Committee for Risk Assessment

RAC

Annex 1

Background document

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

4,4'-sulfonylbisphenol, polymer with ammonium chloride (NH_4Cl), pentachlorophosphorane and phenol

EC Number: 439-270-3
CAS Number: 260408-02-4

CLH-O-0000001412-86-153/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

9 June 2017

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance Name: Phenol, 4,4'-sulfonylbis-, polymer with ammonium chloride ((NH4)Cl), pentachlorophosphorane and phenol

EC Number: **439-270-3**

CAS Number: **260408-02-4**

Index Number: **604-083-00-X**

Contact details for dossier submitter:

BAuA
Federal Institute for Occupational Safety and Health
Federal Office for Chemicals
Friedrich-Henkel-Weg 1-25
D-44149 Dortmund, Germany

Dossier prepared by:
Industry in accordance with Article 37(6) of CLP Regulation

Version number: **2.0 (Post Accordance Check)**

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Phenol, 4,4'-sulfonylbisphenol, polymer with ammonium chloride ((NH 4)Cl), pentachlorophosphorane and phenol***
EC number:	***439-270-3***
CAS number:	***260408-02-4***
Annex VI Index number:	***604-083-00-X***
Degree of purity:	***95 % (w/w)***
Impurities:	***Ca. 5% see confidential information***

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation
Current entry in Annex VI, CLP Regulation	H413 (aquatic chronic 4)
Current proposal for consideration by RAC	Removal of H413
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	no entry

1.3 Proposed harmonised classification and labelling based on CLP Regulation

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				
2.2.	Flammable gases				
2.3.	Flammable aerosols				
2.4.	Oxidising gases				
2.5.	Gases under pressure				
2.6.	Flammable liquids				
2.7.	Flammable solids				
2.8.	Self-reactive substances and mixtures				
2.9.	Pyrophoric liquids				
2.10.	Pyrophoric solids				
2.11.	Self-heating substances and mixtures				
2.12.	Substances and mixtures which in contact with water emit flammable gases				
2.13.	Oxidising liquids				
2.14.	Oxidising solids				
2.15.	Organic peroxides				
2.16.	Substance and mixtures corrosive to metals				
3.1.	Acute toxicity - oral				
	Acute toxicity - dermal				
	Acute toxicity - inhalation				
3.2.	Skin corrosion / irritation				
3.3.	Serious eye damage / eye irritation				
3.4.	Respiratory sensitisation				
3.4.	Skin sensitisation				
3.5.	Germ cell mutagenicity				
3.6.	Carcinogenicity				
3.7.	Reproductive toxicity				
3.8.	Specific target organ toxicity –single exposure				
3.9.	Specific target organ toxicity – repeated exposure				
3.10.	Aspiration hazard				
4.1.	Hazardous to the aquatic environment	none	none	H413 (aquatic chronic 4)	Conclusive but not sufficient .
5.1.	Hazardous to the ozone layer				

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

Labelling: Signal word: Not applicable
Hazard statements: Not applicable
Precautionary statements: Not applicable

Proposed notes assigned to an entry: None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

The Dutch competent authority responsible for the evaluation of the dossier recommended the substance to be classified with R53 and label with R53, S61.

2.2 Short summary of the scientific justification for the CLH proposal

The current classification of the substance is based on the data available at the time on the submission of the dossier and the Dutch competent authority recommend that long-term testing should be performed in order to determine if classification is (un)necessary. In accordance with the recommendation by the authority a *Daphnia magna* Reproduction Test and a Fish Early Life Stage Test was conducted. The test substance did not affect reproduction, growth or survival of *Daphnia magna* at the maximum solubility in test medium after 21 days of exposure. In the ELS test with Fathead minnow, it did not affect time of hatching or the hatching success nor survival, growth or development of the larvae during the post-hatch period at its maximum solubility in test medium. Therefore, it was concluded that the test substance it is not toxic up to the solubility limit in test medium. Based on the outcome of these long-term tests, classification of the substance is deemed unnecessary.

2.3 Current harmonised classification and labelling in Annex VI

H413 (aquatic chronic 4).

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

A change in the existing entry is considered justified due to new data becoming available after the current harmonised classification was agreed.

RAC general comment
The substance phenol, 4,4'-sulfonylbis-, polymer with ammonium chloride ($(NH_4)Cl$), pentachlorophosphorane and phenol, is also known also as SPS-100 and this name is used throughout the current opinion. It is used as a flame-retardant and fire preventing agent consisting of halogen-free compound and is used as an additive for thermoplastic and/or thermosetting polymers.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

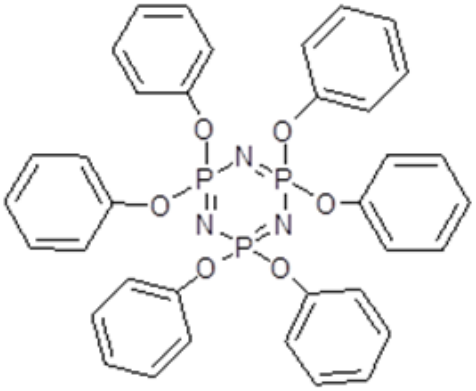
1.1 Name and other identifiers of the substance

The substance Phenol, 4,4'-sulfonylbis-, polymer with ammonium chloride ((NH4)Cl), pentachlorophosphorane and phenol is a polymer having the following characteristics and physical–chemical properties.

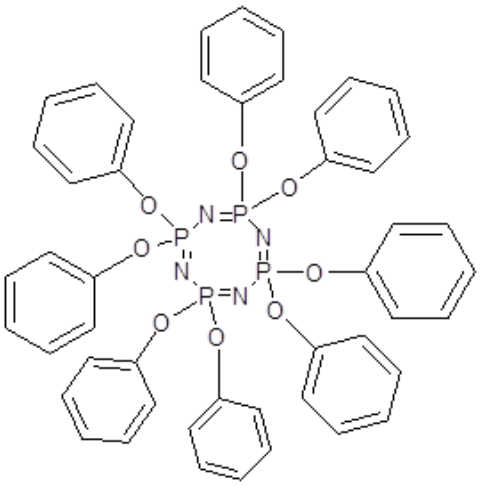
Table 4: Substance identity

EC number:	439-270-3
EC name:	4,4'-sulfonylbisphenol, polymer with ammonium chloride((NH4)Cl), pentachlorophosphorane and phenol
CAS number (EC inventory):	Not available
CAS number:	260408-02-4
CAS name:	Phenol, 4,4'-sulfonylbis-, polymer with ammonium chloride (NH4)Cl), pentachlorophosphorane and phenol
IUPAC name:	4,4'-sulfonylbis-, polymer with ammonium chloride ((NH4)Cl), pentachlorophosphorane and phenol
Other names:	SPS-100 SPB-100 SPE-100
CLP Annex VI Index number:	604-083-00-X
Molecular formula:	C36H30N3O6P3; C48H40N4O8P4
Molecular weight range:	693.6; 924.8

Structural formulas of the two constituents:



Major component 1 (c.a. 65-80%)



Major component 2 (c.a. 10-20%)

1.2 Composition of the substance

Name: Phenol, 4,4'-sulfonylbis-, polymer with ammonium chloride ((NH4)Cl), pentachlorophosphorane and phenol

Current Annex VI entry: H413 (aquatic chronic 4)

Degree of purity (sum of the two constituents): 75 — 100 % (w/w) (typically 95 % (w/w))

The two constituents are mentioned in the below Table.

