Temperature) Purity: 98.4%	Measured

Boiling point	Decomposes from approximately 472 K at 101.17 to 101.20 kPa, no value for boiling temperature could be determined	IUCLID 4.3 Reference: study report, PX-200: Determination of General Physico-Chemical Properties, 519/005, 1999, Hogg, A.S. Method: EU Method A.2 (Boiling Temperature) Purity: 98.4%	measured
Relative density	1.24 at 20°C (+/- 0.5°C).	IUCLID 4.4 Reference: study report, PX-200: Determination of General Physico-Chemical Properties, 519/005, 1999, Hogg, A.S. Method: EU Method A.3 (Relative Density) Purity: 98.4%	measured
Vapour pressure	<4.0E-04 Pa at 25°C, using a vapour pressure balance.	IUCLID 4.6 Reference: study report, PX-200: Determination of Vapour Pressure, 519/007, 1999, Tremain, S.P. Method: EU Method A.4 (Vapour Pressure) effusion method: vapour pressure balance Purity: 98.4%	measured
Surface tension	study scientifically unjustified	IUCLID 4.10	
Water solubility	Insoluble (< 0.1 mg/L)	IUCLID 4.8 Reference: study report, PX-200: Determination of General Physico-Chemical	measured

		Properties, 519/005, 1999, Hogg, A.S. Method: EU Method A.6 (Water Solubility) column elution method Purity: 98.4%	
Partition coefficient n-octanol/water	log10 Pow >6.2	IUCLID 4.7 Reference: study report, PX-200: Determination of General Physico-Chemical Properties, 519/005, 1999, Hogg, A.S. Method: EU Method A.8 (Partition Coefficient) HPLC method Purity: 98.4%	measured
Partition coefficient n-octanol/water	log10 Pow 11.79	IUCLID 4.7 Reference: QSAR, 2011, Green, S Method: Episuite v4 Purity: 98.4%	QSAR estimate
Flash point	study scientifically unjustified	IUCLID 4.11	
Flammability	non flammable	IUCLID 4.13 Reference: study report, PX-200: Determination of Hazardous Physico-Chemical Properties, 519/006, 1999, Tremain, S.P. Method: EU Method A.10 (Flammability (Solids)) Purity: 98.4%	measured

Explosive properties	non explosive	IUCLID 4.14 Reference: study report, PX-200: Determination of Hazardous Physico-Chemical Properties, 519/006, 1999, Tremain, S.P. Method: EU Method A.14 (Explosive properties) Purity: 98.4%	measured
Self-ignition temperature	> 400°C	IUCLID 4.12 Reference: study report, PX-200: Determination of Hazardous Physico-Chemical Properties, 519/006, 1999, Tremain, S.P. Method: EU Method A.15 (Auto-Ignition Temperature (Liquids and Gases)) Purity: 98.4%	measured
Oxidising properties	no oxidising properties	IUCLID 4.15 Reference: study report, PX-200: Determination of Hazardous Physico-Chemical Properties, 519/006, 1999, Tremain, S.P. Method: EU Method A.17 (Oxidising Properties (Solids)) Purity: 98.4%	measured
Granulometry	10.1% having a particle size less than 100 µm	IUCLID 4.5 Reference: study report, PX-200: Determination of General Physico-Chemical Properties,	measured

		519/005, 1999, Hogg, A.S. Method: Particle Size Distribution, Fibre Length and diameter Distribution, June 1996 European Commission technical guidance document. volumetric distribution Purity: 98.4%	
Stability in organic solvents and identity of relevant degradation products	Not determined		
Dissociation constant	Not determined		
Viscosity	Not determined		

2 MANUFACTURE AND USES

2.1 Manufacture

100% of the substance is manufactured outside of the EU.

2.2 Identified uses

All identified uses summarised below take place in closed system

Table 10: Uses by workers in industrial settings

Confidential	IU number	Identified Use (IU) name	Substance supplied to that use	Use descriptors
	1	The substance is used as a fire-preventing agent in prepreg sheets for use in electronic circuit boards for products such as mobile phones, personal computers, televisions and video recorders.	in a mixture	**Process category (PROC):** PROC 0: Other: The neat substance is manufactured outside of the EU. It is imported into the EU as a flame-retardant ingredient of prepregnated sheets (up to 20% by weight) for the manufacture of electronic circuit boards for consumer products such as mobile phones, personal computers, televisions and video recorders. **Sector of end use (SU):** SU 0: Other: Electronic Components.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not considered as part of this proposal.

4 HUMAN HEALTH HAZARD ASSESSMENT

Not considered as part of this proposal.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 11: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Test type: ready biodegradability activated sludge, adapted Standards for testing facility stipulated in Order item No. 3 prescribed the test items for novel chemical substances (Kanpogyo No. 39, Yakuhatsu No. 229 and 59 Kikyoku No. 85, issued March 31, 1984)	Under test conditions no biodegradation observed % Degradation of test substance: > 10.7 — < 17.3 after 28 d (Test mat. analysis) (Average: 13.23%) 0 after 28 d (O2 consumption)	1 (reliable without restriction) key study experimental result **Test material (IUPAC name): Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate**	Gotoh, T. (1995)

5.1.1 Stability

5.1.1.1 Abiotic degradation

5.1.1.1.1 Hydrolysis

Abiotic degradation, hydrolysis as a function of pH could not be determined on the basis of the low water solubility of the substance, due to the limitations of the current methodologies. Given the chemical structure of the substance and the low water solubility of the substance, it is unlikely that abiotic degradation will contribute significantly to the destruction of the substance in the environment.

Reason: study technically not feasible

Justification: In accordance with REACH Annex VIII column 2, the study does not need to be conducted if the substance is readily biodegradable or highly insoluble in water.

5.1.1.1.2 Phototransformation/photolysis

5.1.1.1.2.1 Phototransformation in air

No data available

5.1.1.1.2.2 Phototransformation in water

No data available

5.1.1.1.2.3 Phototransformation in soil

No data available

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

No data available

5.1.2.2 Screening tests

The test results are summarised in the following table:

Table 12: Overview of screening tests for biodegradation in water

Method	Results	Remarks	Reference
Test type: ready biodegradability activated sludge, adapted Standards for testing facility stipulated in Order item No. 3 prescribed the test items for novel chemical substances (Kanpogyo No. 39, Yakuhatsu No. 229 and 59 Kikyoku No. 85, issued March 31, 1984)	under test conditions no biodegradation observed % Degradation of test substance: > 10.7 — < 17.3 after 28 d (Test mat. analysis) (Average: 13.23%) 0 after 28 d (O2 consumption)	1 (reliable without restriction) key study experimental result **Test material (IUPAC name): Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate**	Gotoh, T. (1995)

Test procedure:

The present study was performed according to the degradation test of chemical substances in bacteria stipulated in *Kanpogyo* No. 5, *Yakuhatsu* No. 615 and *49 Kikyoku* No. 392.

Test apparatus

Closed oxygen consumption measuring apparatus (Coulo- Meter No. 7, Ohkura Electric Co., Ltd.)

Test conditions

1) Concentration of test substance: 100 ppm

2) Concentration of standard activated sludge: 30 ppm

3) Test temperature: 25 ±1 °C

4) Test period: 28 days

Results:

Degradation rate based on oxygen consumption

The degradation rate based on oxygen consumption was 0% in each test system. The degradation rate based on the residual amount of the test substance was obtained from the amount prepared, because the recovery of the test substance from the blank sample (test substance + water) was not enough. The degradation rates were 17.3%, 10.7% and 11.7% (average: 13.23%).

Therefore, it was judged that tetrakis(2,6 dimethyl- phenyl)-m-phenylenebisphosphate is not readily biodegradable.

5.1.2.3 Simulation tests

No data available

5.1.3 Summary and discussion of degradation

PX-200 displays a low ready biodegradability in that it achieved 13.23% biodegradation in a 28-day study, indicating that it is unlikely to achieve a half-life of less than 40 or 60 days within fresh water attributed to ready biodegradation alone.

The hydrolysis of PX-200 has not been assessed by testing due to the limitations of the study method with insoluble substances. Furthermore, studies on direct photo transformation in water are not available but it is assumed on the basis of chemical structure that the substance is not degraded by hydrolysis or direct photolysis.

The substance is considered to be persistent in the environment were exposure to occur based on the known lack of ready biodegradation and a perceived likelihood that abiotic processes would not contribute significantly to the depletion of the substance within the environment.

5.2 Environmental distribution

The test substance, PX-200, is a solid under all environmental conditions and is highly insoluble in water (<0.1 mg/l). It has a low volatility (based on a vapour pressure result of <4.0E-04 Pa at 25 °C) and an affinity for soil / sediment (based on the partition coefficient value of Log Pow >6.2 Log Koc as 5.23). As such, any

environmental release will result in virtually all of the substance compartmentalising into soil and water compartments, with little release directly to atmosphere.

This is supported by a Level III fugacity model in the US EPA EPISUITE (Mackay,) which assumes steady-state but not equilibrium conditions. The Level III model in EPI Suite predicts partitioning between air, soil, sediment and water using a combination of default parameters and various input parameters. This model has been used to calculate the theoretical distribution of PX-200 between four environmental compartments (air, water, soil, sediment) at steady state in a unit world.

