CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

2-Phenylhexanenitrile

EC Number: 423-460-8

CAS Number: 3508-98-3

Index Number: 608-039-00-0

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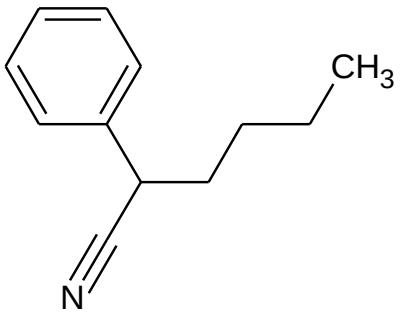
1. IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	2-phenylhexanenitrile / Benzeneacetonitrile, α-butyl-
Other names (usual name, trade name, abbreviation)	Salicynalva
ISO common name (if available and appropriate)	Not applicable
EC number	423-460-8
EC name	2-phenylhexanenitrile
CAS number	3508-98-3
Other identity code (if available)	InChI=1/C12H15N/c1-2-3-7-12(10-13)11-8-5-4-6-9-11/h4-6,8-9,12H,2-3,7H2,1H3
Molecular formula	C12 H15 N
Structural formula	
SMILES notation	N#CC(CCCC)c1ccccc1
Molecular weight	173.12
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	This substance is produced from a non-chiral feed stock originated from the petroleum industry. The chemical transformations to prepare the reference substance are not stereo selective. As the result, the reference substance is racemic and not optically active.
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%)	≥97 - ≤100% (w/w)

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
2-phenylhexanenitrile / Benzeneacetonitrile, α-butyl- (EC number 423-460-8; CAS number 3508-98-3)	≥97 - ≤100% (w/w)	Acute Tox. 4* - H302 Aquatic Acute 1 - H400 Aquatic Chronic 1 - H410	Notified to the C&L Inventory with: Acute Tox. 4 - H302 Aquatic Acute 1 - H400 Aquatic Chronic 1 - H410

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
2-hexenenitrile, 2-phenyl / Benzeneacetonitrile, alpha-butylidene / 2-hexenenitrile, 2-phenyl (CAS number 6519-09-1)	≥0.0 - ≤1% (w/w)	None	Not present in the C&L Inventory	Not relevant for the classification of 2-phenylhexanenitrile
2-phenylacetamide / 2-phenylacetamide / Benzeneacetamide (EC number 203-147-0; CAS number 103-81-1)	≥0.0 - <1% (w/w)	None	Notified to the C&L Inventory with: Acute Tox. 4, H302 Eye Irrit. 2, H319 by 23 notifiers and with Skin Irrit. 2, H315 Eye Irrit. 2, H319 STOT SE 3, H335 by 1 notifier	Not relevant for the classification of 2-phenylhexanenitrile
Unknown impurities: percentage for each impurity is < 1%.	≥0.0 - <1% (w/w)			Not relevant for the classification of 2-phenylhexanenitrile

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
α-tocopherol / 2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-ol (EC number 200-412-2; CAS number 59-02-9)	Stabiliser	≥0 - ≤0.3% (w/w)	None	None	The concentration of α-tocopherol does not influence the classification and labelling of 2-phenylhexanenitrile

Table 5 Test substances (non-confidential information)

Identification of test substance	**Purity**	**Impurities and additives (identity, %, classification if available)**	**Other information**
Salicynalva / 2-Phenylhexanenitrile; Lot/batch No.: 6162H	> 95%	Not reported	Bell, G; Thirkettle, KM and Smith, B (1996a). 2-Phenylhexanenitrile acute toxicity for Rainbow Trout (*Oncorhynchus mykiss*)
Salicynalva / 2-Phenylhexanenitrile; Lot/batch No.: 6162H	> 95%	Not reported	Bell, G; Thirkettle, KM and Smith, B (1996b). 2-Phenylhexanenitrile acute toxicity to *Daphnia magna*
Salicynalva / 2-Phenylhexanenitrile; Lot/batch No.: 6162H	> 95%	Not reported	Bell, G; Thirkettle, KM and Smith, B (1996c). 2-Phenylhexanenitrile algal growth inhibition
2-Phenylhexanenitrile; Lot/batch No.: 6162H	> 95%	Not reported	Bell, G (1996). 2-Phenylhexanenitrile ready biodegradability (closed bottle test).
Salicynalva / 2-phenylhexanenitrile; Lot/batch No.: Test article 97-202-01	> 99%	Not reported	Jordinson, GM and Morris, DS (1998). 2-Phenylhexanenitrile (test article 97-202-01): Determination of biodegradability by EPA OPPTS 835.3220 and modified OECD Test Guideline 303A, draft dated September, 1996.
Salicynalva / 2-Phenylhexanenitrile; Lot/batch No.: 6162H	> 95%	Not reported	Betteley, JMT (1996). 2-Phenylhexanenitrile abiotic degradation: Hydrolysis as a function of pH

2. PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6: The current Annex VI entry and the proposed harmonised classification

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	608-039-00-0	2-phenylhexanenitrile	423-460-8	3508-98-3	Acute Tox. 4* Aquatic Acute 1 Aquatic Chronic 1	H302 H400 H410	GHS07 GHS09 Wng	H302 H410	-	-	-
Dossier submitters proposal	608-039-00-0	2-phenylhexanenitrile	423-460-8	3508-98-3	**Modify** Acute Tox. 4* to Acute Tox 4 and Aquatic Chronic 1 to Aquatic Chronic 2 **Remove** Aquatic Acute 1	**Retain** H302 and **Modify** H410 to H411 and **Remove** H400	**Retain** GHS07 GHS09	**Retain** H302 **Modify** H410 to H411	-	-	-
Resulting Annex VI entry if agreed by RAC and COM	608-039-00-0	2-phenylhexanenitrile	423-460-8	3508-98-3	Acute Tox. 4; Aquatic Chronic 2	H302 H411	GHS07 GHS09	H302 H411	-	-	-