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
2,2,4,4,6,6-Hexaphenoxy-1,3,5,2λ5,4λ5,6λ5-triazatriphosphinine CAS no.: 1184-10-7	ca. 75 % (w/w)	ca. 65 - < 80 % (w/w)	n=3
2,2,4,4,6,6,8,8-Octaphenoxy-1,3,5,7,2λ5,4λ5,6λ5,8λ5-tetrazatetraphosphocine CAS no.: 992-79-0	ca. 20 % (w/w)	> 10 - ca. 20 % (w/w)	n=4

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Phenol	0.05 % (w/w)	0 - 0.2 % (w/w)	
Chlorobenzene	0.05 % (w/w)	0 - 0.2 % (w/w)	
See confidential Annex			

Current Annex VI entry:
Phenol is not classified for the environment according to the CLP regulation.
Chlorobenzene is classified as Aquatic chronic 2 H411.

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

1.2.1 Composition of test material

Three different batches of the test material were used to conduct the studies relevant for classification purposes. The purity of the batches NR-85, 0Y01B, 9E96, 3J84 was equal or greater than 99%. However, the purity of the batch 110806 (used in the long-term Daphnia study) was not reported.

1.3 Physico-chemical properties

Table 8: Summary of physico - chemical properties

Property	Value [1)]	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	–	–	–
Melting/freezing point	–	–	–
Boiling point	–	–	–
Relative density	–	–	–
Vapour pressure	–	–	–
Surface tension	–	–	–
Water solubility	Peak 1: <4 µg/L Peak 2: <28 µg/L Peak 3: <44 µg/L	Brekelmans, M.J.C., 2001a	Estimated based on the limit of detection of the test substance components.
Partition coefficient n-octanol/water	>6.2 (Log Kow)	Brekelmans, M.J.C., 2001b	Measured using the HPLC method
Flash point	–	–	–
Flammability	–	–	–
Explosive properties	–	–	–
Self-ignition temperature	–	–	–
Oxidising properties	–	–	–
Granulometry	–	–	–
Stability in organic solvents and identity of relevant degradation products	–	–	–
Dissociation constant	–	–	–
Viscosity	–	–	–

[1)]Only the values relevant for classification purposes are included in the table.

2 MANUFACTURE AND USES

2.1 Manufacture

No information available on manufacture since the production does not take place within the EU.

2.2 Identified uses

Table 9: Uses at industrial sites

Identifiers	Use descriptors	Other information
IW-1: New chemical	**Process category (PROC):**	

Identifiers	**Use descriptors**	**Other information**
substance - Use category code: 011; Desired effects code: 022; Desired effects non-coded: FLAME RETARDANTS AND FIRE PREVENTING AGENTS	PROC 0: Other: Detailed information on envisaged uses: Flame-retardants and fire preventing agents consisting of halogen-free compound Detailed information on envisaged uses: The substance will be used as an additive for thermoplastic and/or thermosetting pol... **Sector of end use:** SU 0: Other: other (NACE code to be used only): POLYMERS INDUSTRY	
IW-2: Use of Substance by Industry in closed systems: 99 % Use of Substance by Industry in open systems: 1 % Use of Preparation by Industry in closed systems: 99 % Use of Preparation by industry in open systems: 1 %	No data available	

Table 10: Uses by professional workers

Identifiers	Use descriptors	Other information
PW-1: New chemical substance - Use category code: 011; Desired effects code: 022; Desired effects non-coded: FLAME RETARDANTS AND FIRE PREVENTING AGENTS	**Process category (PROC):** PROC 0: Other: Detailed information on envisaged uses: Flame-retardants and fire preventing agents consisting of halogen-free compound Detailed information on envisaged uses: The substance will be used as an additive for thermoplastic and/or thermosetting pol...	
PW-2: Use of Substance by Industry in closed systems: 99 % Use of Substance by Industry in open systems: 1 % Use of Preparation by Industry in closed systems: 99 % Use of Preparation by industry in open systems: 1 %	No data available	

Table 11: Article service life

Identifiers	Use descriptors	Other information
CBI SL-: Article	No data available	Remarks: Estimated maximum content of the substance in the product: < 20 %

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not relevant for the current proposal of revision of the Annex VI classification.

4 HUMAN HEALTH HAZARD ASSESSMENT

Not relevant for the current proposal of revision of the Annex VI classification.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 12: Summary of relevant information on degradation

Method	Results	Remarks	Reference
MITI (I) (OECD 301C)	Not readily biodegradable	A toxicity control was not included	Haruguchi, H., 1998

5.1.1 Stability

Not relevant for the current proposal of revision of the Annex VI classification.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Not relevant for the current proposal of revision of the Annex VI classification.

5.1.2.2 Screening tests

A ready biodegradability test was carried out in accordance with Japanese Industrial Standard (JIS) K 0102-1993-14.1 July 13, 1974, Kanpogyo No. 700, Yakuhatsu No. 615, 49 Kikyoku No. 392 and GLP (Haruguchi, 1998). The test method is essentially the same as OECD 301C, Modified MITI Test (I) (Revised July 17, 1992).

Details on test material

- Name of test material (as cited in study report): SPS-100
- Physical state: Semi-solid
- Expiration date of the lot/batch: Not reported
- Stability under test conditions: Stable
- Storage condition of test material: In refrigerator
- Solubility in water: ≤0.1 wt% (≤1 g/L)

Confidential details on test material

see Confidential Annex

Study design

Oxygen conditions

aerobic

Inoculum or test system

mixture of sewage, soil and basal culture medium

Details on inoculum

- Source of inoculum/activated sludge
Sludge was collected from 10 locations in Japan (March 1998):
Fukogawa city sewage plant (Sapporo-shi Hokkaido)
Kashima industry sewage plant (Kashima-gun Ibaragi)
Nakahama city sewage plant (Osaka-shi Osaka)
Ochiai city sewage plant (Shinjuku-ku Tokyo)
Kitakami river (Ishinomaki-shi Miyagi)
Shinano river (Nishikanbara-gun Niigata)
Yoshino river (Tokushima-shi Tokushima)
Lake Biwa (Otsu-shi Shiga)
Hiroshima bay (Hiroshima-shi Hiroshima)
Dookay bay (Kitakyushu-shi Fukuoka)

- Sampling method
City sewage: Return sludges from sewage plants were collected
Rivers, lake and sea: Surface water and surface soil which are in contact with the atmosphere were collected

- Method of cultivation:
The filtrate (5 L) of the supernatant of the activated sludge cultivated for about 3 months was mixed with the mixed filtrate (5 L) of the supernatant of the sludge collected newly at each location. The mixed filtrate (10 L) was aerated (pre-filtered open air was used) after the pH value was adjusted to 7.0±1.0.
Roughly 30 min. after ceasing aeration of the sludge mixture, about 1/3 of the whole supernatant volume was removed and an equal volume of dechlorinated water was added to the remaining portion. This mixture was aerated and a previously decided amount of synthetic sewage (glucose, peptone and potassium dihydrogenphosphate was dissolved in chlorinated water to obtain 5% (w/v) of the solution for each components, and the pH of the solution was adjusted to 7.0±1.0 with sodium hydroxide) was added to the mixture so that the concentration of the synthetic sewage was 0.1% (w/v) in the volume of the dechlorinated water added. This procedure was repeated once everyday. Cultivation was carried out at 25±2°C.
Microflora in the activated sludge was microscopically observed and the sludge with no abnormal symptoms was used for the test. Date of initiation of use was April 21st 1998.

- Concentration of sludge (as the concentration of suspended solids): 3300 mg/L

Duration of test (contact time)

28 d

Initial test substance concentration

Initial conc.	Based on
100 mg/L	test mat.

Parameter followed for biodegradation estimation

O2 consumption (recorded as BOD)

Details on analytical methods

DETAILS ON PRETREATMENT
- Extraction: Liquid-liquid using ethyl acetate. The ethyl acetate layer was filtrated and dehydrated, followed by evaporation to dryness at 40 °C. 100 mL of tetrahydrofuran were added to the residue.