Partitioning is detailed to be:

- Air 7.55e-006%
- Water 1.3%
- Soil 62 %
- Sediment 36.7 %

It should be noted that as the majority of the substance distributes to the soil compartment and considering the low solubility in water, this indicates that the substance is likely to persist in the soil compartment rather than distribute to the soil pore water.

It is therefore considered likely that very little or no distribution in the environment would occur.

5.2.1 Adsorption/Desorption

The studies on adsorption/desorption are summarised in the following table:

Table 13: Overview of studies on adsorption/desorption

Method	**Results**	**Remarks**	**Reference**
Study type: adsorption (soil/sewage sludge) HPLC estimation method EU Method C.19 (Estimation of the Adsorption Coefficient (KOC) on Soil and Sewage Sludge Using High Performance Liquid Chromatography (HPLC))	Adsorption coefficient: Koc: > 0 log Koc: > 5.63	1 (reliable without restriction) key study experimental result **Test material (IUPAC name): Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate**	Hogg, A.S. (1999)

5.2.2 Volatilisation

No data available.

5.2.3 Distribution modelling

No data available.

5.3 Aquatic Bioaccumulation

Table 14: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Bioaccummulation in Fish study	BCF: < 0.02 (whole body d.w.) (Time of plateau: 56 d)(steady state)	Study conducted using 3%v/v Tween 80-dimethyl formamide dispersing agent	Sewell, I.G. & Bartlett, A.J. (1995)
EPIWIN calculation of BCF	BCF: 8.99 L/kg		S Green (2011a)
CAESAR calculation of BCF	BCF: 6 L/kg (whole body w.w.)		S Green (2011b)

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

Table 15. Overview of estimation on aquatic bioaccumulation

Method	Results	Remarks	Reference
Quantitative Structural-Activity Relationship based upon chemical structure devised from the SMILES code drawn from a database of >40,000 chemicals (called PHYSPROP©) that is included in the EPI Suite™ software. QSAR has been undertaken as the measured BCF data available for this substance has been performed using a dispersing agent that may be considered to affect the uptake of the substance to	BCF: 8.99 L/kg	2 (reliable with restrictions) supporting study (Q)SAR **Test material (IUPAC name): Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate**	S Green (2011a)

Method	**Results**	**Remarks**	**Reference**
the biological organism.			
Computer Assisted Evaluation of industrial chemical Substances According to Regulations (CAESAR), EC funded Project no. 022674 – SSPI, Bioconcentration Factor. Assessment initiated by SMILES code and assessed on structurally related molecules.	BCF: 6 L/kg (whole body w.w.)	2 (reliable with restrictions) supporting study (Q)SAR **Test material (IUPAC name): Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate**	S Green (2011b)

EPIWIN QSAR

US EPA On-Line EPI Suite™ v4.0 model BCFBAF Version 3.2

The BCFBAF method classifies a compound as either ionic or non-ionic. Non-ionic compounds include both alkyl and aryl phosphoric acid esters and aryl phosphates, along with alkyl substituted aromatic rings and aromatic ring structures which are included in the structural fragments on which the estimation is based.

For Log Kow > 7.0 the derived QSAR estimation equation is:

Log BCF = -0.49 Log Kow + 7.554 + Σ correction factors

(n = 35, r^2 = 0.634, Q^2 = 0.57, std dev = 0.538, avg dev = 0.396)

The previous BCFWIN equation:

Log BCF = -1.37 Log Kow + 14.4 + **Σ** correction factors

Certain super-hydrophobic chemicals (Log Kow >7.0) selected from the empirical database had reported BCF values with measured water concentrations that exceed water solubility limits. These BCF values were corrected based on estimates of water solubility limits (Arnot and Gobas, 2006).

The QSAR is initiated by means of SMILES code.

Training Dataset Included:

466 Non-Ionic Compounds (including both alkyl and aryl phosphates)

61 Ionic Compounds

The EPIWIN Output is:

```
============================================================
Whole Body Primary Biotransformation Rate Estimate for Fish:
============================================================
------+-----+-----------------------------------------+---------+---------
 TYPE | NUM | LOG BIOTRANSFORMATION FRAGMENT DESCRIPTION |  COEFF  |  VALUE
------+-----+-----------------------------------------+---------+---------
 Frag |   2 |  Phosphate ester (P=O type)               | -0.6031 | -1.2063
 Frag |   8 |  Alkyl substituent on aromatic ring       |  0.1781 |  1.4245
 Frag |   8 |  Aromatic-CH3                             | -0.0872 | -0.6973
 Frag |  16 |  Aromatic-H                               |  0.2664 |  4.2620
 Frag |   5 |  Benzene                                  | -0.4277 | -2.1386
 L Kow|   * |  Log Kow =  11.79 (KowWin estimate)       |  0.3073 |  3.6225

 MolWt|   * |  Molecular Weight Parameter               |         | -1.7609
 Const|   * |  Equation Constant                        |         | -1.5058
============+=========================================+=========+=========
   RESULT    |        LOG Bio Half-Life (days)         |         |  1.9689
   RESULT    |            Bio Half-Life (days)         |         |   93.09
   NOTE      |  Bio Half-Life Normalized to 10 g fish at 15 deg C  |
============+=========================================+=========+=========
```

The final result should be considered to be suitable for the purposes of a weight of evidence approach, as it is appropriate for the rule base used, and fits the chemical categories employed by the BCFBAF QSAR model.

CAESAR QSAR

Within CAESAR the models were validated by both internal and external validation. The external validation was done in the past [Zhao C, Boriani E, Chana A, Roncaglioni A, Benfenati E: A New Hybrid QSAR Model for Predicting Bioconcentration Factor (BCF). *Chemosphere* 2008, 73:1701-1707.] using about 20% of the original compounds available when modeling started. Here, the model was tested using a new external set obtained by combining the EURAS and the Arnot datasets, excluding the compounds already included in the CAESAR dataset. For the comparison we used the results of predictions for the model developed by Meylan *et al*. [Meylan WM, Howard PH, Aronson D, Printup H, Gouchie S: Improved Method for Estimating Bioconcentration Factor (BCF) from Octanol-Water Partition Coefficient. *SRC TR-97-006 (2nd Update), July 22, 1997; prepared for: Robert S. Boethling, EPA-OPPT, Washington, DC; Contract No. 68-D5-0012; prepared by: ; Syracuse Research Corp., Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212* , Meylan WM, Howard PH, Boethling RS, Aronson D, Printup H, Gouchie S: Improved Method for Estimating Bioconcentration/Bioaccumulation Factor from Octanol/Water Partition Coefficient. *Environ Toxicol Chem* 1999, 18:664-672.] and implemented in the BCFBAF v3.00 included into EPI Suite v4.0 [EPISuite v. 4.0 [http://www.epa.gov/oppt/exposure/pubs/PISuitedl.htm]].

The SDEP was calculated according to:

$$SDEP = \sqrt{\frac{\sum (o_i - p_i)^2}{n}}$$

where o_i are the observed values, p_i the predicted values and n the number of values.

Input was by the SMILES code for the substance

To evaluate the applicability domain CAESAR used three approaches.

First approach: chemical descriptor space

The values for the training set of the eight descriptors in the combined model were used to define their ranges of validity. The CAESAR software gives a warning in this case.

Second approach: rules

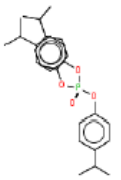
A series of fragments, representing the compounds with greater uncertainty, were manually identified by searching among the structures with highest error (greater than 1 log unit) or misclassified (predicted nB when they are vB, or vice versa). These chemical features have been implemented in our model using short strings called SMARTS to define fragments. In addition, in this case the system gives the user a warning. SMARTS allows the user to specify substructures that are straightforward extensions of SMILES. Thus, flexible and efficient substructure-search specifications can be made in a way that is meaningful to chemists.

Two free programs have been used to do that: MarvinSketch [MarvinSketch, Calculator Plugin and Chemical Terms Demo [http://www.chemaxon.com/ marvin/sketch/index.jsp]] and Daylight Depict SMARTS Match [Daylight Depict SMARTS Match [http://www.daylight.com/daycgi_tutorials/depictmatch.cgi]]. The first is an advanced, Java-based chemical editor for drawing chemical structures, queries and reactions. We used it to draw 11 SMARTS fragments. To check the match between SMARTS and the actual sub-structure of interest, the Daylight Depict SMARTS Match was used, a web application based on Java code. In this program the structure, depicted by a SMILES, is checked to find the fragment represented by the SMARTS.

Third approach: similarity tool

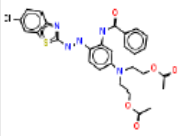
On the basis of several Dragon descriptors encoding different bi-dimensional characteristics of the molecules, a similarity index was developed to retrieve similar compounds from the CAESAR dataset, directly linked to the CAESAR models. More details of these tools are given in the paper on developmental toxicity [Cassano A, Manganaro A, Martin T, Young Y, Piclin N, Pintore M, Bigoni D, Benfenati E: The CAESAR models for developmental toxicity**.** *Chemistry Central Journal* 2010, 4(Suppl 1)**:**S4.], in this issue.