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	*Hazard class not assessed in this dossier*	No
Flammable gases	*Hazard class not assessed in this dossier*	No
Flammable aerosols	*Hazard class not assessed in this dossier*	No
Oxidising gases	*Hazard class not assessed in this dossier*	No
Gases under pressure	*Hazard class not assessed in this dossier*	No
Flammable liquids	*Hazard class not assessed in this dossier*	No
Flammable solids	*Hazard class not assessed in this dossier*	No
Self-reactive substances	*Hazard class not assessed in this dossier*	No
Pyrophoric liquids	*Hazard class not assessed in this dossier*	No
Pyrophoric solids	*Hazard class not assessed in this dossier*	No
Self-heating substances	*Hazard class not assessed in this dossier*	No
Substances which in contact with water emit flammable gases	*Hazard class not assessed in this dossier*	No
Oxidising liquids	*Hazard class not assessed in this dossier*	No
Oxidising solids	*Hazard class not assessed in this dossier*	No
Organic peroxides	*Hazard class not assessed in this dossier*	No
Corrosive to metals	*Hazard class not assessed in this dossier*	No
Acute toxicity via oral route	*Proposed harmonised classification: Acute Tox 4*	Yes
Acute toxicity via dermal route	*Hazard class not assessed in this dossier*	No
Acute toxicity via inhalation route	*Hazard class not assessed in this dossier*	No
Skin corrosion/ irritation	*Hazard class not assessed in this dossier*	No
Serious eye damage/eye irritation	*Hazard class not assessed in this dossier*	No
Respiratory sensitisation	*Hazard class not assessed in this dossier*	No
Skin sensitisation	*Hazard class not assessed in this dossier*	No
Germ cell mutagenicity	*Hazard class not assessed in this dossier*	No
Carcinogenicity	*Hazard class not assessed in this dossier*	No
Reproductive toxicity	*Hazard class not assessed in this dossier*	No
Specific target organ toxicity-single exposure	*Hazard class not assessed in this dossier*	No

Specific target organ toxicity-repeated exposure	*Hazard class not assessed in this dossier*	No
Aspiration hazard	*Hazard class not assessed in this dossier*	No
Hazardous to the aquatic environment	Proposed harmonised classification: Aquatic Chronic 2 - H411	Yes
Hazardous to the ozone layer	*Hazard class not assessed in this dossier*	No

3. HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Environmental hazards: According to the criteria outlined in Annex VI of EU Directive 67/548/EEC (DSD), 2-phenylhexanenitrile was classified as very toxic for the aquatic environment (N, R50/53: Very toxic to the aquatic environment, may cause long-term adverse effects in the aquatic environment) based on the endpoint algal biomass inhibition (EbC50). The substance was included in the Annex I of Directive 67/548/EEC.

The corresponding classification translation to Regulation (EC) 1272/2008 (CLP) is Aquatic Acute cat. 1 and Aquatic Chronic cat. 1 (H400: Very toxic to aquatic life and H410: Very toxic to aquatic life with long lasting effects). This environmental classification is included in the current entry of the substance in the Annex VI, Table 3.1 of CLP.

4. JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Justification that action is needed at Community level is required: *Change in existing entry due to changes in the criteria.*

The current classification and labelling in Annex VI, Table 3.1 and 3.2 to Regulation (EC) 1272/2008 should be updated if the proposed harmonised classification and labelling is agreed by RAC and the European Commission.

According to article 36(3) of Regulation (EC) 1272/2008 where a substance does not fulfil the criteria for respiratory sensitisation cat. 1, germ cell mutagenicity cat. 1A/1B/2, carcinogenicity cat. 1A/1B/2 and reproductive toxicity cat. 1A/1B/2 and does not fulfil the plant protection and biocidal active substance definition, a harmonised classification and labelling in accordance with article 37 may also be added to Annex VI on a case-by-case basis, if justification is provided demonstrating the need for such action at Community level.

The need for such action is stated in the Recital (53) of Regulation (EC) 1272/2008 *'…the specific substances listed in Annex I of Directive 67/548/EEC, all existing harmonised classifications should be converted into new harmonised classification using the new criteria (...). By subjecting all future harmonisations of classifications to this Regulation, inconsistencies in harmonised classifications of the same substance under the existing and the new criteria should be avoided.'*

The harmonised classification as R50/53 (Very toxic to the aquatic environment, may cause long-term adverse effects in the aquatic environment) of 2-phenylhexanenitrile included in the Annex I of Directive 67/548/EEC (DSD) was based on the algal biomass endpoint (EbC50). The corresponding classification translation to Regulation (EC) 1272/2008 was Aquatic Acute 1 and Aquatic Chronic 1. However, according to Annex I, Table 4.1.0, of Regulation (EC) 1272/2008, and the ECHA Guidance

document on CLP, the classification shall be based on the algal growth rate endpoint (ErC_x, NOErC), where this is available, instead of the biomass endpoint (EbC_x, NOEbC). This is because growth rate is not dependent on the test design, whereas biomass inhibition depends on the growth rate of the test species as well as test duration and other elements of the test design.

For 2-phenylhexanenitrile, reliable ErC50 and NOErC values are available for algae (*Pseudokirchneriella subcapitata* 72h-ErC50 2.58 mg/L, 72h-NOErC 0.26 mg/L). When taking into account these toxicity values for algae instead of the biomass endpoint, the lowest acute toxicity value of the substance is the 48h-EC50 of 1.6 mg/L for *Daphnia magna*. This is above the classification limit of 1 mg/L, and thus 2-phenylhexanenitrile does not meet the criteria for classification as Aquatic Acute 1.