IDENTIFICATION AND QUANTIFICATION OF PARENT COMPOUND
- Separation method: HPLC-UV
- Conditions
Instrument: HPLC, pump type 880-PU (Japan spectroscopic Co., Ltd) and detector type 870-UV (Japan spectroscopic Co., Ltd)
Column: Asahipak GS-310H, 25 cm x 7.6 mm I.D. stainless steel
Eluent: tetrahydrofuran
Flow rate: 1.0 ml/min
Injection volume: 20 µL
- LOD: 2.9 mg/L
- Detection method: UV at 260 nm
- Linearity range: 75 and 300 mg/L (3 point calibration curve)
- Extraction recovery
Average recovery rate of test solutions containing water and test substance: 95.5%
Average recovery rate of test solutions containing sludge and test substance: 95.3%

- The concentration of the test substance in the sample for HLPC analysis was proportionally calculated by comparing the peak area of the chromatogram of the sample for HPLC analysis with that on the chromatogram of 300 mg/L of the standard solution.

Details on study design

TEST CONDITIONS
- Composition of medium (basal culture medium): 3 mL of each solution A, B, C and D (prescribed in JIS K 0102-1993-21) made up to 1000 mL with purified water (pH was adjusted to 7.0).
- Test temperature: 25±1°C
- pH: 7.0
- pH adjusted: no
- Aeration of dilution water: yes
- Suspended solids concentration: 30 mg/L
- Continuous darkness: Not reported
- Other: Test solution was stirred by magnetic stirrer.

TEST SYSTEM
- Culturing apparatus: Closed system oxygen consumption measuring apparatus
- Measuring equipment
Coulometer: Ohkura Electric Co., Ltd.
Data sampler: Asahi Techneion Co., Ltd.
- Number of culture flasks/concentration: 3 for the test substance + sludge and 1 for the controls (water + test substance; sludge plus aniline; sludge without test substance)
- Details of trap for CO2: Soda lime

SAMPLING
- Sampling frequency: at days 7, 14, 21 and 28

CONTROL AND BLANK SYSTEM
- Inoculum blank: yes
- Abiotic control: yes
- Reference substance control: yes

Reference substance

aniline

Results and discussions

% Degradation of test substance

%Degr.	St. dev.	Parameter	Sampling time	Remarks
2	1	O2 consumption	28 d	Average of three vessels
0		Test mat. analysis (HPLC)	28 d	

Details on results

- Degradation of test substance: Test was conducted using three vessels with an average biodegradation of 2% by BOD after 28 d. Degradation ranged between 0-1 % after 7 d, 1-2 % after 14 d, 0-3 % after 21 d and 1-3% after 28 d.

BOD5 / COD results

Results with reference substance

Reference substance degradation: 54 % after 7 d, 69 % after 14 d, 72 % after 21 d and 74 % after 28 d

Applicant's summary and conclusion

Validity criteria fulfilled

yes according to OECD 301C (1992)

Interpretation of results

not readily biodegradable

Conclusions

SPS-100 was not biodegraded by microorganisms under the present test conditions.

Executive summary

A ready biodegradability study of SPS-100 was performed for 28 days using a method similar to MITI (I) (OECD 301C). The initial concentration of the test substance was 100 mg/L. At the end of the test period (28 d) the average biodegradation of SPS-100 was 2%. Thus, the criterion for ready biodegradability (at least 60% biodegradation) was not met. The study is considered to be reliable with restrictions since a toxicity control was not included.

The study on biodegradation in water (screening test) is summarized in the following table:

Table 13: Screening tests for biodegradation in water

Method	Results	Remarks	Reference
Test type: ready biodegradability mixture of sewage, soil and basal culture medium	Not readily biodegradable % Degradation of test substance:	2 (reliable with restrictions) experimental result	Haruguchi, H (1998)

Method	Results	Remarks	Reference
In accordance with Japanese Industrial Standard (JIS) K 0102-1993-14.1 July 13, 1974, Kanpogyo No. 700, Yakuhatsu No. 615, 49 Kikyoku No. 392 This test method is essentially the same as OECD 301C, Modified MITI Test (I) (Revised July 17, 1992)	2 after 28 d (O2 consumption) (Average of three vessels) 0 after 28 d (Test mat. analysis (HPLC))	Test material (IUPAC name): Phenol, 4,4'-sulfonylbis-, polymer with ammonium chloride (NH4Cl), pentachlorophosphorane and phenol	

5.1.2.3 Simulation tests

Not relevant for the current proposal of revision of the Annex VI classification.

5.1.3 Summary and discussion of degradation

The following information is taken into account for any hazard/ persistency assessment:

A ready biodegradability study of SPS-100 was performed for 28 days using a method similar to MITI (I) (OECD 301C). At the end of the test period (28 d) the average biodegradation of SPS-100 was 2%. Thus, the criterion for ready biodegradability (at least 60% biodegradation) was not met. The study is considered to be reliable with restrictions since a toxicity control was not included.

Biodegradation in water: under test conditions no biodegradation observed.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

The study on adsorption/desorption is summarized in the following table:

Table 14: Studies on adsorption/desorption

Method	Results	Remarks	Reference
Study type: adsorption/desorption expert statement The adsorption/desorption of SPS-100 has been calculated using the method described in the Technical Guidance Document on Risk Assessment (1996): Log Koc = 0.81 log Po/w + 0.10 > 5.12	Adsorption coefficient: log Koc: > 5.12	2 (reliable with restrictions) (Q)SAR Test material (IUPAC name): Phenol, 4,4'-sulfonylbis-, polymer with ammonium chloride (NH4Cl), pentachlorophosphorane and phenol	Nederveen, M (2001)

5.2.2 Volatilisation

Based on a low vapour pressure (0.015 Pa at 20°C, Krips 2001) it is concluded that the substance has a low potential for volatilization. It can be assumed that the test substance did not evaporate from the test vessels in the aquatic toxicity studies. Distribution modelling

5.3 Aquatic Bioaccumulation

Table 15: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Similar to OECD 305	BCF: < 21.3 (whole body d.w.) (steady state) (Determined by authors but considered unreliable.) BCF: < 2.1 (whole body d.w.) (steady state) (Determined by authors but considered unreliable	The study should be considered unreliable. Although the test was performed based on a method similar to OECD 305 and according to GLP principles, the test concentrations of the three components that were analysed were above the maximal water solubility limit of (any component of) the test substance. The report does not contain any information of the concentration of the truly dissolved test substance and no substance was detected in fish. Furthermore relevant details on test conditions (e.g. photoperiod, mortality in control and treated fish) were not reported and the documentation of the analytical method and results is poor.	Maihara, A (2000)

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

Not relevant for the current proposal of revision of the Annex VI classification.

5.3.1.2 Measured bioaccumulation data

A BCF test was carried out in accordance with 1974, Kanpogyo No. 5, Yakuhatu No. 615, 49 Kikyoku No. 392 and GLP (Maihara, 2000). The test method is essentially the same as OECD 305 (1996).

Details on test material

- Name of test material (as cited in study report): SPS-100
- Physical state: Semi-solid (light yellow color)
- Stability under test conditions: Stable
- Storage condition of test material: In the dark at room temperature (in desiccator)
- Stability under storage conditions: Stable
- Solubility in water: <0.1 wt% (<1 g/L)

Confidential details on test material

see Confidential Annex

Details on sampling

WATER SAMPLES

- Sampling intervals/frequency for water samples: About 300 mL were sampled twice a week.