The following chemicals similar to the query compound have been identified in the CAESAR database:



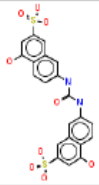
Dataset id: 482
SMILES: O=P(Oc1ccc(cc1)C(C)C)(Oc1ccc(cc1)C(C)C)Oc1ccc(cc1)C(C)C
Similarity: 0.76

Experimental Log BCF: 1.50
Predicted Log BCF: 1.29



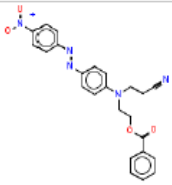
Dataset id: 507
SMILES: O=C(c1ccccc1)Nc1c(ccc(c1)N(CCOC(=O)C)CCOC(=O)C)N=NC1=Nc2ccc(cc2S1)Cl
Similarity: 0.625

Experimental Log BCF: 1.22
Predicted Log BCF: 0.50



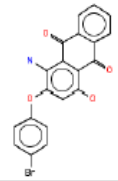
Dataset id: 495
SMILES: O=C(Nc1cc2cc(cc(c2cc1)O)S(=O)(=O)O)Nc1cc2cc(cc(c2cc1)O)S(=O)(=O)O
Similarity: 0.59

Experimental Log BCF: 0.26
Predicted Log BCF: 0.26



Dataset id: 448
SMILES: N#CCCN(c1ccc(cc1)N=Nc1ccc(cc1)[N+](=O)[O-])CCOC(=O)c1ccccc1
Similarity: 0.564

Experimental Log BCF: 0.83
Predicted Log BCF: 0.81



Dataset id: 508
SMILES: O=C1c2ccccc2C(=O)c2c1c(cc(c2N)Oc1ccc(cc1)Br)O
Similarity: 0.562

Experimental Log BCF: 2.05
Predicted Log BCF: 1.27

Dataset id: 480
SMILES: O=P(Oc1ccccc1)(Oc1ccccc1)OCCCCCCCCC(C)C
Similarity: 0.557

Experimental Log BCF: 2.83
Predicted Log BCF: 1.76

The structural analogues are considered to adequately fall within the same domain to at least support a weight of evidence approach.

The final result should be considered to be suitable for the purposes of a weight of evidence approach, as it is appropriate for the rule base used, and has been prepared and evaluated in conjunction with the European Chemicals Agency for the purposes of REACH registration.

5.3.1.2 Measured bioaccumulation data

Table 16. Overview of studies on aquatic bioaccumulation

Method	Results	Remarks	Reference
Cyprinus carpio aqueous (freshwater) flow-through Total uptake duration: 56 d Total depuration duration: 0 d Details of method: BASIS INFORMATION - Measured/calculated log Pow: >6.2 - Results from toxicokinetic study: Please see section 7.1.1 - Results from residue study: No data available. - Monitoring data: No data. BASIS FOR CALCULATION OF BCF - Estimation software: Not used. Please see results section for equation used to calculate BCF. - Result based on measured log Pow of: Not applicable. - Result based on calculated log Pow of: Not applicable.	BCF: < 0.2 (whole body d.w.) (Time of plateau: 56 d)(steady state) (Study conducted using 3%v/v Tween 80-dimethylformamide dispersing agent) BCF: < 0.02 (whole body d.w.) (Time of plateau: 56 d)(steady state) (Study conducted using 3%v/v Tween 80-dimethylformamide dispersing agent) Lipid content: 0 mg/kg bw d.w. (start of exposure) (Solvent control group) 0 mg/kg bw d.w. (end of exposure) (Solvent control group) < 0.2 mg/kg bw d.w. (Day 14) (0.10 mg/l group) < 0.2 mg/kg bw d.w. (end of exposure) (0.10 mg/l group) < 0.02 mg/kg bw d.w. (Day 14) (1.0 mg/l group) < 0.02 mg/kg bw d.w. (end of exposure) (1.0 mg/l group)	1 (reliable without restriction) key study experimental result **Test material (IUPAC name): Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate**	Sewell, I.G. & Bartlett, A.J. (1995)

Method	Results	Remarks	Reference
OECD Guideline 305 C (Bioaccumulation: Test for the Degree of Bioconcentration in Fish)			

Methods

A study was performed to assess the bioaccumulation of the test material in common carp *(Cyprinus carpio).* The method followed was that described in OECD Guideline No. 305C "Bioaccumulation: Test for the Degree of Bioaccumulation in Fish" and the requirements of the Japanese Ministry of International Trade and Industry's Chemical Substance Control law Clause No. 117, 1973).

Procedures

Following a preliminary acute killifish study, common carp were exposed, in groups of 25, to an aqueous dispersion of the test material at concentrations of 0.10 and 1.0 mg/1 for a period of 56 days under dynamic test conditions. Samples of test fish were taken from the solvent control, and 0.10 and 1.0 mg/1 test groups on days 14, 28, 42, 49 and 56, and the concentration of test material in the fish tissues determined.

Results

The 48-hour LC_{50} from the exposure of killifish to PX-200 was estimated to be greater than 100 mg/1.

The Bioconcentration Factors (BCFs) for PX-200, in common carp, after 56 days were calculated to be less than 0.20 at a concentration of 0.10 mg/1 and less than 0.020 at a concentration of 1.0 mg/1.

ASSESSMENT OF THE BIOACCUMULATION OF PX-200 IN COMMON CARP

TABLE OF BIOCONCENTRATION FACTORS (BCFs)

Nominal Concentration (mg/1)	Bioconcentration Factor				
	Days				
	14	28	42	49	56
0.10	<0.20	<0.20	<0.20	<0.20	<0.20
1.0	<0.020	<0.020	<0.020	<0.020	<0.020

Analysis of the test solutions on days 0, 2, 6, 8, 13, 15, 20, 22, 27, 29, 34, 36, 41, 43, 48, 50, 54 and 56 showed the measured test concentrations to be near nominal.

5.3.2 Summary and discussion of aquatic bioaccumulation

The partition coefficient of the substance was measured to be >6.2 by means of the HPLC method. The limit value is due to the limitation of the method. It was therefore considered appropriate to undertake an estimation of the partition coefficient by means of QSAR estimation based upon the SMILES code of the molecule using US EPA KOWWIN v1.67 of the EPI Suite v4. Based upon structural fragmentation drawn from a database of >40,000 substances, including aryl phosphate esters and aromatic species predicted for the fragmentation estimates, the log Pow is estimated to be 11.79.

Based on these data, the substance may be considered to be of concern as potential for bioaccumulation, according to screening criteria for bioaccumulation in ECHA guidance (Chapter R.11 PBT Assessment). The likely reliability of the log Pow is, however, considered to diminish above a value of 6, as noted in Appendix R.11-1 Annex 1 of ECHA guidance on PBT Assessment. Substances with log Pow between 4.5 and 6 are considered likely to be highly accumulating; however no substantial bioconcentration is assumed for compounds having log Pow with values less than 4.5 or greater than 6. For compounds having log Pow greater than 6, a gradual decrease of the BCF is observed and it has been hypothesised within the published literature that a high log Pow is more an effect of solubility than a tendency of the substance to be lipophilic.

Considering that the measured log Pow is a limit value at 6.2 and has been estimated based on structure to be 11.79, it is considered that the results indicate that the substance is likely to be non-bioaccumulating based on the partition coefficient.

A fish bioaccumulation study has been conducted using common carp and according to the OECD 305 test guideline which concludes that the BCF is <0.02 based on whole body weight after 56 days. The study was, however, conducted primarily for notification in Japan and, due to the low water solubility, the test formulations were prepared using a dispersing agent of 3%v/v Tween 80-dimethylformamide. The use of a dispersing agent is considered to affect the uptake of the test item to the fish reducing the reliability of the result.

In support of these data, two separate *in silico* QSAR estimations have been undertaken using the EPIWIN and CAESAR database systems. The EPIWIN QSAR estimates that the BCF for the substance is 8.99 L/Kg while the CAESAR QSAR estimates the BCF for the substance to be 6 L/Kg. These data support the consideration that the use of the dispersing agent affects the uptake of the substance to the test organism but suggest that the BCF for the substance is below the threshold of concern.

Summary:

Based on a weight of evidence approach of study data with reduced reliability and two separate QSAR estimation techniques, PX-200 is considered to be not bioaccumulative with a BCF <100. It is, therefore, considered to not meet the DSD criteria of ≥ 100 nor the CLP criteria of ≥ 500.

5.4 Aquatic toxicity

Table 17: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
96 h fish LC50	>0.027 mg/l (measured)	No response at limit of solubility	Wetton, P.M. & Mullee, D.M. (2000)
48 h Daphnia EC50	>0.032 mg/l (measured)	No response at limit of solubility	Wetton, P.M & Mullee, D.M.