Chronic toxicity data is only available for algae. Therefore, the substance is classified for long-term hazards based on both the available chronic and acute data in combination with the environmental fate data of the substance, and the most stringent outcome is selected. Based on the available chronic toxicity data being in the range of >0.1 to ≤ 1 mg/L (72h-NOErC of 0.26 mg/L of *Pseudokirchneriella subcapitata*) and the substance not being rapidly degradable, 2-phenylhexanenitrile is classified as Aquatic Chronic 2. The substance receives the same chronic classification based on the acute toxicity data on fish, aquatic invertebrate and algae, and the substance not being rapidly degradable (the lowest acute toxicity value, 48h-EC50 of 1.6 mg/L for *Daphnia magna*, being within the range of >1 to ≤ 10 mg/L). Consequently, 2-phenylhexanenitrile meets the criteria for classification as Aquatic Chronic 2.

5. IDENTIFIED USES

2-phenylhexanenitrile is used as an ingredient in fragrance mixtures.

6. DATA SOURCES

The CLH dossier is based on the data available in the REACH registration dossier of 2-phenylhexanenitrile.

7. PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Liquid (colourless) at 20°C and 101.3 kPa	Donoso, A (2005)	According to analytical data from the owner company
Freezing point	Lower than -25 °C at 101.3 kPa	Dulichan, A (2006)	According to the OECD TG 102 and EU Method A.1 (Melting / Freezing Temperature)
Boiling point	265°C to 276°C at 101100 to 101300 Pa	Dulichan, A (2006)	Distillation method according to OECD Guideline 103 (Boiling point/boiling range) and EU Method A.2 (Boiling Temperature)
Relative density	0.94706 at 20°C	Dulichan, A (2006)	Pycnometer method according to OECD Guideline 109 (Density of Liquids and Solids) and EU Method A.3 (Relative Density)
Vapour pressure	6.4 ± 0.5 Pa at 25°C	Taylor, N. (1996)	Isoteniscope method according to OECD Guideline 104 (Vapour Pressure Curve) and EU Method A.4 (Vapour Pressure)
Surface tension	59.5 mN/m at 20°C and 33 mg/L According to EU method A.5 the substance is surface active because the value is just below 60 mN/m. This surface tension is not expected to have any impact on the risk assessment of the substance.	Dulichan, A. (2006)	OECD harmonised ring method according to OECD Guideline 115 and EU Method A.5
Water solubility	37.7 mg/L at 20°C	Dulichan, A. (2006)	Flask method according to OECD Guideline 105 and EU Method A.6
Partition coefficient n-octanol/water	Log Kow = 3.14 at 21°C	Dulichan, A. (2006)	Shake-flask method according to OECD Guideline 107 (Partition Coefficient (n-octanol / water), Shake Flask Method) and EU Method A.8 (Partition Coefficient)
Flash point	128.5°C at 102400 Pa	Dulichan, A. (2006)	Closed cup method according to EU Method A.9 (Flash-Point)
Flammability	The substance has a high flashpoint (128.5°C) not indicating flammability. In addition, the substance does not contain groups that might lead to the evolution of a dangerous amount of flammable gas when coming into contact with water or damp air. No metals, transition metals, boron or silicon are present. Also, handling during the water solubility test confirms this conclusion. With regards to pyrophoric properties, evaluation of the structural features of the substance revealed that the substance does not contain any chemical group that might lead to spontaneous ignition a short time after coming into contact with air.		Justified approach using Column 2 of REACH Annex VII

Explosive properties	Non explosive	Dulichan, A. (2006)	According to EU Method A.14 (Explosive properties)
Self-ignition temperature	406°C at 102300 Pa	Dulichan, A. (2006)	According to EU Method A.15 (Auto-Ignition Temperature (Liquids and Gases))
Oxidising properties	The substance does not possess oxygen or halogen atoms that are chemically bound to nitrogen or oxygen atoms.		Justified approach using Column 2 of REACH Annex VII
Granulometry	The substance is a liquid and is thus marketed in a non-solid or granular form.		Justified approach using Column 2 of REACH Annex VII
Stability in organic solvents and identity of relevant degradation products	No data available		
Dissociation constant	No data available		
Viscosity	No data available		

8. EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Not relevant for this dossier.

8.2 Flammable gases

Not relevant for this dossier.

8.3 Aerosols

Not relevant for this dossier.

8.4 Oxidising gases

Not relevant for this dossier.

8.5 Gases under pressure

Not relevant for this dossier.

8.6 Flammable liquids

Not relevant for this dossier.

8.7 Flammable solids

Not relevant for this dossier.

8.8 Self-reactive substances

Not relevant for this dossier.

8.9 Pyrophoric liquids

Not relevant for this dossier.

8.10 Pyrophoric solids

Not relevant for this dossier.

8.11 Self-heating substances

Not relevant for this dossier.

8.12 Substances which in contact with water emit flammable gases

Not relevant for this dossier.

8.13 Oxidising liquids

Not relevant for this dossier.

8.14 Oxidising solids

Not relevant for this dossier.

8.15 Organic peroxides

Not relevant for this dossier.

8.16 Corrosive to metals

Not relevant for this dossier.

9. TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not relevant for this dossier.

10. EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Table 9: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute toxicity (oral) – Fixed dose procedure (EU method B.1 bis), GLP	Rat, Sprague-Dawley (CD), 5 animals/sex/group	2-Phenylhexanenitrile (Salicynalva) Purity > 95%	2000, 500 and 50 mg/kg bw, oral gavage, single dose	No LD_{50} can be derived	Salicynalva. Acute oral toxicity to the rat (IFF Study No. 94-208). Huntingdon Life Sciences Ltd. (1996)

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

The oral acute toxicity of 2-phenylhexanenitrile was determined in Sprague-Dawley rats following the fixed dose method procedure (EU method B.1 bis). The study was conducted in 1996 according to the old method as published in the former EU legislation. Therefore, the level of doses in the study does not correspond to the ones used in the current updated method, equivalent to OECD TG (2001).