- Sample treatment procedure: 100 mL of the low test concentration solution was sampled to a graduated cylinder. 10 mL of high test concentration solution and solvent control solution were diluted with dilution water to a final volume of 100 mL. The solutions were added to Empore extraction disk C18HD at a flow rate of 4 mL/min using a digital pump. 5 mL of Hexane/Ethyl acetate (1:1,v/v) and 1 mL of pure water were added o each test tube, samples were then shake for 10 min and centrifuged for 5 min at 3000 rpm. The entire organic layer was transferred into a new test tube and evaporated to dryness under a stream of nitrogen gas at 40 °C. The residue was dissolved in 1 mL of tetrahydrofuran. A 100 µL aliquot of the resulted solution was injected into the HPLC system (GPC analysis for HPLC). This solution was evaporated to dryness under a stream of nitrogen gas at 40 °C. The residue was dissolved in 0.5 mL of acetonitrile. A 50 µL aliquot of the resulted solution was injected into the HPLC system (Gradient analysis for HPLC).

FISH TISSUE SAMPLES

- Sampling intervals/frequency for test organisms: The concentration of the test substance in the test fish was measured with the HPLC analytical method. Three fish were sampled for analysis from the lowest and the highest test concentration groups plus the solvent control group after 2, 4, 6 and 8 weeks. The fish were sacrificed after sampling, and their length and weight were measured. In two fish of the low and high concentration groups the test substance concentration was measured and one fish of the solvent control group was used to measure the lipid content. To measure the test substance concentration in the test blank (blank of the recovery test), three fish before the exposure period started and two fish in the solvent control group after 8 weeks were used. The remaining fish were frozen and preserved until the completion of the study.

- Sample treatment procedure: The excess moisture in the fish was removed after sacrifice, the length and weight were measured, and the fish were homogenize one by one. 5 g of the homogenized fish were used and 30 mL of Ethyl acetate/Acetonitrile (7:3, v/v) were added, the homogenate was shaken for 10 min and centrifuged for 10 min at 3000 rpm. The entire upper layer was transferred into a 100 mL flask. This procedure was repeated twice. The extracted solution was adjusted to 100 mL with 30 mL of Ethyl acetate/Acetonitrile (7:3, v/v). Next, 5 mL of this solution was sampled and evaporated to dryness under a stream of nitrogen gas at 40 °C. The residue was dissolved in 0.5 mL of acetonitrile. 50 µL aliquot of the resulting solution was injected into the HPLC system (Gradient analysis).

Details on analytical methods

WATER SAMPLES

Analytical conditions of HPLC

Method 1 - GPC analysis for HPLC
Equipment: HPLC-UV with auto-sampler, system controller, column oven, and data processor.

Measurement conditions
Analytical column: Shim-Pack GPC-802 (8.0 mm I.D. x 30 cm)
Guard column: Shim-Pack GPC-800P
Mobile phase: Tetrahydrofuran
Flow rate: 1.0 mL/min
Column temperature : 35 °C
Detection wavelength : 260 nm
Chart speed: 5 mm/min
Injection volume: 100 µL

- Standard solutions for calibration curve: 5.0, 10.0, 15.0, 20.0 and 25.0 mg/L in tetrahydrofuran.

- Calibration curve: Linearity (r) was >0.99 and intra-assay precision (CV) was between 0.3 - 1.0%.

Method 2 - Gradient analysis for HPLC
Equipment: HPLC-UV with auto-sampler, system controller, column oven, and data processor.

Measurement conditions
Analytical column : Inertsil ODS-3 (4.6 mm I.D. x 250 mm) (GL science)

Mobile phase
A solution: Acetonitrile
B solution: Acetonitrile/Pure water = 1:1 (v/v)
The gradient analysis was done by the following time program:
Time (min): 0, 40, 80, 90, 100 and 110;
Func.: B. Conc.;
Value: 100, 10, 0, 0 and 100, respectively.

Flow rate :1.0 mL/min
Column temperature : 40 °C
Detection wavelength : 260 nm
Chart speed : 1 mm/min
Injection volume : 50 µL

- Standard solutions: prepared at concentrations of 0.5, 1.0, 5.0 and 10.0 mg/L in acetonitrile. The 0.5 mg/L concentration was not detectable.

- Recovery samples: A 10 mg of the test substance and a 1 g of HCO-40 were weighed, and dissolved in acetone. Acetone was evaporated at 40 °C. The residue was dissolved in 100 mL of pure water as the stock solution of 100 mg/L. This stock solution was diluted in dilution water, and

made the recovery samples at 1.0 and 0.1 mg/L. The recovery in the gradient analysis for HPLC was 94.9±0.9%.

- Calculation method: The concentration of the test solution was calculated from the peak area value by the GPC analysis and the gradient analysis for HPLC. However, the concentration of the test solution by the gradient analysis was used for the calculation of the bioconcentration factor. The concentration of the test substance (Peak No.1) in the fish samples was calculated from the peak area with gradient analysis for HPLC.

- LOQ: 0.01 mg/L (taking into account concentration factor). For analysis of standards it was 0.1 mg/L

FISH TISSUE SAMPLES

- Analytical conditions of HPLC - The analytical conditions of the GPC analysis for HPLC and the gradient analysis for HPLC were the same used for the water samples (see details above)

- Standard solutions of SPS-100 (Gradient analysis for HPLC): Standard solution of 100 mg/L (GPC analysis for HPLC) was diluted to 5.0 mg/L with tetrahydrofuran. This standard solution was evaporated to dryness under a stream of nitrogen gas at 40 °C. The residue was dissolved in 0.5 mL of acetonitrile and used as the test substance standard solution (Gradient analysis for HPLC).

- Standard solution of SPS-100 (Addition for fish): A 10.0 mg aliquot of the test substance was accurately weighed and dissolved in acetone to make exactly 20 ml, that was used as a 500 mg/L of the standard solution.

- Recovery samples: The fish not used for the test were homogenized. 5 g of homogenized fish were sampled (n=3). 100 µL of 500 mg/L standard solution was added to fish and made to the sample for the recovery test. This concentration corresponds to 10 µg/g of fish concentration, 10 times the high concentration test solution, 100 times the low concentration test solution. The blank test prepared by adding 100 µL of acetone to 5 g of fish without test substance was treated similarly (n=3).

- Recoveries: Recovery sample was prepared by adding 100 µL of 500 mg/L standard solution to 5 g of homogenized fish. The recovery was calculated using 3 analytical samples. The recovery 101.1 ± 1.3%.

- Calculation method: The concentration of the test substance was calculated from the peak area value using gradient analysis for HPLC.

Vehicle

yes

Details on preparation of test solutions

- Test substance stock solutions: 8 g of the test substance and 800 g of HCO-40 were weighed, and dissolved in acetone. Acetone was evaporated at 40 °C. The residue was dissolved in 8.0 L of pure water to obtain a stock solution of 1000 mg/L for the highest test concentration. The stock solution

of 100 mg/L used to prepare the lowest test concentration was made by adding 7.2 L of pure water to 800 mL of the previously prepared stock solution.
The solvent control stock solution was prepared by weighing 800 g of HCO-40 which was dissolved in acetone. Acetone was evaporated at 40 °C and the residue was dissolved in 8.0 L of pure water. The stock solutions of the test substance and solvent control were diluted 1000 times in dilution water.

Test organisms

Cyprinus carpio

Details on test organisms

TEST ORGANISM
- Common name: Carp
- Age at the start of the test: Not reported
- Source: Yamaguchi Fish Farm. 1-6-7, Shimorokumanji, Higashiosaka, Osaka, Japan. Lot No. K98K-9902.
- Length at study initiation (mean and SD): 9.0±0.4 cm (n=60)
- Weight at study initiation (mean and SD): 19.1±2.4 g (n=60)
- Lipid content (%): 4.5±0.5
- Feeding during test: Yes
- Food type: Fodder
- Amount: The fish were fed with an amount corresponding to 2% of their weight
- Frequency: Once or twice a day in the morning and/or evening.