			(2008)
21 d Daphnia NOEC	≥0.00077 mg/L (measured)	No response at limit of solubility	Makiko Anai (2010)
	≥0.0011 mg/L (measured) by read across	No response at limit of solubility	Makiko Anai (2011)
72 h algal EC50	> 0.031 mg/l (measured)	No response at limit of solubility	Mead, C. & Mullee, D.M. (2008)
long-term effects on sediment organisms	EC50 (28 d): > 1000 mg/kg sediment dw test mat. (nominal) based on: emergence rate of *Chironomus riparius* by read across	No response at maximum dose	Goodband T. / Mullee D.M. (2011a)
	EC50 (28 d): > 1000 mg/kg sediment dw test mat. (nominal) based on: emergence rate of *Lumbriculus variegatus* by read across	No response at maximum dose	Goodband T. / Mullee D.M. (2011b)
Acute Toxicity to Earthworm	LC50 (14 d): > 1000 mg/kg soil dw test mat. (nominal) by read across	No response at maximum dose	Goodband T. (2011)
Toxicity to terrestrial plants	EC50 (21 d): > 1000 mg/kg soil dw test mat. (nominal) based on growth of *Glycine max (G. soja)* by read across	No response at maximum dose	Goodband T.J. / Mullee D.M. (2011)
	EC50 (21 d): > 1000 mg/kg soil dw test mat. (nominal) based on growth of *Lycopersicon esculentum* by read across	No response at maximum dose	
	EC50 (21 d): > 1000 mg/kg soil dw test mat. (nominal) based on growth of *Avena sativa* by read across	No response at maximum dose	
Effects on soil micro-organisms (nitrogen transformation)	EC50 (28 d): > 1000 mg/kg soil dw test mat. (nominal) based on nitrate formation rate by read across	No response at maximum dose	Clarke N. (2011)

No effects were observed in any of the toxicity tests at the limit of solubility in the test systems.

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

The results are summarised in the following table:

Table 18. Overview of short-term effects on fish

Method	Results	Remarks	Reference
Oncorhynchus mykiss freshwater semi-static OECD Guideline 203 (Fish, Acute Toxicity Test) EU Method C.1 (Acute Toxicity for Fish)	LC50 (96 h): > 0.8 mg/L test mat. (nominal) LC50 (96 h): > 0.027 mg/L test mat. (meas. (TWA))	1 (reliable without restriction) key study experimental result **Test material (IUPAC name): Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate**	Wetton, P.M. & Mullee, D.M. (2000)

Methods

A study was performed to assess the acute toxicity of the test material to rainbow trout *(Oncorhynchus mykiss).* The method followed that described in the OECD Guidelines for Testing of Chemicals (1992) No 203, "Fish, Acute Toxicity Test" referenced as Method C.l of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

Procedures

Following a preliminary range-finding study fish were exposed, in two groups of ten, to an aqueous dispersion of the test material, at a single concentration of 0.80 mg/1 for a period of 96 hours under semi-static test conditions. The number of mortalities and any sub-lethal effects of exposure in each test and control vessel were determined 3 and 6 hours after the start of exposure and then daily throughout the study until termination after 96 hours.

Results

The 96-Hour LC_{50} based on nominal test concentrations was greater than 0.80 mg/1 and correspondingly the No Observed Effect Concentration was 0.80 mg/1.

The test concentration of 0.80 mg/1 was the highest attainable test concentration due to the limited solubility of the test material in water and auxiliary solvent, and having due regard for the amount of auxiliary solvent permitted in the test under the OECD Guidelines.

Preliminary solubility work and analysis showed that at a test concentration of 0.80 mg/1, a proportion of the test material remained undissolved. Therefore, analysis was performed on samples of filtered and unfiltered test media during the definitive study. The filtered samples indicated the concentration of test material in solution and hence bioavailable to the test fish. Analysis of the fresh and old test media preparations over the 96 hour study duration showed the measured concentrations to be within the range of 80% to 101% of nominal for the unfiltered test media (a single exception was noted) and within a range of 1% to 16% of nominal for the filtered test samples.

The measured concentrations of the unfiltered media indicate both the amount of dispersed and dissolved test material in the test system, whereas the measured values from the filtered media show the amount of test material which was in solution and hence bioavailable to the test fish. It was therefore considered appropriate to base the results on the time-weighted mean measured test concentrations of the filtered test media in order to give a "worst case" analysis of the data.
The 96-Hour LC_{50} based on the time-weighted mean measured test concentration of the filtered test media was greater than 0.027 mg/1 and correspondingly the No Observed Effect Concentration was 0.027 mg/1.

5.4.1.2 Long-term toxicity to fish

No data available.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

The results are summarised in the following table:

Table 19. Overview of short-term effects on aquatic invertebrates

Method	Results	Remarks	Reference
Daphnia magna freshwater static OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) EU Method C.2 (Acute Toxicity for Daphnia)	EC50 (48 h): > 0.032 mg/L test mat. (meas. (TWA)) based on: immobilisation	1 (reliable without restriction) key study experimental result **Test material (IUPAC name): Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate**	Wetton, P.M & Mullee, D.M. (2008)

Methods

Preliminary solubility work and analysis showed that at a test concentration of 0.80 mg/1 a proportion of the test material remained undissolved. Therefore analysis was performed on samples of filtered and unfiltered test media during the definitive study. The filtered samples indicated the concentration of test material in solution and hence bioavailable to the test organisms.

Analysis of the unfiltered media showed measured values of 72% and 67% of nominal at 0 hours and
82% and 80% at 48 hours. The low results shown for the 0 hour test samples were considered to be due to the problems associated with sampling and analysis of dispersions

at low test concentrations in a complex biological system. Analysis of frozen duplicate samples showed lower measured values from those originally obtained. This was considered to be due to losses during storage and thawing prior to analysis. Analysis of the filtered test media showed measured test concentrations of 3% of nominal at 0 hours and 6% of nominal at 48 hours.

The results shown from analysis of the unfiltered media indicate both the amount of dispersed and dissolved test material in the test system, whereas the measured values from the filtered media indicate the amount of dissolved test material which was bioavailable to the test organisms. It was therefore considered appropriate to base the results on the time-weighted mean measured test concentrations of the filtered test media in order to give a "worst case" analysis of the data.

The 48-Hour EC_{50} based on the time-weighted mean measured test concentration of the filtered test media was greater than 0.032 mg/1 and correspondingly the No Observed Effect Concentration was
0.032 mg/1

5.4.2.2 Long-term toxicity to aquatic invertebrates

The results are summarised in the following table:

Table 20. Overview of long-term effects on aquatic invertebrates

Method	Results	Remarks	Reference
Daphnia magna brackish water semi-static OECD Guideline 211 (Daphnia magna Reproduction Test)	NOEC (21 d): >= 0.00077 mg/L test mat. based on: mortality	1 (reliable without restriction) key study experimental result **Test material (IUPAC name): Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate**	Makiko Anai (2010)
Daphnia magna *freshwater* *semi-static* *OECD Guideline 211 (Daphnia magna Reproduction Test)*	NOEC (21 d): >= 0.0011 mg/L test mat. (meas. (TWA)) based on: mortality	1 (reliable without restriction) key study experimental result Test material (CAS number): 5945-33-5	Makiko Anai (2011)

Data for Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate

Test conditions

Test item PX-200	
Test organism	Daphnia magna
Dilution water	Reconstituted water described in ASTM

Test concentration	A test concentration around the solubility in dilution water and control
Preparation of test solution (nominal	Test sample and dilution water were mixed to prepare 100 mg/L concentration), and they were stirred for 48 hours. Then, the mixed solution was filtered through a glass fiber filter to produce test solution.
Type of test	Semi static regime (three times renewal per week)
Exposure duration	21 days
Replicate	10 replicates/test level
Number of organism	10 daphnids/test level (one daphnid/test vessel)
Volume of test solution	800 ml/test level (80 mL/test vessel)
Temperature of test solutions	20.1 to 20.3 °C
Irradiation condition	Artificial light of white fluorescent lamp, 16-hour light at intensity not exceeding 15-20 f/E • m^{-2} • s^{-1} /8-hour dark
Feeding	Feeding Chlorella vulgaris daily at ration level between 0.1 and 0.2 mg C (amount of organic carbon) /daphnia/day
Aeration	No aeration
Analysis of concentration of test item in test solution	HPLC analysis (triple repetitions of weekly measurement on sample from the same solution when freshly prepared and at before renewal or at end of the exposure)

Results

Measured concentration of test item dissolved in test solution	
At preparation	0.000088 to 0.00074 mg/L
At before renewal or end of exposure	0.00047 to 0.0030 mg/L
Measured concentration of test item in test solution used for exposure	
At preparation	0.0066 to 0.045 mg/L
At before renewal or end of exposure	0.0043 to 0.037 mg/L
21-day EC50 (concentration causing 50 per cent reduction in reproduction)	>0.00077mg/L
21-day LC50 for parent daphnid (median lethal concentration)	>0.00077 mg/L
NOEC (no observed effect concentration)	>0.00077 mg/L

[The values shown in (3), (4) and (5) were based on the time-weighted mean of measured dissolved concentration]

Data for CAS number: 5945-33-5

- Name of test material: 4,4'-(isopropylidene diphenyl) bis (diphenyl phosphate)

- Molecular formula: C39H34O8P2

- Molecular weight: 692

- Smiles notation:
C1=CC=C(C=C1)OP(OC(C=CC1C(C)(C)C(=CC=C2OP(=O)(OC(=CC=C3)C=C3)OC(C=CC3)=CC=3)C=C2)=CC=1)(OC(=CC=C1)C=C1)=O

The read across substance is similar in structure, being an aryl phosphate of similar molecular weight to PX-200. The substance also displays similar physical chemistry, having extremely low volatility, high Pow and highly insoluble in water with high Koc. The activity of the two substances in the aquatic environment is, therefore, considered to be very similar, with both substances likely to have a tendency to bind to sediments and soils in the environment with

limited tendency to the aqueous or the air compartments. The data are, therefore, considered to be adequately representative of PX-200.