A preliminary study was carried out by dosing one female rat at 500 mg/kg bodyweight. Only piloerection was observed and recovery was complete by Day 7.

In the main study, groups of ten rats (five males and five females) was treated at 2000, 500 and 50 mg/kg bw.

Five deaths occurred (one male at 500 mg/kg and two males and two females at 2000 mg/kg) between 24 and 48 hours of dosing. Macroscopic examination revealed congestion in the major organs and tissues.

Clinical signs among rats treated at 500 and 2000 mg/kg included piloerection, abnormal body carriage, abnormal gait, decreased respiratory rate, pallor of the extremities, increased salivation, walking on toes and unsteadiness. In addition, animals of the highest dose showed lethargy, increased urine production and hair loss. In rats dosed at 50 mg/kg only piloerection was recorded. In general, recovery was complete with the exception of hair loss that persisted at the end of the observation period (Day 15) in one surviving rat of the high-dose group.

Bodyweight gain was decreased in several animals dosed at 2000 mg/kg bw, but no differences were observed at the end of the study. No abnormalities were recorded at the macroscopic examination on Day 15.

As a result, authors established the discriminating dose, that is the highest of the pre-set dose levels which can be administered without causing mortality, of 2-phenylhexanenitrile at 50 mg/kg bw.

10.1.2 Comparison with the CLP criteria

The EU guideline for fixed dose procedure method includes flow charts that allow conclusions to be drawn with respect to classification. Therefore, taking into account the results of the available study, a classification as Acute Tox. 4, H302 is proposed according to the test guideline, since the substance 2-phenylhexanenitrile caused one animal death at the dose of 500 mg/kg bw.

As it has been mentioned previously, the study was conducted in 1996 according to the old guideline. Although the new guideline uses the dose of 300 mg/kg bw instead of 500 mg/kg bw, it is noted that

there were four deaths at 2000 mg/kg bw that would also lead to category 4 according to the current test method.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

The substance 2-phenylhexanenitrile is included in Annex VI classified as Acute Tox. 4*, H302 (minimum classification). After the revision of the animal data available for the determination of the acute toxicity by oral route, the current classification as Acute Tox. 4 with the hazard statement H302 (Harmful if swallowed) is supported.

10.2 Acute toxicity - dermal route

Not relevant for this dossier.

10.3 Acute toxicity - inhalation route

Not relevant for this dossier.

10.4 Skin corrosion/irritation

Not relevant for this dossier.

10.5 Serious eye damage/eye irritation

Not relevant for this dossier.

10.6 Respiratory sensitisation

Not relevant for this dossier.

10.7 Skin sensitisation

Not relevant for this dossier.

10.8 Germ cell mutagenicity

Not relevant for this dossier.

10.9 Carcinogenicity

Not relevant for this dossier.

10.10 Reproductive toxicity

Not relevant for this dossier.

10.11 Specific target organ toxicity-single exposure

Not relevant for this dossier.

10.12 Specific target organ toxicity-repeated exposure

Not relevant for this dossier.

10.13 Aspiration hazard

Not relevant for this dossier.

11. EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 10: Summary of relevant information on rapid degradability

Method	Results	Key or Supportive study	Remarks	Reference
According to EEC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC (0.1. No. L383A, 29.12.92) Part C, Method 4-E "Determination of ready biodegradability, Closed Bottle Test" and the OECD Guideline for Testing of Chemicals No. 301D "Ready Biodegradability: Closed Bottle Test".	Not readily biodegradable: No biodegradation observed after 28 days.	Key	Reliability: Klimisch 1 (reliable without restriction).	Bell, G (1996)
According to OECD TG 303A with appropriate modifications to ensure compliance with EPA "Public Draft" Guideline OPPTS 835.3220.	In view of the similar removal between the reference substance, sodium benzoate, and the test substance it can be expected that a significant level of ultimate biodegradation/removal was obtained but no final conclusion on ultimate biodegradation can be made based on this study.	Supportive	Reliability: Klimisch 1 (reliable without restriction).	Jordinson, GM and Morris, DS (1998)
Similar to OECD TG 111 and in compliance with EEC Methods for the determination of ecotoxicity, Directive 92/69/EEC (OJ No. L383A, 29. 12. 92), Part C, Method C7. Degradation: Abiotic degradation: hydrolysis as a function of pH).	Hydrolytically stable for classification purposes: Half-life times in water at 25°C: ▪ 25.9 days at pH 4; ▪ 15.4 days at pH 7; ▪ 4.7 days at pH 9.	Key	Reliability: Klimisch 1 (reliable without restriction).	Betteley, JMT (1996)

11.1.1 Ready biodegradability

Title: Salicynalva (2-phenylhexanenitrile) ready biodegradability (closed bottle test)

Author: Bell, G

Year: 1996

Reliability: Klimisch 1 (reliable without restriction).