ACCLIMATION
- Acclimation period: The acclimation was begun with the water tank of flow-through without sterilization and disinfection on June 29, 1999. The water temperature of the acclimation tank was kept at 25 ± 2 °C.
- Acclimation conditions (same as test or not): yes
- Type of food: Fodder with 7% of cod-liver oil.
- Amount: The fish were fed with an amount corresponding to 2% of their weight.
- Feeding frequency: As a rule, the fish (common carp) were fed every day or every other day.
- Health during acclimation (any mortality observed): No weakened or dead fish were found in the 7 days prior to the start of the exposure period.

Study design

Route of exposure

aqueous

Test type

flow-through

Water media type

freshwater

Total exposure / uptake duration

56 d

Test conditions

Test temperature

24.7 - 25.9 °C

pH

7.1 - 8.0

Dissolved oxygen

6.9 - 8.1 mg/L

Nominal and measured concentrations

- Nominal concentrations: 0.1 mg/L (low concentration) and 1 mg/L (high concentration)
- Measured concentrations (average)
Low concentration: 0.094 mg/L (range: 0.094 - 0.096 mg/L)
High concentration: 0.96 mg/L (range: 0.96 - 1.03 mg/L)

Details on test conditions

TEST SYSTEM
- Test vessel: 100 L capacity, filled with 100 L of test solution
- Aeration: Not reported
- Type of flow-through: Not reported
- Renewal rate of test solution (flow rate)
Solvent control and high test concentration: 500 mL/min
Low test concentration: 0.5 mL/min
- No. of organisms per vessel: 20
- No. of vessels per concentration (replicates): 1
- No. of vessels per control (replicates): 1
- Biomass loading rate (at the start)
Solvent control: 3.98 g/L
Low test concentration: 3.86 g/L
High test concentration: 3.64 g/L

- Observations: The behaviour and appearance of the fish were observed during the exposure period.

TEST MEDIUM / WATER PARAMETERS
- Source/preparation of dilution water: Dechlorinated city water (fully aerated)
- Chlorine: Residual chlorine concentration was below 0.02 mg/L
- Holding medium different from test medium: No
- Intervals of water quality measurement: Water quality parameters (temperature, dissolved oxygen and pH) were measure several days per week throughout the test period.

OTHER TEST CONDITIONS
- Adjustment of pH: No
- Photoperiod: Not reported

Reference substance (positive control)

no

Details on estimation of bioconcentration

The bioconcentration factor was calculated using the following equation:

Bioconcentration factor (CFn) =(Fn-FB)/Wn

CFn - Bioconcentration factor after n weeks

Fn -The concentration of the test substance in the test fish after n weeks (p,g)g)
Wn - Mean concentration of the test substance n the test· solution with gradient analysis for HPLC after n weeks (mg/L)
FB - Mean concentration of the test substance in the test fish in blank test before or after the exposure period (p,gjg)

Results and discussions

Lipid content

4.5 %

Time point Average

Remarks range (%): 4.1- 5.2. ; time period: test week 2-8.

Bioaccumulation factor

Conc. in environment / dose	Type	Value	Basis	Time of plateau	Calculation basis	Remarks
0.1 mg/L (nominal)	BCF	< 21.3	whole body d.w.		steady state	Determined by authors, considered unreliable as the concentration in water exceeds the solubility limit and no test substance was detected in fish.
1 mg/L (nominal)	BCF	< 2.1	whole body d.w.		steady state	Determined by authors, considered unreliable as the concentration in water exceeds the solubility limit and no test substance was detected in fish.

Kinetic parameters

- Depuration (loss) rate constant (k2): No depuration phase in the study.

Details on results

- Method of calculation: Steady state . The steady state concentration in fish was < 2 µg/g after 56 d and the steady state uptake period was 3 d (no. of samples was 56)

- The concentration of the test substance (peak No. 1) in the test fish of the low and high concentration groups was < 2 µg/g. In addition the concentration of Peak No.2, 3, and 4 was measured with the gradient analysis for HPLC in the test fish, no peaks were detected.

- Mortality of test organisms: Not reported

- Behavioural abnormalities: No abnormalities were observed during the exposure period.

- Organ specific bioaccumulation: the concentration of the test substance was not measured in each part of the fish body

Any other information on results incl. tables

Measuring concentration of the test substance in the test solution at the bioconcentration test (Gradient analysis for HPLC - Peak No.1). The concentration of the standard used was 5 mg/L.

	Test week		Recovery	Fish weight (g)	Concentration in the test fish (µg/g)
Solvent	8	I	101.1	5.08	<2
		II		5.05	<2
Low concentration	2	I	101.1	4.97	<2
		II		5.01	<2
	4	I		5.02	<2
		II		5.02	<2
	6	I		5.01	<2
		II		5.02	<2
	8	I		4.97	<2
		II		5.01	<2
High concentration	2	I	101.1	5.00	<2
		II		5.01	<2
	4	I		4.99	<2
		II		4.98	<2
	6	I		5.02	<2
		II		5.01	<2
	8	I		5.07	<2
		II		5.06	<2

Bioconcentration factors during the exposure period (reported by authors)

		Test week			
		2	4	6	8
Low concentration	I	<20.8	<21.1	<21.1	<21.3
	II	<20.8	<21.1	<21.1	<21.3
High concentration	I	<1.9	<2.0	<2.0	<2.1
	II	<1.9	<2.0	<2.0	<2.1

Remarks on results including tables and figures

- Comments: At test week 8, the bioconcentration factor was < 21.3 at the low concentration exposure and < 2.1 at the high exposure concentration. Because the BCF determined with whole fish was so low, the concentration of the test substance was not measured in each part of the fish body and the depuration phase test were not performed.

Applicant's summary and conclusion

Validity criteria fulfilled

yes (According to OECD 305 (1996). However, The mortality or other adverse effects/disease in both control and treated fish was not reported.)

Conclusions

No reliable BCF can be determined from this study, because the test concentrations of the three components that were analysed were above the maximal water solubility limit of (any component

of) the test substance. The report does not contain any information of the concentration of the truly dissolved test substance and no substance was detected in fish.

Executive summary

A bioconcentration study for SPS-100 was performed in common carp (Cyprinus carpio) based on a method similar to OECD 305 and according to GLP principles. Following a 56 day (8 weeks) uptake period, steady state bioconcentration factor (BCF) was determined. A depuration phase was not performed since the determined BCFs were low. The study was performed at nominal concentrations of 100 and 1000 µg/L. During the exposure phase, samples from the test solutions were analysed by HPLC, with quantification of the concentration of Peak no. 1. From the report it is not clear which component is indicated by peak 1.The water solubility of SPS-100 was determined based on the analysis of three components: the water solubility of these components were concluded to be <4 µg/L, <28 µg/L and <44 µg/L, respectively. In the bioconcentration study, the measured concentrations of peak 1 in the test solutions were 94-96 µg/L and 960-1030 µg/L for the low and high level, respectively. These concentrations were therefore much higher than the water solubility of (any component of) the test substance. The report does not contain any information of the concentration of truly dissolved test substance. In fish, the concentration of peak 1 was always <2 µg/g, irrespective of the nominal concentration. Furthermore relevant details on test conditions (e.g. photoperiod, mortality in control and treated fish) were not reported and the documentation of the analytical method and results is poor. Therefore the study is considered to be unreliable.In a worst-case approach, it may be assumed that the actual concentration of peak 1 in the test solutions was <4 µg/L. In line with the OECD guidance document on difficult test substances, the concentration in the test solution is taken to be half the LOD (of 4 µg/L), i.e. 2 µg/L. This leads to a provisional BCF value of <2 [µg/g] / 2 [µg/L] = <1 L/g or <1000 L/kg. The current-day OECD 305 guideline for a bioconcentration test in fish indicates that for substances with very low solubility in the aquatic environment, exposure via water may be of limited importance in comparison to the dietary route. This is also indicated by ECHA in IR/CSR Guidance Chapter R.11. Thus, for SPS-100, the bioconcentration study is of limited value.

