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**ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

**REPORT OF THE PILOT EXERCISE ON CLASSIFICATIONS FOR SELECTED CHEMICALS
ASSESSED AT COCAM**

ANNEX 3: COLLATED CLASSIFICATION PROPOSALS FOR COCAM 4 AND COCAM 5

**Series on Testing and Assessment
No. 210**

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OECD Environment, Health and Safety Publications

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IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among **FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD**

Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris 2014

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or contact:

**OECD Environment Directorate,
Environment, Health and Safety Division
2 rue André-Pascal
75775 Paris Cedex 16
France**

Fax: (33-1) 44 30 61 80

E-mail: ehscont@oecd.org

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This annex lists all of the submissions made by participating member countries and BIAC to the pilot exercise to suggest classifications according to the Globally Harmonised System (GHS) for four chemicals assessed in the OECD Cooperative Chemicals Assessment Programme (CoCAP). Individual submissions, given here as they were made, are ordered firstly by phase (phase 1 = CoCAM 4; phase 2 = CoCAM 5) and, within each phase, by chemical, in the same order as the chemicals and submitting countries are covered in the report, as shown in the table of contents below.

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CoCAM 4 – Phase One of the Exercise***Classifications for 2,4-Dimethylaniline (CAS. 95-68-1)****Notified classification for 2,4-Dimethylaniline (CAS. 95-68-1)*

Notificati ons	Asp. Tox.	Acute Tox.	Skin Irrit.	Eye Irrit.	Skin sentic.	STOT SE	STOT RE	Muta.	Carc.	Repro.	Aqua. Acute	Aquatic Chronic	of noti-
ECHACHEM 1		Acute Tox. 4 (o)(d) Acute tox. 2 (i)		eye Irrit. 2			STOT RE 1 STOT RE 2					Aqua. Chro. 2	3 0
ECHACHEM 2		Acute tox. 3 (o)(d)(i)					STOT RE 2					Aqua. Chro. 2	2 8
ECHACHEM 3		Acute tox. 3 (o)(d)(i)					STOT RE 2					Aqua. Chro. 2	2 4
ECHACHEM 4		Acute tox. 3 (o)(d)(i)					STOT RE 2					Aqua. Chro. 2	4
ECHACHEM 5 (n)		Acute tox. 3 (o)(d)(i)					STOT RE 2					Aqua. Chro. 2	2
ECHACHEM 6 (n)		Acute tox. 3 (o)(d)(i)					STOT RE 2					Aqua. Chro. 2	2
ECHACHEM 7		Acute tox. 4 (o) Acute tox. 3 (d)(i)	Skin Irrit. 2	eye Irrit. 2			STOT RE 2	Muta. 2				Aqua. Chro. 2	1
ECHACHEM 8		Acute tox. 3 (o)(d)(i)					STOT RE 2					Aqua. Chro. 2	1
ECHACHEM 9 (n)		Acute tox. 3 (o)(d)(i)					STOT RE 2					Aqua. Chro. 2	1
GHS-J		Acute tox. 4 (o)(i)		eye Irrit. 2		STOT SE 1 (b)	STOT RE 1 (b)				Aqua. Acute 2	Aqua. Chro. 2	
HSNO CCID		6.1E (o)	6.5B								9.1B (fish)	9.1B (fish)	

Note:

(i): inhalation (o): oral (d): dermal (b): blood system

RIVM-NL classification proposal for 2,4-Dimethylaniline (CAS. 95-68-1)

	Asp. Tox.	Acute Tox.	Skin Irrit.	Eye Irrit.	Skin sentiz.	STOT SE	STOT RE	Muta.	Carc.	Repro.	Aqua. Acute	Aquatic Chronic	of noti-
Proposed draft classification - RIVM	NCP	Acute Tox 3 (o) NCP other routes	NCP	NCP	NCP	NCP	STOT RE 1	Muta 2	NCP	NCP	Aquatic Acute 2	Aquatic Chronic 1*	

Note:

(i): inhalation (o): oral (d): dermal (b): blood system

NCP: No classification possible, see further remarks in the justification on the reason why no classification could be made

*: based on read-across. Aquatic Chronic 2 if based on available data on the substance self.