Method: A study was performed to assess the ready biodegradability of 2-phenylhexanenitrile. The study was conducted in accordance with EEC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC (0.1. No. L383A, 29.12.92) Part C, Method 4-E "Determination of ready

biodegradability, Closed Bottle Test" and the OECD Guideline for Testing of Chemicals No. 301D "Ready Biodegradability: Closed Bottle Test". The test substance (purity > 95%) was dissolved in chloroform to give a stock solution of 560 mg/10 mL. Then, 10 µL aliquots of stock solution were placed on individual pieces of glass filter paper and the solvent allowed to evaporate to dryness. Sealed bottles containing the test substance (adsorbed onto glass filter paper) at a concentration of 2 mg/L and inorganic nutrient medium were inoculated with activated sewage sludge bacteria (collected from a sewage treatment plant predominantly receiving domestic sewage) and incubated for up to 28 days at 20 ± 1 °C. On days 0, 4, 7, 11, 14, 18, 21, 25 and 28 duplicate bottles were taken and dissolved oxygen measurements were performed electrochemically. Percentage biodegradation values were determined by comparing the extent of oxygen depletion with the Theoretical Oxygen Demand (2.77 mg O2/mg). Additional bottles, containing both the test substance and a readily biodegradable standard substance were prepared in order to provide additional information on the inhibitory effect of the test substance.

Results: 2-phenylhexanenitrile attained no biodegradation after 28 days and it was not found to be inhibitory to activated sewage sludge bacteria under the conditions of this test. Degradation (%) of test substance (O2 consumption): 0 after 4 d; 1 after 7 d; 3 after 14 d; 1 after 21 d and 0 after 28 d. Sodium benzoate (reference substance) attained 80% biodegradation within 28 days.

Conclusion: Based on the results of the ready biodegradability screening test (0% degradation after 28 days) 2-phenylhexanenitrile is considered as not readily biodegradable.

11.1.2 BOD_5/COD

No data available

11.1.3 Hydrolysis

Title: Salicynalva (2-phenylhexanenitrile) abiotic degradation: Hydrolysis as a function of pH

Author: Betteley, JMT

Year: 1996

Reliability: Klimisch 1 (reliable without restriction).

Method: The hydrolysis of the substance has been tested using a protocol similar to OECD TG 111 and in compliance with EEC Methods for the determination of ecotoxicity, Directive 92/69/EEC (OJ No. L383A, 29. 12. 92), Part C, Method C7. Degradation: Abiotic degradation: hydrolysis as a function of pH). Hydrolysis was evaluated at 50 °C in aqueous solutions at pH 4, 7 and 9. The starting concentration of 2-phenylhexanenitrile (purity > 95%) was circa 15 mg/L. At each time point, including 0 hours, 2.4 hours and 5 days, an aliquot of test solution was diluted with mobile phase and the concentration of the substance determined by HPLC analysis.

Results: Under the preliminary test condition, the substance was found to undergo hydrolysis at pH 4, 7 and 9, which indicated a half-life of between 1 day and 1 year at 25 °C and therefore a full study was carried out. Under the test conditions in the first test at 50 °C, the substance was found to undergo a pseudo first order reaction at pH 4, 7 and 9 and therefore the final test was performed at 60 and 70 °C. The half-life times of 2-phenylhexanenitrile in water were determined to be 25.9 days at pH 4, 15.4 days at pH 7 and 4.7 days at pH 9 at 25°C.

Conclusion: The half-life times of 2-phenylhexanenitrile in water are 25.9 days at pH 4, 15.4 days at pH 7 and 4.7 days at pH 9 at 25°C. The substance is considered as hydrolytically stable under

environmental conditions for classification purposes since the longest half-life is above 16 days at pH range 4-9 (see ECHA Guidance on the Application of the CLP Criteria, v. 4.1, June 2015).

11.1.4 Other convincing scientific evidence

11.1.4.1. Field investigations and monitoring data (if relevant for C&L)

No data available.

11.1.4.2. Inherent and enhanced ready biodegradability tests

No data available.

11.1.4.3. Water, water-sediment and soil degradation data (including simulation studies)

Title: Salicynalva (2-phenylhexanenitrile) (test article 97-202-01): Determination of biodegradability by EPA OPPTS 835.3220 and modified OECD Test Guideline 303A, draft dated September, 1996

Author: Jordinson, GM and Morris DS

Year: 1998

Reliability: Klimisch 1 (reliable without restriction).

Method: The biodegradation and/or removal of 2-phenylhexanenitrile within sewage treatment processes was investigated using the OECD Test Guideline 303A with appropriate modifications to ensure compliance with EPA "Public Draft" Guideline OPPTS 835.3220. 2-phenylhexanenitrile (purity > 99%) fresh test solutions were prepared daily at a nominal concentration of 31 mg/L (26 mg/L as carbon; from the empirical formula supplied by the Sponsor, 2-phenylhexanenitrile was calculated to consist of 83% carbon). The activated sludge is retained in a porous polythene cylinder into which the test substance is dosed together with a mixture of domestic and synthetic sewage (predominantly domestic sewage; adaptation not specified but assumed to be non-adapted). Treated effluent percolates through the porous liner (pore size 50-70 µm) and overflows to a collection vessel. The use of a porous reactor wall eliminates the need for a sludge settlement chamber. The methods of analysis used to determine biodegradability and removal of the test substance were DNPOC analysis and specific test substance analysis using gas chromatography.

Results: The test substance attained a level of primary biodegradation/removal in this study of 88% and 89%, as shown by the parent compound analytical data. These high levels of test substance removal were already attained on day 4 of the equilibrium period and remained as such during the whole test. The level of ultimate biodegradation/removal achieved, provided by an assessment of the level of dissolved organic carbon removed, was 87% (attained already on day 9 of the equilibrium period and remained as such during the whole test). Whilst this figure was shown to be statistically different to the levels of DOC removal observed in the control and reference substance pots, the statistical analyses also showed significant differences between duplicate control and reference substance pots. Therefore, including the small data set, undue emphasis should not be placed on the statistical results obtained. The overall difference, in terms of percentage carbon removal, between the test pots and the control and reference substance pots, during the definitive period, was only 3%. Although there was apparent adsorption of the test substance to the test solution feed lines, there was no notable amount of adsorption to either the sludge solids or the porous pot apparatus. A final

reasoning considering primary degradation and ultimate degradation is therefore not possible. Transformation products were not measured, as this is not required for this study. A substantial amount of nitrification was observed within all of the porous pots as shown by the analytical data for NH_4-N and total oxidised nitrogen. The test substance appeared to cause no detrimental effect to this important and sensitive environmental process.