The study on aquatic bioaccumulation are summarized in the following table:

Table 16: Studies on aquatic bioaccumulation

Method	Results	Remarks	Reference
Cyprinus carpio aqueous (freshwater) flow-through Total uptake duration: 56 d 1974, Kanpogyo No. 5, Yakuhatu No. 615, 49 Kikyoku No. 392. This test	BCF: < 21.3 (whole body d.w.) (steady state) (Determined by authors, considered irreliable as the concentration in water exceeds the solubility limit and no test substance was detected in fish.)	3 (not reliable) experimental result Test material (IUPAC name): Phenol, 4,4'-sulfonylbis-, polymer with ammonium	Maihara, A (2000)

Method	Results	Remarks	Reference
method is essentially the same as OECD 305 (1996)	BCF: < 2.1 (whole body d.w.) (steady state) (Determined by authors, considered irreliable as the concentration in water exceeds the solubility limit and no test substance was detected in fish.) Lipid content: 4.5 % (Average) (range (%): 4.1- 5.2. ; time period: test week 2-8.)	chloride (NH4Cl), pentachlorophosphorane and phenol	

5.3.2 Summary and discussion of aquatic bioaccumulation

A bioconcentration study for SPS-100 was performed in common carp (*Cyprinus carpio*) based on a method similar to OECD 305 and according to GLP principles. Following a 56 day (8 weeks) uptake period, steady state bioconcentration factor (BCF) was determined. A depuration phase was not performed since the determined BCFs were low. The study was performed at nominal concentrations of 100 and 1000 µg/L that were achieved via dispersion with emulsifier HCO-40. During the exposure phase, samples from the test mixtures were analyzed by HPLC, with quantification of the concentration of Peak no. 1. From the report it is not clear which component is indicated by Peak no. 1. The water solubility of SPS-100 was determined based on the analysis of three components: the water solubility of these components were concluded to be <4 µg/L, <28 µg/L and <44 µg/L, respectively. In the bioconcentration study, the measured concentrations of Peak no. 1 in the test solutions were 94-96 µg/L and 960-1030 µg/L for the low and high level, respectively, confirming the nominal concentration. These concentrations were therefore much higher than the water solubility of (any component of) the test substance. The report does not contain any information of the concentration of truly dissolved test substance. In fish, the concentration of Peak no. 1 was always <2 µg/g, irrespective of the nominal concentration. Furthermore relevant details on test conditions (e. g. photoperiod, mortality in control and treated fish) were not reported and the documentation of the analytical method and results is poor. Therefore the study is considered to be unreliable. In a worst-case approach, it may be assumed that the actual concentration of Peak no. 1 in the test solutions was <4 µg/L. In line with the OECD guidance document on difficult test substances, the concentration in the test solution is taken to be half the LOD (of 4 µg/L), i. e. 2 µg/L. This leads to a provisional BCF value of <2 [µg/g] / 2 [µg/L] = <1 L/g or <1000 L/kg. The current-day OECD 305 guideline for a bioconcentration test in fish indicates that for substances with very low solubility in the aquatic environment, exposure via water may be of limited importance in comparison to the dietary route. This is also indicated by ECHA in IR/CSR Guidance Chapter R.11. Thus, for SPS-100, the current bioconcentration study is of limited value.

The following information is taken into account for any hazard / bioaccumulation assessment:

No reliable BCF can be determined from this study, because the test concentrations were above the maximum water solubility limit of (any component of) the test substance. The report does not contain any information of the concentration of the truly dissolved test substance and no substance was detected in fish.

5.4 Aquatic toxicity

Table 17: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
Acute fish toxicity: Method 71 of JIS K0102. Test was performed as part of a bioconcentration study similar to OECD Guidelines for Testing of Chemicals "Bioconcentration : Flow-Through Fish Test; 305C, Modified MITI Test"	LC50 (96 h): > 100 mg/L test mat. (nominal) based on: mortality	The recoveries of the analytical method were not reported and the final dispersant (HCO-40) concentrations were above the maximum mentioned on OECD 203 guideline.	Maihara, A (1999)
OECD Guideline 210 (Fish, Early-Life Stage Toxicity Test) (2013) EPA OPPTS 850.1400 (Fish Early-life Stage Toxicity Test) .	NOEC (33 d): based on: embryo development, number hatched, time to hatch and larval development (Since no effects were observed in a WSF prepared at a loading rate of 10 mg/L, the NOEC is considered to be equal to the maximum soluble test substance concentration in test medium.)		Migchielsen, M.H.J. (2014)
OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	EC50 (48 h): based on: mobility (No acute toxicity up to solubility limit)		Migchielsen, MHJ (2001a)
OECD Guideline 211 (Daphnia magna Reproduction Test) (2008)	NOEC (21 d): based on: parental body length, reproduction, growth, survival (Did not affect reproduction, growth or survival up to solubility limit);		Migchielsen, MHJ (2012)
OECD Guideline 201 (Alga, Growth Inhibition Test) (1984)	EC50 (72 h): based on: growth rate (No acute toxicity up to solubility limit); NOEC (72 h): based on: growth rate (No acute toxicity up to solubility limit)		Migchielsen, MHJ (2001b)
OECD Guideline 209 (Activated Sludge, Respiration Inhibition Test) (1984)	EC50 (30 min): > 100 mg/L test mat. (nominal) based on: respiration rate.		Desmares-Koopmans, MJE (2001)

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

In a 96-h acute toxicity study conducted according to method 71 of JIS K0102, ricefish (*Oryzias Iatipes*) were exposed to SPS-100 under semi-static conditions at the following nominal concentrations: blank-control, solvent control, 10, 18, 32, 56, 100 mg/L. Test substance induced no visible or lethal effects in ricefish at any of the concentrations tested. The 96 h LC50 was >100 mg/L

based on analytically confirmed nominal concentrations. The study is considered to be reliable with restrictions since the recoveries of the analytical method were not reported and the final dispersant (HCO-40) concentrations were above the maximum mentioned on OECD Guideline No. 203.

The results are summarized in the following table:

Table 18: Short-term effects on fish

Method	Results	Remarks	Reference
Oryzias latipes freshwater semi-static equivalent or similar to Test method according to method 71 of Japanese Industrial Standards (JIS) K0102. This test method is essentially the same as that in the OECD Guidelines for Testing of Chemicals "Bioconcentration : Flow-Through Fish Test; 305C, Modified MITI Test".	LC50 (96 h): > 100 mg/L test mat. (nominal) based on: mortality	2 (reliable with restrictions) experimental result Test material (IUPAC name): Phenol, 4,4'-sulfonylbis-, polymer with ammonium chloride (NH4Cl), pentachlorophosphorane and phenol	Maihara, A (1999)

5.4.1.2 Long-term toxicity to fish

A fish, early-life stage toxicity test under semi-static conditions was performed with SPS-100 in order to assess its possible lethal and sub-lethal effects during the embryonic and early larval development of the fathead minnow. The study was conducted in accordance with OECD 210 and in compliance with GLP. A WSF was prepared by stirring SPS-100 at10 mg/L for 3 days and filtration through rough filter paper. Analytical measurements showed that concentrations in the WSF were variable and ranged between 6.7 and 178 µg/L for day 0 until day 9 (the embryonic and early larval stage which are the most sensitive life stages of the fathead minnow) and ranged between < LOD (< 2.3 µg/L) and 29.1 µg/L from day 14 until day 33 (later larval stages). SPS-100 did not induce any significant, visible effects on the development of fathead minnow embryos at its maximum solubility in test medium. The test substance did not affect time of hatching or the hatching success nor survival, growth or development of the larvae during the post-hatch period at its maximum solubility in test medium. Hence, the NOEC of SPS-100 for the early life stages of fish equals the maximum soluble concentration in test medium. The study was reliable without restrictions.