Test conditions	
Test organism	Daphnia magna
Dilution water	Reconstituted water described in ASTM
Test concentration	A test concentration around the solubility in dilution water [below the determination limit (<0.0011 mg/L): measured value in preliminary study] and a control
Preparation of test solution	Test sample dissolved in acetone was added in a preparation container. After the acetone was evaporated, dilution water was added to the container to prepare 100 mg/L (nominal concentration). This suspension was stirred for 48 hours and then filtered with a glass fiber filter by suction to produce test solution.
Type of test Exposure	Semi-static regime (three times renewal per week)
Type of test Exposure duration	21days
Replicate	10 replicates/test level
Number of organism	10 daphnids/test level (one daphnid/test vessel)
Volume of test solution	800 ml/test level (80 ml/test vessel)
Temperature of test solutions	19.8 - 20.2°C
Irradiation condition	Artificial light of white fluorescent lamp, 16-hour light at intensity not exceeding 15-20μE·m^{-2}·s^{-1}/8-hour dark
Feeding	Chlorella vulgaris daily at ration level between 0.1 and 0.2 mg C (amount of organic carbon) /Daphnia/day
Aeration	No aeration
Analysis of concentration of test item in test solution	HPLC analysis (triple repetitions of weekly measurement on sample from the same solution when freshly prepared and before the renewal or at the end of the exposure)

Results

(1) Measured concentration of test item dissolved in test solution
  At preparation — n.d. (<0.0011 mg/L)
  Before renewal or at the end of exposure — n.d. - 0.0036 mg/L
(2) Measured concentration of test item in test solution used for exposure
  At preparation — 0.0034 - 0.072 mg/L
  Before renewal or at the end of exposure — 0.0020 - 0.064 mg/L
(3) 21-day EC_{50} (concentration causing 50% reduction in reproduction) — >0.0011 mg/L
(4) 21-day LC_{50} for parent Daphnia (median lethal concentration) — >0.0011 mg/L
(5) NOEC (no observed effect concentration) — ≥0.0011 mg/L

[The values shown in (3), (4) and (5) were based on the time-weighted mean of measured dissolved concentration.]

5.4.3 Algae and aquatic plants

The results are summarised in the following table:

Table 21. Overview of effects on algae and aquatic plants

Method	Results	Remarks	Reference
Scenedesmus subspicatus (new name: Desmodesmus	EC50 (72 h): > 0.031 mg/L test mat. (meas. (TWA)) based	1 (reliable without	Mead, C. & Mullee, D.M.

Method	Results	Remarks	Reference
subspicatus) (algae) freshwater static OECD Guideline 201 (Alga, Growth Inhibition Test) EU Method C.3 (Algal Inhibition test)	on: growth rate NOEC (72 h): 0.031 mg/L test mat. (meas. (TWA)) based on: growth rate	restriction) key study experimental result **Test material (IUPAC name): Tetrakis(2,6-dimethylphenyl)-m-phenylene biphosphate**	(2008)

Methods

A study was performed to assess the effect of the test material on the growth of the green alga *Scenedesmus subspicatus.* The method followed that described in the OECD Guidelines for Testing of Chemicals (1984) No 201, "Alga, Growth Inhibition Test" referenced as Method C.3 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

Procedure

Following a preliminary range-finding study, *Scenedesmus subspicatus* was exposed to an aqueous dispersion of the test material at a concentration of 0.80 mg/1 (six replicate flasks) for 72 hours, under constant illumination and shaking at a temperature of 24 ± 1°C.

Samples of the algal populations were removed daily and cell concentrations determined for each control and treatment group, using a Coulter® Multisizer II Particle Counter.

Results

Exposure of *Scenedesmus subspicatus* to the test material gave EC_{50} values of greater than 0.80 mg/1 and correspondingly the No Observed Effect Concentration was 0.80 mg/1.

The test concentration of 0.80 mg/1 was the highest attainable test concentration that could be prepared due to the limited solubility of the test material in water and auxiliary solvent and having due regard to the amount of auxiliary solvent permitted in the test under the OECD Guidelines.

During preliminary solubility and range-finding work it was difficult to determine whether all of the test material had dissolved in the test medium (by visual inspection) or whether a micro-dispersion may have formed. Pre-study samples approximately equivalent to the test concentration to be used in the definitive study were analysed directly and after filtration through 0.2 µm filters. The results showed a loss of test material from the filtered samples thereby indicating that at a test concentration of 0.80 mg/1 the test material formed a micro-dispersion. Therefore analysis was performed on samples of filtered and unfiltered test media during the study.

Analysis of the test solutions at 0 hours showed measured values of 104% and 87% of nominal for the unfiltered samples and 3% and 4% of nominal for the filtered samples (replicates R_1–R_3 and R_4 – R_6 respectively).

Analysis of the test solutions at 72 hours showed measured values of 65% and 87% of nominal for the unfiltered samples and 4% and 6% of nominal for the filtered samples (replicates R_1 – R_3 and R_4 – R_6 respectively).

The measured concentrations of the unfiltered samples indicate the amount of test material which is both dispersed and dissolved in the test system. The filtered samples indicate the amount of test material in solution and thus bioavailable to the algae. It was therefore considered justifiable to base the results on the time-weighted mean measured test concentration of the filtered test material in order to give a "worst case" analysis of the data. The EC_{50} values based on the time-weighted mean measured test concentrations were greater than 0.031 mg/1 and correspondingly the No Observed Effect Concentration was 0.031 mg/1.

5.4.4 Other aquatic organisms (including sediment)

5.4.4.1 Sediment organisms

The results are summarised in the following table:

Table 22. Overview of long-term effects on sediment organisms

Method	Results	Remarks	Reference
Chironomus riparius freshwater long-term toxicity (laboratory study) static OECD Guideline 218 (Sediment-Water Chironomid Toxicity Test Using Spiked Sediment)	NOEC (28 d): >= 1000 mg/kg sediment dw test mat. (nominal) based on: emergence rate EC50 (28 d): > 1000 mg/kg sediment dw test mat. (nominal) based on: emergence rate	1 (reliable without restriction) key study experimental result **Test material (CAS number): 5945-33-5**	Goodband T. / Mullee D.M. (2011a)
Lumbriculus variegatus freshwater long-term toxicity (laboratory study) OECD Guidelines for the Testing of Chemicals (2004), "Sediment-water Lumbriculus Toxicity Test using Spiked Sediment", OECD Guideline No. 225, October 2007.	NOEC (28 d): >= 1000 mg/kg sediment dw test mat. (nominal) based on: reproduction EC50 (28 d): > 1000 mg/kg sediment dw test mat. (nominal) based on: reproduction	1 (reliable without restriction) key study experimental result **Test material (CAS number): 5945-33-5**	Goodband T. / Mullee D.M. (2011b)

Chironomus riparius study

- Name of test material: 4,4'-(isopropylidene diphenyl) bis (diphenyl phosphate)

- Molecular formula: C39H34O8P2

- Molecular weight: 692

- Smiles notation:
C1=CC=C(C=C1)OP(OC(C=CC1C(C)(C)C(=CC=C2OP(=O)(OC(=CC=C3)C=C3)OC(C=CC3)=CC=3)C=C2)=CC=1)(OC(=CC=C1)C=C1)=O

The read across substance is similar in structure, being an aryl phosphate of similar molecular weight to PX-200. The substance also displays similar physical chemistry, having extremely low volatility, high Pow and highly insoluble in water with high Koc. The activity of the two substances in the aquatic environment is, therefore, considered to be very similar, with both substances likely to have a tendency to bind to sediments and soils in the environment with limited tendency to the aqueous or the air compartments. The data are, therefore, considered to be adequately representative of PX-200.

Methods. Following a preliminary range-finding test, 120 larvae of *Chironomus riparius* (six replicates of 20 larvae) were exposed to formulated sediment spiked with test item at a single concentration of 1000 mg/kg (dry weight of sediment) for a period of 28 days. The numbers of emerged adult midges were recorded daily.

A further 40 larvae (two replicates of 20 larvae) of each test group were prepared and sacrificed on Day 10 of the exposure period to determine the 10-Day larval survival and growth data.

Results. The 28-Day EC_{50} (reduction in emergence) based on nominal test concentrations was greater than 1000 mg/kg. The No Observed Effect Concentration was equal to or greater than 1000 mg/kg.

The EC_{50} (development rate) based on nominal test concentrations was greater than 1000 mg/kg.

Analysis of the test sediment on Day -7 (i.e. before overlying water was placed above sediment) showed a measured concentration to be 92% of nominal, thereby confirming correct dosing of the sediment.

Analysis of the sediment on Day 0 of the test (i.e. after 7 days equilibration period) showed the measured concentration to be 93% of nominal. Analysis of the overlying water on Day 0 showed a measured concentration of 0.00382 mg/l. Analysis of the interstitial water on Day 0 showed a measured concentration of 1.99 mg/l.