Conclusion: In view of the similar removal between the reference substance, sodium benzoate, and the test substance it can be expected that a significant level of ultimate biodegradation/removal was obtained but no final conclusion on ultimate biodegradation can be made based on this study. It is stated in the OECD 303A Guideline that, if a high initial removal has taken place, the simulation test cannot differentiate between biological and abiotic elimination processes. In such cases, the ready biodegradation screening test is more appropriate to evaluate biological processes.

Furthermore, according to Annex II, section II.2.3.5 of the Guidance on the Application of the CLP Criteria (version 4.1, June 2015), results from tests simulating the conditions in a sewage treatment plant (STP) e.g. the OECD Test Guideline 303 cannot be used for assessing the degradation in the aquatic environment. The main reasons for this are that the microbial biomass in a STP is significantly different from the biomass in the environment, that there is a considerably different composition of substrates, and that the presence of rapidly mineralized organic matter in waste water may facilitate degradation of the test substance by co-metabolism.

The substance 2-phenylhexanenitrile is regarded as not readily biodegradable as a worst-case approach for further risk assessment and classification purposes.

11.1.4.4. Photochemical degradation

No data available.

11.2 ENVIRONMENTAL TRANSFORMATION OF METALS OR INORGANIC METAL COMPOUNDS

Not relevant for this dossier.

11.3 BIOACCUMULATION

11.3.1 Estimated bioaccumulation

No data available.

11.3.2 Measured partition coefficient and bioaccumulation test data

Measured partition coefficient

Title: Salicynalva (2-phenylhexanenitrile) Physio-chemical properties

Author: Dulichan, A

Year: 2006

Reliability: Klimisch 1 (reliable without restriction).

Method: A study was performed to determine the Partition Coefficient of 2-phenylhexanenitrile according to OECD Guideline 107 (Partition Coefficient (n-octanol / water), Shake Flask Method) and EU Method A.8 (Partition Coefficient). The purity of the test substance was > 95% (Lot No: 6162H).

Test 1: octanol stock solution (10 mL) and octanol saturated water (20 mL) were combined and shaken mechanically for 15 minutes. The test was performed in duplicate; Test 2: as for test 1 except that octanol stock solution (20 mL) was used; Test 3: as for test 1 except that octanol stock solution (40 mL) was used; Blank test: as for test 2 except that water saturated octanol was used in place of the octanol stock solution. This was carried out in duplicate. The analytical method used was High Performance Liquid Chromatography (HPLC).

Results: The mean value for the partition coefficient from all determinations was: P = 1383.5 and Log P = 3.14 at 21°C. Standard deviations of individual P values about their mean is 537.18. The range of Log P values obtained was 0.49 within the repeatability criterion of ± 0.3 log units required by the directive. The mass balance is calculated with recoveries in the range 100.0% - 102.2%

Conclusion: Based on the measured partition coefficient (n-octanol/water) (Log P = 3.14 at 21°C) 2-phenylhexanenitrile has a low potential for bioaccumulation.

Bioaccumulation test data

No data available.

11.4 ACUTE AQUATIC HAZARD

Table 11: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Key or Supportive study	Remarks	Reference

According to EEC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC (O.J. No. L383A, 29. 12.92) Part C, Method 1 "Acute toxicity for fish" and the OECD Guideline for Testing of Chemicals No. 203 "Fish, Acute Toxicity Test".	*Oncorhynchus mykiss*	2-phenylhexanenitrile; Purity: > 95%; Lot/batch No.: 6162H	96h-LC50: 2.2 mg/L (mean measured)	Key	Reliability: Klimisch 1 (reliable without restriction) .	Bell, G; Thirkettle, KM and Smith, B (1996a)
According to EEC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC (O.J. No. L383A, 29.12.92) Part C, Method 2 "Acute toxicity for Daphnia" and the OECD Guideline for Testing of Chemicals No. 202, Part I "Daphnia sp., Acute Immobilisation Test"	*Daphnia magna*	2-phenylhexanenitrile; Purity: > 95%; Lot/batch No.: 6162H	48h-EC50: 1.6 mg/L (mean measured)	Key	Reliability: Klimisch 1 (reliable without restriction) .	Bell, G; Thirkettle, KM and Smith, B (1996b)
According to EEC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC (O.J. No. L383A, 29.12.92) Part C, Method 3 "Algal inhibition test" and the OECD Guideline for Testing of Chemicals No. 201 "Alga, Growth Inhibition Test"	*Selenastrum capricornutum* (new name: *Pseudokirchneriella subcapitata*)	2-phenylhexanenitrile; Purity: > 95%; Lot/batch No.: 6162H	72h-ErC50: 2.58 mg/L (mean measured) 72h-NOErC: 0.26 mg/L (mean measured)	Key	Reliability: Klimisch 1 (reliable without restriction) .	Bell, G; Thirkettle, KM and Smith, B (1996c)

11.4.1 Acute (short-term) toxicity to fish

Title: Salicynalva (2-phenylhexanenitrile) acute toxicity for Rainbow Trout (*Oncorhynchus mykiss*)

Author: Bell, G; Thirkettle, KM and Smith, B

Year: 1996

Reliability: Klimisch 1 (reliable without restriction).