The results are summarized in the following Table:

Table 19: Long-term effects on fish

Method	Results	Remarks	Reference
Pimephales promelas freshwater early-life stage: reproduction, (sub)lethal effects semi-static OECD Guideline 210 (Fish, Early-Life Stage Toxicity Test) (2013) EPA OPPTS 850.1400 (Fish Early-life Stage Toxicity Test) Guidance document on aquatic toxicity testing of difficult substances and mixtures, OECD series on testing and assessment number 23, December 14, 2000.	NOEC (33 d): test matbased on: embryo development, number hatched, time to hatch and larval development (The NOEC corresponds to a WSF prepared at a loading rate of 10 mg/L which equals the maximum soluble test substance concentration in test medium)	1 (reliable without restriction) experimental result Test material (IUPAC name): Phenol, 4,4'-sulfonylbis-, polymer with ammonium chloride (NH4Cl), pentachlorophosphorane and phenol	Migchielsen, M.H.J. (2014)

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

In a 48-h acute toxicity study, water fleas (*Daphnia magna*) were exposed to the substance at nominal concentrations of 0 (blank-control), 0.1% (filtrate), 1% (filtrate), 10% (filtrate), 100% (filtrate) and 100 mg/L (unfiltered) under static conditions. The test substance was not toxic for *Daphnia magna*. No toxicity was observed at any of the test concentrations, including the maximum solubility of the substance in test medium. Therefore, it was concluded that the test substance it is not acutely toxic up to the solubility limit. The study is considered to be reliable without restrictions according to OECD Guideline No. 202.

The results are summarised in the following table:

Table 20: Short-term effects on aquatic invertebrates

Method	Results	Remarks	Reference
Daphnia magna freshwater static ISO 6341 (Water quality - Determination of the Inhibition of the Mobility of Daphnia magna Straus (Cladocera, Crustacea) - Acute toxicity test (1996) OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) (1984) EU Method C.2 (Acute Toxicity for Daphnia) (1992)	EC50 (48 h): based on: mobility (No acute toxicity up to solubility limit)	1 (reliable without restriction) experimental result Test material (IUPAC name): Phenol, 4,4'-sulfonylbis-, polymer with ammonium chloride (NH4Cl), pentachlorophosphorane and phenol	Migchielsen, MHJ (2001a)

5.4.2.2 Long-term toxicity to aquatic invertebrates

In a 21-day semi-static reproduction study, the toxicity of SPS-100 to aquatic invertebrate *Daphnia magna* was assessed according to OECD Guideline No. 211 (2008) and GLP principles. Nominal concentration was prepared at a loading rate of 100 mg/L and the resulting average concentration measured in the filtered test solutions was 0.2 mg/L. SPS-100 did not affect reproduction, growth or survival of *Daphnia magna* at the maximum solubility in test medium (0.2 mg/L) after 21 days of exposure. Therefore, it was concluded that the test substance it is not toxic up to the solubility limit. All criteria for acceptability of the test were met according to OECD guideline No. 211 and the study is considered to be reliable without restrictions.

The results are summarized in the following table:

Table 21: Long-term effects on aquatic invertebrates

Method	Results	Remarks	Reference
Daphnia magna freshwater semi-static OECD Guideline 211 (Daphnia magna Reproduction Test) (2008) EU Method C.20 (Daphnia magna Reproduction Test) (2008) ISO International Standard - 10706 (2000)	NOEC (21 d): based on: parental body length, reproduction, growth, survival (Did not affect reproduction, growth or survival up to solubility limit)	1 (reliable without restriction) experimental result **Test material (IUPAC name): Phenol, 4,4'-sulfonylbis-, polymer with ammonium chloride (NH4Cl), pentachlorophosphorane and phenol**	Migchielsen, MHJ (2012)

5.4.3 Algae and aquatic plants

In a 72 h toxicity study, freshwater algae (*Selenastrum capricornutum*) were exposed to SPS-100 at: 0 (control); 0.1; 1; 10 and 100% of a filtrate (5 µm) prepared at a nominal loading rate of 100 mg/L. The EC50 for both algal growth inhibition and growth rate reduction exceeded the maximum solubility of the substance. Hence, as a consequence of the extremely low solubility of the substance, concentration levels that might be toxic for algae could not be reached. Therefore, it was concluded that the test substance it is not toxic up to the solubility limit. The present toxicity study is classified as reliable without restrictions according to the OECD guideline No. 201.

The results are summarized in the following table:

Table 22: Effects on algae and aquatic plants

Method	Results	Remarks	Reference
Selenastrum capricornutum (new name: Pseudokirchnerella subcapitata) (algae) freshwater static ISO 8692 (Water Quality - Fresh Water Algal Growth Inhibition Test with Scenedesmus subspicatus and Selenastrum capricornutum) (1989) EU Method C.3 (Algal Inhibition test) (1992) OECD Guideline 201 (Alga, Growth Inhibition Test) (1984)	EC50 (72 h): based on: growth rate (No toxicity up to solubility limit) NOEC (72 h): based on: growth rate (No toxicity up to solubility limit)	1 (reliable without restriction) experimental result Test material (IUPAC name): Phenol, 4,4'-sulfonylbis-, polymer with ammonium chloride (NH4Cl), pentachlorophosphorane and phenol	Migchielsen, MHJ (2001b)

5.4.4 Other aquatic organisms (including sediment)

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

CLP regulation (EC No 1272/2008)

The proposed classification of the test substance was done in accordance with the criteria set in the 'Safety net' classification - Chronic Category 4. In the acute aquatic toxicity tests, the test substance was not acutely toxic up to the water solubility limit. As the substance is not readily biodegradable and has a log Kow >6.2 (cut-off value: log Kow ≥4), it indicates a potential for bioaccumulation. However, based on the fact that chronic NOECs to fish, daphnia and algae are above the water solubility limit of the substance, no classification for environmental hazards is deemed necessary

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

In accordance with the 2nd ATP of Regulation (EC) No 1272/2008 (CLP), the classification of the substance is deemed unnecessary since chronic toxicity NOECs to fish, daphnia and algae are greater than the water solubility of the test substance.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Currently, SPS-100 has an harmonised classification in Annex VI of CLP as Aquatic Chronic 4. The dossier submitter (DS) proposed to remove the current classification based on new data available after the current harmonised classification was agreed.

WATER SOLUBILITY: The substance is constituted by 3 components, which give 3 peaks in the HPLC: Peak 1: < 4 µg/L, Peak 2: < 28 µg/L, Peak 3: < 44 µg/L; the water solubility was estimated based on the limit of detection of the components (Brekelmans, 2001a).

LOG K_{OW}: The partition coefficient Log K_{OW} > 6.2, was measured using the HPLC method (Brekelmans, 2001b).

DEGRADATION: The substances degradation was investigated in a screening test (ready biodegradability – Japanese Industrial Standard) performed in 1998 and considered in accordance with OECD TG 301C. The test was performed at 25°C, without a toxicity control.

After 28 days, a 2% and 0% degradation was observed, measured by O_2 consumption and test material analysis (HPLC), respectively. As the test was performed significantly above water solubility, the lack of degradation might reflect limited bioavailability/dissolution.