Analysis of the sediment on Day 28 of the test showed the measured concentration to be 74% of nominal. Analysis of the overlying water on Day 28 showed a measured concentration of 0.00294 mg/l. Analysis of the interstitial water on Day 28 showed a measured concentration of 1.02 mg/l.

Lumbriculus variegatus Study

- Name of test material: 4,4'-(isopropylidene diphenyl) bis (diphenyl phosphate)

- Molecular formula: C39H34O8P2

- Molecular weight: 692

- Smiles notation:
C1=CC=C(C=C1)OP(OC(C=CC1C(C)(C)C(=CC=C2OP(=O)(OC(=CC=C3)C=C3)OC(C=CC3)=CC=3)C=C2)=CC=1)(OC(=CC=C1)C=C1)=O

The read across substance is similar in structure, being an aryl phosphate of similar molecular weight to PX-200. The substance also displays similar physical chemistry, having extremely low volatility, high Pow and highly insoluble in water with high Koc. The activity of the two substances in the aquatic environment is, therefore, considered to be very similar, with both substances likely to have a tendency to bind to sediments and soils in the environment with limited tendency to the aqueous or the air compartments. The data are, therefore, considered to be adequately representative of PX-200.

Methods. Following a preliminary range-finding test, 60 *Lumbriculus variegatus* (6 replicates of 10 worms) were exposed to formulated sediment spiked with test item at a concentration of 1000 mg/kg (dry weight of sediment) for a period of 28 days. The numbers of worms and the dry weight data of these worms were recorded at the end of the test.

Further replicates were prepared for the solvent control and each test group and sacrificed on Days 0 and 28 for chemical analysis of the sediment and overlying water.

A positive control conducted approximately every six months used pentachlorophenol sodium salt (PCP-Na salt) as the reference item. *Lumbriculus variegatus* was exposed to formulated sediment spiked with test item at concentrations of 1.0, 3.2, 10, 32 and 100 mg/kg (dry weight of sediment) for a period of 28 days. The numbers of worms and the dry weight data of these worms were recorded at the end of the test.

Results. The Day 28 EC_{50} (reproduction) based on nominal test concentrations was greater than 1000 mg/kg. The No Observed Effect Concentration was equal to or greater than 1000 mg/kg.

Analysis of the test sediment on Day -7 (i.e. before overlying water was placed above sediment) showed a measured concentration to be 90% of nominal, thereby confirming correct dosing of the sediment.

Analysis of the sediment on Day 0 of the test (i.e. after 7 days equilibration period) showed the measured concentration to be 101% of nominal. Analysis of the overlying water on Day 0 showed a measured concentration of 0.0000561 mg/l. Analysis of the interstitial water on Day 0 showed a measured concentration of 2.09 mg/l.

Analysis of the sediment on Day 28 of the test showed the measured concentration to be 83% of nominal. Analysis of the overlying water on Day 28 showed a measured concentration of 0.00122 mg/l. Analysis of the interstitial water on Day 28 showed a measured concentration of 1.39 mg/l.

5.4.4.2 Toxicity to soil macro-organisms

The results are summarised in the following table:

Table 23. Overview of effects on soil macro-organisms

Method	Results	Remarks	Reference
Eisenia fetida (annelids) short-term toxicity (laboratory study) Substrate: artificial soil OECD Guideline 207 (Earthworm, Acute Toxicity Tests)	NOEC (14 d): >= 1000 mg/kg soil dw test mat. (nominal) based on: mortality LC50 (14 d): > 1000 mg/kg soil dw test mat. (nominal) based on: mortality	1 (reliable without restriction) key study experimental result **Test material (CAS number): 5945-33-5**	Goodband T. (2011)

- Name of test material: 4,4'-(isopropylidene diphenyl) bis (diphenyl phosphate)

- Molecular formula: C39H34O8P2

- Molecular weight: 692

- Smiles notation:
C1=CC=C(C=C1)OP(OC(C=CC1C(C)(C)C(=CC=C2OP(=O)(OC(=CC=C3)C=C3)OC(C=CC3)=CC=3)C=C2)=CC=1)(OC(=CC=C1)C=C1)=O

The read across substance is similar in structure, being an aryl phosphate of similar molecular weight to PX-200. The substance also displays similar physical chemistry, having extremely low volatility, high Pow and highly insoluble in water with high Koc. The activity of the two substances in the aquatic environment is, therefore, considered to be very similar, with both substances likely to have a tendency to bind to sediments and soils in the environment with limited tendency to the aqueous or the air compartments. The data are, therefore, considered to be adequately representative of PX-200.

Methods. Following a preliminary range-finding test, 60 earthworms (six replicates of 10 worms) were exposed to a single concentration of 1000 mg/kg of soil for a period of 14 days at a temperature of 19°C to 23°C. The number of mortalities was determined after 7 and 14 days exposure. A positive control using chloroacetamide, conducted approximately every 6 months, is reported for reference purposes.

Results. The 14-Day LC_{50} for the test item to earthworms *(Eisenia foetida)* based on the nominal test concentration was greater than 1000 mg/kg. The No Observed Effect Concentration was equal to or greater than 1000 mg/kg.

The result of the positive control gave a 14-Day LC_5o for chloroacetamide of 43 mg/kg with 95% confidence limits of 41 - 45 mg/kg. The No Observed Effect Concentration was 18 mg/kg.

5.4.4.3 Toxicity to terrestrial plants

The results are summarised in the following table:

Table 24. Overview of effects on terrestrial plants

Method	Results	Remarks	Reference
Glycine max (G. soja) (Dicotyledonae (dicots)) *Lycopersicon esculentum* (Dicotyledonae (dicots)) *Avena sativa* (Monocotyledonae (monocots)) short-term toxicity (laboratory study) seedling emergence toxicity test Substrate: natural soil OECD Guideline 208 (Terrestrial Plants Test: Seedling Emergence and Seedling Growth Test)	*Glycine max (G. soja)*: EC50 (21 d): > 1000 mg/kg soil dw test mat. (nominal) based on: seedling emergence *Glycine max (G. soja)*: NOEC (21 d): >= 1000 mg/kg soil dw test mat. (nominal) based on: seedling emergence *Glycine max (G. soja)*: EC50 (21 d): > 1000 mg/kg soil dw test mat. (nominal) based on: growth *Glycine max (G. soja)*: NOEC (21 d): >= 1000 mg/kg soil dw test mat. (nominal) based on: growth *Lycopersicon esculentum*: EC50 (21 d): > 1000 mg/kg soil dw test mat. (nominal) based on: seedling emergence *Lycopersicon esculentum*: NOEC (21 d): >= 1000 mg/kg soil dw test mat. (nominal) based on: seedling emergence *Lycopersicon esculentum*: EC50 (21 d): > 1000 mg/kg soil dw test mat. (nominal) based on: growth *Lycopersicon esculentum*: NOEC (21 d): >= 1000 mg/kg soil dw test mat. (nominal) based on: growth	1 (reliable without restriction) key study experimental result **Test material (CAS number): 5945-33-5**	Goodband T.J. / Mullee D.M. (2011)

Method	Results	Remarks	Reference
	Avena sativa: EC50 (21 d): > 1000 mg/kg soil dw test mat. (nominal) based on: seedling emergence *Avena sativa*: NOEC (21 d): >= 1000 mg/kg soil dw test mat. (nominal) based on: seedling emergence *Avena sativa*: EC50 (21 d): > 1000 mg/kg soil dw test mat. (nominal) based on: growth *Avena sativa*: NOEC (21 d): >= 1000 mg/kg soil dw test mat. (nominal) based on: growth		

- Name of test material: 4,4'-(isopropylidene diphenyl) bis (diphenyl phosphate)

- Molecular formula: C39H34O8P2

- Molecular weight: 692

- Smiles notation:
C1=CC=C(C=C1)OP(OC(C=CC1C(C)(C)C(=CC=C2OP(=O)(OC(=CC=C3)C=C3)OC(C=CC3)=CC=3)C=C2)=CC=1)(OC(=CC=C1)C=C1)=O

The read across substance is similar in structure, being an aryl phosphate of similar molecular weight to PX-200. The substance also displays similar physical chemistry, having extremely low volatility, high Pow and highly insoluble in water with high Koc. The activity of the two substances in the aquatic environment is, therefore, considered to be very similar, with both substances likely to have a tendency to bind to sediments and soils in the environment with limited tendency to the aqueous or the air compartments. The data are, therefore, considered to be adequately representative of PX-200.

Methods. Following a preliminary range-finding test, three plant species; two dicotylendonous species, soybean *(Glycine max)* and tomato *(Lycopersicon esculentum)* and one monocotylendonous species, oat *(Avena sativa)* were exposed to a single concentration of 1000mg/kg. The number of seedlings emerged and any mortalities and/or morphological abnormalities were determined daily for 21 days after 50% emergence in the control for each species.

Results. Analysis of the 25 g/250 ml solvent stock solution used to prepare the test concentrations on Day 0 showed a measured test concentration of 102% of nominal value and so the results are based on nominal test concentrations only.