Method: A study was performed to assess the acute toxicity of 2-phenylhexanenitrile to rainbow trout (*Oncorhynchus mykiss*) under semi-static conditions. The study was conducted in accordance with EEC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC (O.J. No. L383A, 29. 12.92) Part C, Method 1 "Acute toxicity for fish" and the OECD Guideline for Testing of Chemicals No. 203 "Fish, Acute Toxicity Test". The test substance (purity > 95%) was dissolved in Tween 80 acetone (20: 80 v/v) to give an initial stock solution of 100 mg/mL. The test solutions were prepared by serial dilutions of this stock and the solvent control contained 100 µL solvent per liter. Groups of ten juvenile fish were exposed to a range of concentrations of 2-phenylhexanenitrile dissolved in water. The initial fish loading was 0.71 g bodyweight/litre (static volume). Observations were made on the numbers of dead fish and the incidence of sub-lethal effects after 3, 6, 24, 48, 72 and 96 hours exposure.

Results: Nominal test concentrations: 0.10, 0.22, 0.46, 1.0, 2.2, 4.6 and 10 mg 2-phenylhexanenitrile/L; mean measured test concentrations: 0.068, 0.15, 0.37, 0.79, 1.7, 3.7 and 8.6 mg 2-phenylhexanenitrile/L. Measured concentrations ranged from 76 to 102% of nominal in freshly prepared solutions and from 51 to 81 % of nominal in 24 hour old expired solutions. All results are expressed in terms of mean measured concentration. No mortality was observed at 0.79 mg 2-phenylhexanenitrile/L, while at 8.6 mg/L it was 100%. In both controls no mortality was observed and environmental parameters (pH, temperature and dissolved oxygen) remained within acceptable limits throughout the duration of the study.

Conclusion: The 96h-LC50 value for 2-phenylhexanenitrile with rainbow trout was 2.2 mg/L. (95% confidence limits: 1.5 – 3.2 mg/L)

11.4.2 Acute (short-term) toxicity to aquatic invertebrates

Title: Salicynalva (2-phenylhexanenitrile) acute toxicity to *Daphnia magna*

Author: Bell, G; Thirkettle, KM and Smith, B

Year: 1996

Reliability: Klimisch 1 (reliable without restriction).

Method: A study was performed to assess the acute toxicity of 2-phenylhexanenitrile to *Daphnia magna*. The study was conducted in accordance with EEC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC (O.J. No. L383A, 29.12.92) Part C, Method 2 "Acute toxicity for Daphnia" and the OECD Guideline for Testing of Chemicals No. 202, Part I "Daphnia sp., Acute Immobilisation Test". The test substance (purity > 95%) was dissolved in 20% Tween 80 : acetone (20:80 v/v) to give an initial stock solution of 100 mg/mL. The test solutions were prepared by serial dilutions of this stock and the solvent control contained 100 µL solvent per liter. Groups of twenty, first instar Daphnia (less than 24 hours old) were exposed for 48 hours to seven concentrations of 2-phenylhexanenitrile dissolved in Elendt M7 medium. The incidence of immobilisation was recorded for each test and control groups at 24 hours and at 48 hours.

Results: Nominal test concentrations: 0.156, 0.313, 0.625, 1.25, 2.5, 5.0 and 10 mg 2-phenylhexanenitrile/L; mean measured test concentrations: 0.193, 0.364, 0.695, 1.26, 2.5, 4.8 and 9.3 mg 2-phenylhexanenitrile/L. Measured concentrations ranged from 98 - 127 % of nominal at 0 hours and 86 - 121 % of nominal at 48 hours. All results are expressed in terms of mean measured concentration. At 48 hours no immobilization was observed at 0.695 mg 2-phenylhexanenitrile/L, while at 4.8 mg/L and higher it was 100%. In both controls no immobilization was observed and environmental parameters (pH, temperature and dissolved oxygen) remained within acceptable limits throughout the duration of the study.

Conclusion: The 48h-EC50 (immobilisation) value for 2-phenylhexanenitrile with *Daphnia magna* was 1.6 mg/L. (95% confidence limits: 1.3 – 1.9 mg/L)

11.4.3 Acute (short-term) toxicity to algae or other aquatic plants

Title: Salicynalva (2-phenylhexanenitrile) algal growth inhibition

Author: Bell, G; Thirkettle, KM and Smith, B

Year: 1996

Reliability: Klimisch 1 (reliable without restriction).

Method: A study was performed to assess the inhibitory effect of 2-phenylhexanenitrile on the growth of the unicellular green alga *Selenastrum capricornutum* (new name: *Pseudokirchneriella subcapitata*) according to EEC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC (O.J. No. L383A, 29.12.92) Part C, Method 3 "Algal inhibition test" and the OECD Guideline for Testing of Chemicals No. 201 "Alga, Growth Inhibition Test". The test substance (purity > 95%) was dissolved in the auxiliary solvent Tween : acetone (20:80 v/v) to give an initial stock solution of 100 mg/mL. The test solutions were prepared by serial dilutions of this stock and the solvent control contained 100 µL solvent per liter. Algal cultures exposed to six test concentrations of 2-phenylhexanenitrile plus one untreated control and one solvent control, were incubated on an orbital shaker under continuous illumination at 24 ± 1°C for 72 hours. Growth was monitored daily by measuring the number of cells in each culture.

Results: Nominal test concentrations: 0.22, 0.46, 1.0, 2.2, 4.6 and 10 mg 2-phenylhexanenitrile/L; mean measured test concentrations (geometric mean of the measured concentrations at 0 and 72 hours): 0.10, 0.26, 0.64, 1.8, 3.5 and 5.1 mg 2-phenylhexanenitrile/L. Values ranged from 82 - 101 % of nominal at 0 hours, apart from the lowest exposure level which was 57% of nominal, and 30 - 65 % of nominal at 72 hours. Test concentrations decreased in the course of this study which is attributed to adsorption both to the algae and to the glassware or volatilisation, since losses were observed in all treatments including that where no algae was present, but in these flasks the losses were only ca. 40 % of that observed in the flasks containing the algae. The following results were found based on mean measured concentrations: EbC50 (72 h): 0.81 mg/L; ErC50 (72 h): 2.58 mg/L and NOErC (72 h): 0.26 mg/L. All test and control cultures were inspected microscopically at 72 hours. At the 0.64 mg/L exposure level all cultures contained turgid cells, and at test concentrations of 3.5 and 5.1 mg/L all cultures showed evidence of cell debris with only a few small cells remaining intact.