Result: Not readily biodegradable
Test quality: 2 (reliable with restrictions) – Haruguchi, 1998

AQUATIC BIOACCUMULATION

The substances bioaccumulation potential was measured using a Japanese standard method equivalent to OECD TG 305 (Maihara, 2000).

Cyprinus carpio were exposed to the test item at nominal concentrations of 0.1 and 1 mg/L, achieved via dispersion with the emulsifier HCO-40, using a flow-through system, with a total uptake duration of 56 d. No depuration phase was took place in the study. The effects of growth dilution are unknown. The steady state concentration in fish from both low and high concentration solutions after 56 d was measured via HPLC. Only peak 1 was detected to be < 2 µg/g, however it is not clear from the report to which of the 3 components this peak corresponds to.

Results: **BCF: < 21.3** (whole body d.w.) with 0.1 mg/L (nominal) (steady state)

BCF: < 2.1 (whole body d.w.) with 1 mg/L (nominal) (steady state)

According to the DS, the study should be considered unreliable: the report does not contain any information on the concentration of the truly dissolved test substance (the nominal concentration in water of both solutions (100 and 1000 µg/L) exceeds the solubility limit of the 3 components which have different solubilities (< 4 µg/L, < 28 µg/L and < 44 µg/L respectively)). Additionally, in fish, the concentration of peak 1 was always < 2 µg/g,

irrespective of the nominal concentration. Relevant details on test conditions (e.g. photoperiod, mortality in control and treated fish) were not reported and the documentation of the analytical method and results are poor.

AQUATIC TOXICITIES

The DS included studies for all thropic levels for both acute and chronic toxicity conduceted using SPS-100.

1. Acute fish toxicity with *Oryzias latipes* (ricefish) – Method 71 of JIS K0102, which is equivalent or similar to OECD TG 305C "Bioconcentration: Flow-Through Fish Test; 305C, Modified MITI Test". The ricefish were exposed for 96h to 5 concentrations up to 100 mg/L, which were analytically confirmed. A dispersant (HCO-40) was used to reach the concentrations over water solubility.

Result: LC_{50} (96h) >100 mg/L based on mortality (nominal test material concentration).

The study (Maihara, 1999) is considered to be reliable with restrictions (Klimisch score 2) because the recoveries of the analytical method were not reported and the final dispersant (HCO-40) concentrations were above the maximum mentioned in the OECD TG 203.

2. Chronic fish test with *Pimephales promelas* – OECD TG 210 (Fish, Early-Life Stage Toxicity Test), EPA OPPTS 850.1400 (Fish Early-life Stage Toxicity Test). The study (Migchielsen, 2014) is considered reliable without restriction (1).

Result: NOEC (33d) Maximum soluble test substance (based on: embryo development, number hatched, time to hatch and larval development). Since no effects were observed in a water soluble fraction (WSF) prepared at a loading rate of 10 mg/L, the NOEC is considered to be equal to the maximum soluble test substance concentration in test medium.

Maximum soluble concentration of the test substance: Analytical measurements showed that concentrations in the WSF were variable and ranged between 6.7 and 178 µg/L for day 0 until day 9 (the embryonic and early larval stage which are the most sensitive life stages of the fathead minnow) and ranged between < LOD (< 2.3 µg/L) and 29.1 µg/L from day 14 until day 33 (later larval stages).

3. Acute invertebrate test with *Daphnia sp.* based on immobilisation according to OECD TG 202.

Result: EC_{50} (48h) No acute toxicity up to solubility limit based on mobility was observed. The test (Migchielsen, 2001a) is considered reliable (Klimisch score 1), without restriction.

4. Chronic invertebrate test with *D. magna* based on reproduction OECD TG 211

Result: NOEC (21d) No inhibition of reproduction, growth or survival up to solubility limit, based on: parental body length, reproduction, growth, survival. The Migchielsen study (2012) is considered relaible without restriction (1).

5. Algae, growth inhibition test with *Pseudokirchnerella subcapitata*, OECD TG 201

- *Acute result:* EC_{50} (72h) No acute toxicity up to solubility limit, based on growth rate;

- *Chronic result:* NOEC (72h) No acute toxicity up to solubility limit based on growth rate. The Migchielsen study (2001b) is considered reliable without restriction (1).

6. Respiration inhibition with activated sludge, OECD TG 209

Result: EC_{50} (30 min): >100 mg/L (nominal test material concentration) based on respiration rate (Desmares-Koopmans, 2001).

Comments received during public consultation

Two Member States Competent Authorities (MSCA) commented, one of them agreed, the other disagreed with the classification proposed by the DS. The one which agreed had the same argument as DS, the opposing one sent in two comments:

1. "The substance fulfils the criteria of classification Aquatic Chronic 4; H413, ...moreover it is potentially toxic for environmental organisms" – evidence was not added to support these statements by the commenter.

2. "Fish tests (short and long term) are not reliable" – Contradictory to this statement the chronic fish test is classified in the CLH dossier as reliable without restriction (1) and the acute fish test as reliable with restriction (2). The commenter's classification of the test results as not reliable was not justified.

"..only nominal concentrations are reported" – The DS replied that this statement is correct, but does not influence the evidences, because the results are not given in concentrations, but, – as the criterion for not classification is the solubility limit, –they are reported as follow: "NOEC is greater than the water solubility of the substance". The RAC considers this reporting as sufficient and correct.

Assessment and comparison with the classification criteria

Discussion of environmental fate and comparison with criteria

Water solubility: was measured with HPLC and determined to be very low for all three components which have different solubility ranging from < 4 µg/L and < 44 µg/L.

Degradability: Ready biodegradability study of SPS-100 was performed for 28 days using a method similar to MITI (I) (OECD TG 301C). At the end of the test period the average biodegradation was 2%. Thus, the criterion for ready biodegradability (at least 60% biodegradation) was not met. The study is considered to be reliable with restrictions since a toxicity control was not included.

Bioaccumulation: the test result is considered unreliable. The OECD TG 305 (bioconcentration test in fish) indicates that for substances with very low solubility in the aquatic environment, exposure via water may be of limited importance in comparison to the dietary route. In addition, other experimental shortcomings were reported in the study, as discussed above.

A worst case interpretation of the data suggests a BCF at or below 1000 L/kg. Both this predicted BCF and the high Kow indicate that SPS-100 may have the potential to bioaccumulative. Nevertheless the decision-making pathways in CLP Annex I, Table 4.1.0

allows to exclude bioaccumulative substances from classification, on the ground that it has no acute or chronic toxicity on the aquatic ecosystem.

Aquatic toxicity: All acute and chronic test results show that no acute and no chronic toxicity occured up to solubility limit.

According to the CLP regulation, Aquatic Chronic category 4 is appropriate when (i) poorly soluble substances for which no acute toxicity is recorded at levels up to the water solubility, and (ii) which are not rapidly degradable and (iii) have an experimentally determined BCF ≥ 500 (or, if absent, a log Kow ≥ 4), unless other scientific evidence exists showing classification to be unnecessary. Such evidence includes chronic toxicity NOECs >water solubility or >1 mg/L.

SPS-100 fulfills criteria (i) to (iii), however the removal of the environmental classification is supported by newly performed long-term test results, which confirmed that classification is unnecessary since chronic toxicity NOECs to fish, daphnia and algae are greater than the water solubility of the test substance – in accordance with the 2nd ATP to Regulation (EC) No 1272/2008 (CLP).

Overall, RAC agrees with the DS proposal to remove the classification as Aquatic Chronic 4; H413 of phenol, 4,4'-sulfonylbis-, polymer with ammonium chloride ($(NH_4)Cl$), pentachlorophosphorane and phenol.

6 OTHER INFORMATION

Not relevant for the current proposal of revision of the Annex VI classification.

7 REFERENCES

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8 ANNEXES

Not relevant for the current proposal of revision of the Annex VI classification.