The EC_{50} (emergence) and EC_{50} (growth based on final dry weight) for the test item based on nominal test concentrations for the three species tested were as follows:

Species	**EC50 (emergence) (mg/kg)**	95% **Confidence limits (mg/kg)**	**No Observed Effect Concentration (mg/kg)**	**EC_{50} (growth) (mg/kg)**	95% **Confidence limits (mg/kg)**	**No Observed Effect Concentration (mg/kg)**
Soybean	>1000		>1000	>1000	-	>1000
Tomato	>1000	-	>1000	>1000	-	>1000
Oat	>1000	-	>1000	>1000	-	>1000

No Observed Effect Concentration (growth) based on the concentration where no significant effect was observed for dry weight compared to the solvent control and no morphological abnormalities were observed

5.4.4.4 Toxicity to soil micro-organisms

The results are summarised in the following table:

Table 25. Overview of effects on soil micro-organisms

Method	**Results**	**Remarks**	**Reference**
Species/Inoculum: soil OECD Guideline 216 (Soil Microorganisms: Nitrogen Transformation Test) EU Method C.21 (Soil Microorganisms: Nitrogen Transformation Test)	NOEC (28 d): >= 1000 mg/kg soil dw test mat. (nominal) based on: nitrate formation rate EC50 (28 d): > 1000 mg/kg soil dw test mat. (nominal) based on: nitrate formation rate	1 (reliable without restriction) key study experimental result **Test material (CAS number): 5945-33-5**	Clarke N. (2011)

- Name of test material: 4,4'-(isopropylidene diphenyl) bis (diphenyl phosphate)

- Molecular formula: C39H34O8P2

- Molecular weight: 692

- Smiles notation:
C1=CC=C(C=C1)OP(OC(C=CC1C(C)(C)C(=CC=C2OP(=O)(OC(=CC=C3)C=C3)OC(C=CC3)=CC=3)C=C2)=CC=1)(OC(=CC=C1)C=C1)=O

The read across substance is similar in structure, being an aryl phosphate of similar molecular weight to PX-200. The substance also displays similar physical chemistry, having extremely low volatility, high Pow and highly insoluble in water with high Koc. The activity of the two

substances in the aquatic environment is, therefore, considered to be very similar, with both substances likely to have a tendency to bind to sediments and soils in the environment with limited tendency to the aqueous or the air compartments. The data are, therefore, considered to be adequately representative of PX-200.

Methods. Following a preliminary range-finding test soil microorganisms were exposed to the test item at a single concentration of 1000 mg/kg for 28 days at a temperature of approximately 21 °C, in the dark with the addition of powdered Lucerne-green-grass meal to act as a respiratory substrate.

The inhibitory effect of the test item on nitrogen transformation was assessed by the determination of nitrate concentration in the soil samples on Days 0, 7 and 28 and compared to data obtained from control soil samples.

Results. The effect of the test item on the nitrogen transformation activity of the soil microorganisms gave an EC_{50} of greater than 1000 mg/kg. Correspondingly the No Observed Effect Concentration (NOEC) was equal to or greater than 1000 mg/kg.

5.4.4.5 Overview of Toxicity

The water solubility of this aryl phosphate ester is extremely low, making exposure to aquatic species problematic. The substance has, nevertheless, been tested up to the limit of solubility in each test system for acute exposure to fish, Daphnia and algae with no toxic effects. The substance has also been tested up to the limit of solubility for chronic exposure to Daphnia with no toxic effects. A closely related structural analogue has also been tested to its limit of solubility for chronic exposure to Daphnia, again with no toxic effects.

Based on the fugacity model estimated for the substance and presented in section 5.2, any environmental exposure of the substance is likely to result in low partitioning of the substance to the air or water compartments and is likely be absorbed to the soil and sediment compartments.

Using read across to a closely related structural analogue that displays similar chemical characteristics, data are available to suggest no toxicity to sediment dwelling species, earthworms or terrestrial plants and no inhibition of nitrogen transformation in the soil up to the maximum required doses in each test guideline.

The substance is therefore considered not toxic in the environment.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

5.1 Degradation:
The test substance is not considered to be readily biodegradable.

5.2 Environmental distribution:
Based on the fugacity modelling available, the substance is likely to have low partitioning to the air or water compartments and is likely to be absorbed to soil and sediment compartments.

5.3 Aquatic bioaccumulation:
Experimental data
Bioaccumulation test of PX-200 in carp (Sewell, I.G. & Bartlett, A.J. (1995))
BCF range: <0.2
The study was conducted using a dispersing agent to assist exposure of the substance to the test media. While this might be considered to increase by availability by increased water

solubility, it is also considered to have the potential to effect uptake of the substance by the test species and the result of the experimental data alone is therefore considered not wholly conclusive.

QSAR data
In support of the experimental data and to assist in interpretation of the data, the substance was further assessed by means of QSAR using the tool established by US EPA and the tool established in cooperation with ECHA.
EPIWIN QSAR estimates that the BCF for the substance is 8.99 L/Kg.
CAESAR QSAR estimates the BCF for the substance to be 6 L/Kg.
The difference between to the two estimates is considered likely to the various algorithms used by each system and the available data on which the two systems rely. The QSAR data are, nevertheless, considered to be reliable for the purpose of considering a weight of evidence.

The bioaccumulation criteria (BCF values) that indicate a potential to bioaccumulate are:
DSD: BCF >100
CLP: BCF >500
It is not possible to determine adequately the effect of the dispersing agent on the experimental result of BCF. The two QSAR estimates both provide comparable results and, when taken in conjunction of the high Pow value, are considered to indicate a low potential to bioaccumulate.
Based upon the total weight of evidence, it is considered that the substance does not bioaccumulate.

5.4 Aquatic Toxicity (including sediment and terrestrial data)
Acute toxicity studies:
No acute toxicity recorded up to levels of water solubility (LC50/EC50 values therefore not identified and studies concluded as limit tests).

Chronic Toxicity studies:
Chronic toxicity studies in daphnia available for the substance itself and a related aryl phosphate substance both showed an absence of chronic toxicity effects at the solubility limit and chronic toxicity NOEC values were determined to be greater than the water solubility limit.

Sediment organism studies:
The sediment organism study data available by read across on a representative and related aryl phosphate show no toxicity to *Chironomus riparius* or *Lumbriculus variegatus* achieving the EC50 and NOEC values as limit values greater than the maximum required dose for the test guideline.

Soil macro-organisms study:
The earthworm toxicity study available by read across on a representative and related aryl phosphate showed no toxicity to earthworms, achieving the EC50 and NOEC values as limit values greater than the maximum required dose for the test guideline.

Soil micro-organisms:
The nitrogen transformation study available by read across on a representative and related aryl phosphate showed no inhibition of the nitrate formation rate, achieving the EC50 and NOEC values as limit values greater than the maximum required dose for the test guideline.

Toxicity to terrestrial plants:
The terrestrial plant toxicity study data available by read across on a representative and related aryl phosphate show no inhibition to growth of the soybean, tomato and oat seedlings achieving the EC50 and NOEC values as limit values greater than the maximum required dose for the test guideline.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

PX-200 is considered to not fulfil any criteria for classification and labelling for environmental hazard. It is therefore proposed that the existing classification, Aquatic Chronic 4 (R53), is removed.

CLP: Not classified based on available data.

DSD: Not classified based on available data.

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

Tetrakis (2,6-dimethylphenyl)-m-phenylene biphosphate (PX-200) has a harmonised classification as Aquatic Chronic 4, H413 according to Regulation (EC) No 1272/2008 (CLP), and R53 according to Directive 67/548/EEC (DSD). This classification was based on its low water solubility, lack of biodegradation and high n-octanol/water partition coefficient (P_{ow}). The dossier submitter (DS) proposes to remove the classification Aquatic Chronic 4, H413 and R53, justified by the absence of ecotoxic effects in all available studies and a mainly QSAR-based re-consideration of PX-200's bioaccumulation potential.

Degradation

Biodegradation of PX-200 was studied in a ready biodegradability test. After 28 days 13.23% biodegradation was observed by test material analysis and no biodegradation was observed by oxygen consumption. Due to the low water solubility no hydrolysis test was performed. Based on the chemical structure, the DS assumed that PX-200 is not degraded by direct photolysis.

Bioaccumulation

A measured and an estimated value of Pow were provided in the CLH report. The measured log P_{OW} of PX-200 was > 6.2 (HPLC method). The QSAR estimate resulted in a log P_{ow} of 11.79 (US EPA KOWWIN v 1.67 of EPI Suite v4). The DS states that for such high values the reliability of the applied methods for log P_{OW} estimates are considered to diminish. Moreover, the DS argues that with increasing log P_{ow} values a decrease of the bioconcentration factor (BCF) can be observed and it has been hypothesized in the literature that in these cases the high log P_{ow} is more an effect of solubility than lipophilicity.

A fish bioaccumulation study (OECD TG 305, *Cyprinus carpio*) on PCX-200 was summarised in the CLH report. The concentration of the test material was of 0.1 mg/l and 1 mg/l. A dispersing agent (3% v/v Tween 80-dimethylformamide) was used and the DS considered it to potentially affect the uptake of the test item to the fish and so reducing the reliability of the result. After 56 days, fish bioconcentration factors (BCFs) of < 0.2 (0.1 mg/l) and < 0.02 (1 mg/l) were determined.