In the control groups the biomass increased exponentially by a factor of at least 16 within the 72-hour test period.

Conclusion: The ErC50 (72 h) of 2-phenylhexanenitrile for algae is 2.58 mg/L (95% confidence limits: 2.44 – 2.74 mg/L) and the NOErC (72 h) is 0.26 mg/L.

11.4.4 Acute (short-term) toxicity to other aquatic organisms

No data available.

11.5 -LONG-TERM AQUATIC HAZARD

No long-term aquatic toxicity data are available for this substance, except the 72h NOErC value for algae (see section 11.4.3).

11.6 COMPARISON WITH THE CLP CRITERIA

According to CLP and the ECHA guidance on the application of the CLP criteria (2015), the algal growth rate reduction (ErC50 or NOErC), and not biomass (EbC50 or NOEbC), is the preferred endpoint because it is not dependent on the test design, whereas the endpoint biomass (growth) inhibition depends on the growth rate of the test species as well as test duration and other elements of the test design. Therefore, 2-phenylhexanenitrile should be classified based on the algal growth rate reduction endpoint.

Short-term toxicity tests for fish, aquatic invertebrates and algae are available for 2-phenylhexanenitrile. The LC50 and E(r)C50 values are between 1 and 10 mg/L. In addition, one reliable NOErC value of 0.26 mg/L for algae is available for long-term toxicity. Based on the available information, the substance is not rapidly degradable (not readily biodegradable and hydrolytically stable) and has a low potential for bioaccumulation (log Kow of 3.14).

Based on this information, 2-phenylhexanenitrile meets the criteria for classification as Aquatic Chronic cat. 2 (H411: Toxic to aquatic life with long lasting effects) according to Regulation (EC) 1272/2008 (CLP).

In table 11 and 12 the results of the available studies on aquatic toxicity and environmental fate are compared to the classification criteria of CLP regulation (1272/2008).

11.6.1 Acute aquatic hazard

Table 12: Comparison of the effect levels (L(E)C50) obtained in the short-term aquatic toxicity studies with the CLP criteria for acute aquatic hazard

Results	CLP criteria
▪ 96h-LC_{50} fish (*Oncorhynchus mykiss)*: 2.2 mg/L ▪ 48h-EC_{50} aquatic invertebrates (*Daphnia magna*): 1.6 mg/L ▪ 72h-ErC50 algae (*Pseudokirchneriella subcapitata)*: 2.58 mg/L	- Acute (short-term) aquatic hazard: Not classified since the lowest L(E)C_{50} is 1.6 mg/L (cut off value: ≤1 mg/L)

11.6.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Table 13: Comparison of the effect levels (L(E)C50 and NOEC) obtained in the short-term aquatic toxicity studies and the environmental fate data with the CLP criteria for long-term aquatic hazard

Results	CLP criteria
• Biodegradability: Not readily biodegradable (0 % degradation after 28 days) • Hydrolysis: Half-lives of 25.9 days at pH 4, 15.4 days at pH 7 and 4.7 days at pH 9 at 25°C • Bioaccumulation potential: log Kow of 3.14 • 96h-LC_{50} fish (*Oncorhynchus mykiss)*: 2.2 mg/L • 48h-EC_{50} aquatic invertebrates (*Daphnia magna*): 1.6 mg/L • 72h-ErC50 algae (*Pseudokirchneriella subcapitata)*: 2.58 mg/L • 72h-NOErC algae (*Pseudokirchneriella subcapitata)*: 0.26 mg/L	- Based on the available information, 2-phenylhexanenitrile is not readily biodegradable and is considered as hydrolytically stable under environmental conditions for classification purposes as the longest half-life is above 16 days at pH 4-9. Therefore, the substance is considered as not rapidly degradable and has a low potential for bioaccumulation (log Kow < 4) for classification purposes. - Reliable chronic toxicity data is available only for algae. According to Figure 4.1.1 of the CLP Regulation, if chronic toxicity data is not available for all trophic levels, the long-term aquatic hazard should be assessed according to criteria given in both Table 4.1.0 (b) (i) or (ii) and in Table 4.1.0 (b) (iii), and then the substance should be classified according to the most stringent outcome: Based on the 72h-NOErC of 0.26 mg/L and the substance being not rapidly degradable, 2-phenylhexanenitrile is classified as Aquatic Chronic 2 (cut off value: >0.1 to ≤ 1 mg/L; Table 4.1.0(b)(i) of the CLP Regulation). Based on the 96h-LC50 fish (2.2. mg/L), the 48h-EC50 aquatic invertebrates (1.6 mg/L) and the 72h-ErC50 algae (2.58 mg/L) being within the cut off values: >1 to ≤ 10 mg/L, and the substance is not rapidly degradable, 2-phenylhexanenitrile is classified as Aquatic Chronic 2 (Table 4.1.0(b)(iii) of the CLP Regulation) - Long-term aquatic hazard: Aquatic Chronic 2 H411.

11.7 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

2-phenylhexanenitrile meets the criteria for classification as Aquatic Chronic 2, H411, according to Regulation (EC) 1272/2008/EC (CLP).

12. EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

No data available.

13. ADDITIONAL LABELLING

Not relevant for this dossier.

14. REFERENCES

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