Two QSAR assessments were performed to support the measured BCF. The calculations resulted in BCFs of 8.99 l/kg (EPIWIN -BCFBAF method) and 6 l/kg (CAESAR). The DS considered the results to be suitable for the purpose of a weight of evidence approach. The DS also considered in their assessment of bioaccumulation potential that the uptake of the substance to the test organism was affected by the dispersing agent in the OECD TG 305 study. In a weight-of-evidence approach the DS concludes that the BCF is below the threshold of concern.

Aquatic toxicity

Several acute and chronic aquatic toxicity studies were provided in the CLH report but no effects were observed up to the highest test concentrations in any study. Due to the very poor solubility of PX-200 (< 0.1 mg/l), test solutions were difficult to prepare. The highest achievable concentrations varied considerably between studies and were well below 0.1 mg/L.

For fish the 96 h LC_{50} based on the time-weighted mean measured concentration was > 0.027 mg/L. No data on chronic fish toxicity was provided in the CLH report.

The short-term *Daphnia magna* study resulted in a 48 h EC_{50} > 0.032 mg/L based on the time-weighted mean measured concentration of filtered test media.

In a long-term *Daphnia magna* study with PX-200 the 21 d NOEC based on mortality was ≥ 0.00077 mg/L and read across for a related aryl phosphate substance (bisphenol A polyphosphate, EC No: 425-220-8 and CAS No 5945-33-5) gave ≥ 0.0011 mg/L.

Toxicity to algae was examined using only one test concentration and no growth inhibition was observed, resulting in a 72 h growth rate based EC_{50} > 0.031 mg/L and a NOEC of 0.031 mg/L for filtered test media (time-weighted mean measured concentration; the concentrations in the filtered solutions were around 5% of nominal at both the start and the end of the test, suggesting that the organisms were exposed to dissolved concentrations far lower than the nominal concentration of 0.8 mg/L).

Moreover, the DS provides additional information on short- and long-term effects on sediment organisms and terrestrial plants for a related aryl phosphate substance based on read across which shows that no response was seen at the maximum dose of ≥1000 mg/kg soil dw test material.

Comments received during public consultation

Comments were received from five Member States (MS). Four MS agreed with the DS to remove the harmonised classification Aquatic Chronic 4, H413.

One MS did not agree with the DS's conclusion that the substance is not bioaccumulative. The QSAR estimated log P_{OW} of 11.79, which is the basis for the EPIWIN BCF estimation, was not regarded as reliable by the MS because insufficient measured data for log P_{ow} > 9 were within the training dataset of the model. They also criticise the result of the CAESAR estimation because the similarities of the compounds in the CAESAR database were in the range of 0.557-0.76, while similarities above 0.85 are recommended for sufficient reliability.

The experimental BCF was not regarded as reliable by the MS, because the test concentrations in the OECD TG 305 study were higher than the water solubility of the substance and the use of a dispersing agent in stock solution preparation may have resulted in precipitation of the substance in the test vessels, meaning that the reported BCF values may actually be underestimates. The MS concluded that assessment of bioaccumulation should be based on the measured log P_{ow} of > 6.2. Referring to a particular publication (Nendza M & Müller M 2010. *SAR and QSAR in Environmental*

Research, 21, 495-512), the MS argued that log P_{OW} > 10 indicate BCFs < 2000, but do not sufficiently indicate that the BCF is < 500. Therefore, according to the MS, relevant bioaccumulation potential cannot be excluded for PX-200.

Responding to these comments, the DS agreed that the available bioaccumulation data and information is of limited reliability. In a weight-of-evidence assessment the DS puts particular emphasis on the observation that substances with log P_{ow} values above 6 often show decreasing bioaccumulation, referring to literature and ECHA guidance documents Chapter R. 11 and Part C. PBT Assessment.

One MS requested clarification on the limit of determination of the test substance in the long-term *Daphnia magna* study. The DS clarified this technicality in the annexed RCOM.

Assessment and comparison with the classification criteria

Classification according to CLP

With the category 'Aquatic Chronic 4' the CLP regulation provides an option to assign a "safety net" for substances not meeting the classification for categories 1, 2, or 3 but still giving some grounds for concern. Chronic 4 is for example triggered if no acute toxicity is recorded at the solubility limit for a poorly soluble substance, which shows a BCF of ≥ 500 (or if absent a log P_{ow} ≥ 4) and is not rapidly degradable, unless other scientific evidence exists showing classification to be unnecessary.

Classification according to DSD

Classification R53 according to the DSD was based on available evidence concerning the persistence, potential to bio-accumulate and predicted or observed environmental fate and behaviour. R53 is for example assigned if a substance is not readily biodegradable and has potential for bioaccumulation as shown by a fish BCF≥ 100 (or if absent a log P_{ow} ≥ 3), unless other scientific evidence exist showing classification to be unnecessary.

Degradation

In a ready biodegradability test PX-200 only degraded by 13.23% in 28 days. Hence, PX-200 does not meet the criteria for being rapidly degradable. Due to limitations of the study method regarding poorly soluble substances no hydrolysis tests have been carried out. Nevertheless, RAC assumes that PX-200 is not rapidly degraded by hydrolysis. RAC agrees with the DS that PX-200 is unlikely to undergo direct photolysis, owing to its chemical structure.

In conclusion RAC considers PX-200 not to meet the criteria for rapid degradability by biotic or abiotic degradation.

Bioaccumulation

The log n-octanol/water partition coefficient has been measured to be > 6.2 using the HPLC method. Since the experimental result is a limit value, a value of 11.79 has been additionally calculated by means of QSAR based on the SMILES-code of the substance. However, in this case the QSAR analysis is subject to some uncertainties as the underlying dataset of the model does not contain sufficient measured data for log P_{ow} values greater than 9. Even if a decrease of the BCF has been observed for substances with a log P_{ow} > 6.2, bioaccumulation cannot be ruled out, especially if the log P_{ow} is not reliable.

One experimental BCF study using common carp determined a BCF < 0.02 based on

whole body weight after 56 days. However, RAC agrees with the DS that this is not reliable due to the use of a dispersing agent and nominal test concentrations are above the reported water solubility in pure water, so the actual dissolved concentration of the test material is unknown.

In addition, the BCF value was calculated using two different QSAR approaches. By means of EPIWIN QSAR a BCF of 8.99 l/kg was estimated while with CAESAR the derived value was 6 l/kg. In the case of the CAESAR QSAR approach, RAC notes that the chemicals in the datasets used to estimate the BCF show only moderate similarities.

While the experimental and calculated BCFs do not suggest bioaccumulation above the threshold values in the classification criteria (DSD: ≥ 100, CLP: ≥500), the reliability of the methods and results is very limited. Considering the overall deficient information package, RAC does not see sufficient evidence for disregarding the bioaccumulation potential with a view to the safety net concept of the category Aquatic Chronic 4.

Aquatic Toxicity

Studies are available for both acute and chronic aquatic toxicity. RAC notes particular uncertainties about real exposure to the test substance, as the measured highest achievable concentrations varied considerably, both across different tests and over test durations.

Acute toxicity
No toxicity was found at the maximum achievable test concentration in the acute tests for fish, daphnids and algae. RAC does not consider PX-200 acutely toxic for any taxonomic group tested.

Chronic toxicity

No effects were observed at maximum achievable test concentrations in the two available studies with PX-200, one standard algal growth inhibition study and one standard daphnid reprotoxicity study. Data on long-term fish toxicity are not available. RAC notes that for the related aryl phosphate, i.e. bisphenol A polyphosphate (EC Number: 425-220-8, CAS No: 5945-33-5), one single effect has been found in a daphnid reprotoxicity study at the highest measured test concentration (growth reduction at 1.4 mg/l = LOEC, NOEC = 1.2 mg/l).

Conclusion on classification

PX-200 is considered not rapidly degradable. In addition RAC does not see sufficient conclusive evidence for absence of bioaccumulation potential, based on the available information on partition coefficient and QSAR-based BCF estimates. However, meaningful test data would only be expected from fish feeding studies, considering the very poor water solubility of PX-200. Overall, the uncertainties associated with all experimental data generated in aquatic test systems are considerable. Moreover, RAC notes that the DS's approach to read across from related aryl phosphates is rather weakly justified. Preferably, read across from more structurally similar substances should have been attempted, to provide increased confidence in the conclusions.

Two available chronic toxicity studies (for daphnids and algae) show no effects up to the maximum achievable test concentration. RAC does not expect that in an additional extended or chronic fish study with PX-200 any effects would be seen up to the practical water solubility limit of ~30 µg/l in the test medium (it is noted that although the actual level of exposure is unknown, no toxic effects were observed in the fish bioaccumulation

test, and the substance does not show any classifiable chronic toxic effects in other vertebrates). In conclusion RAC considers the available chronic data as sufficient evidence that a safety net classification in category Aquatic Chronic 4 is not warranted, and agrees with the DS to delete the corresponding entry in Annex VI, table 3.1, of the CLP Regulation. Regarding DSD criteria, RAC concludes that the available chronic data sufficiently indicate absence of aquatic toxicity, thus providing evidence for removing the classification R53. Thus, in spite of the very poor solubility, not ready biodegradability, and absence of conclusive evidence on bioaccumulation potential, RAC agrees with the DS's proposal to delete the corresponding entry in Annex VI, table 3.2, of the CLP Regulation.

6 OTHER INFORMATION

None

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