Key Studies and Justification for proposed draft classification

Acute toxicity

Key information: In an experiment for the single oral dose toxicity of dimethylanilines in rats and mice, the following LD₅₀ values (in mg/kg bw) were determined: 2,4-dimethylaniline: rats 470, mice 250. No reliable information is available for the dermal route, and no information is available for the inhalation route regarding acute toxicity

Conclusion: Acute Tox 3 (oral) based on LD50 of 250 mg/kg in mice. Although the rat is the preferred species, the mouse data are valid and give rise to more conservative classification. Classification not possible for the dermal and inhalation routes due to lack of data

Corrosion/irriation skin

Key information: 2,4-Dimethylaniline has a weak irritant effect on the skin of rabbits.

Conclusion: Classification is not possible due to lack of details on scoring, observation time, number of rabbits etc.

Corrosion/irriation eye

Key information: 2,4-Dimethylaniline has an irritant effects on the eyes of rabbits.

Conclusion: Classification is not possible due to lack of details on scoring, observation time, number of rabbits etc.

Sensitisation (skin & respiratory)

Key information: No information was available concerning skin sensitisation in animals for any of the dimethylaniline isomers.

Conclusion: Classification not possible due to lack of data

STOT-SE

Key information: None. No information on the acute studies or the first treatments in the repeated dose studies.

Conclusion: No classification possible due to lack of information/details

STOT-RE

Key information: The most general target for dimethylanilines was the blood. Increased methemoglobin resulted in hemolysis, reduction of hemoglobin and erythrocyte concentration and cyanosis at sufficiently high doses of ≥ 50 mg/kg bw/day for 2,6-, 2,3-, 2,5-, 3,4- and 3,5-dimethylanilines while hematological changes were observed at 10 mg/kg bw/day for 2,4-dimethylaniline (OECD TG 422 and 407). Hemosiderin deposition in the liver, kidney and spleen were similarly observed as a secondary effect to the loss of functional erythrocytes. Extramedullary haematopoiesis, increased size of erythrocytes and swelling of the spleen occurred as compensatory actions. Additionally, the hemolysis led to changes in other blood parameters like WBC count, or increase of reticulocytes (OECD TG 422 and 407). Also, high urine volumes along with a decrease of the specific density, reduced pH and reduction of urinary levels of protein and ketone bodies generally occurred in treated with any category substances, generally at high doses (TG 422 and 407).

Effects on the kidney such as papillary necrosis, dilatation of renal tubules, and/or hyaline droplets were observed at 10 mg/kg bw/day in animals treated with 2,4-dimethylaniline. 2,4-Dimethylaniline also showed stronger renal effects comparing to 2,6-dimethylaniline dosing (6-month feeding study).

There were increased relative and/or absolute weights of the liver (enlarged liver) or hypertrophy at 10 mg/kg bw/day in animal treated with 2,4- dimethylaniline.

Conclusion: It is difficult to conclude on the relevant classification for STOT-RE, since no details are available on the quantity or severity of the effects, and, for most effects, at which dose and which duration they are observed. However, based on the effects on kidney (papillary necrosis, dilatation of renal tubules, high urine volume, reduced pH, protein and ketone bodies), but, depending on the severity probably also based on the effects on blood (increased methemoglobin, hemolysis, decreased Hb and erythrocyte conc and cyanosis), and liver (absolute weight, hypertrophy) in rats at 10 mg/kg in a 28 day study following OECD TG 407 classification as STOT RE 1 would be justified (guidance value STOT-RE1 for 28 day study is <30 mg/kg bw/day).

Mutagenicity

Key information: In bacterial reverse mutation assays with multiple strain of *S. typhimurium* and *E. coli* (OECD 471), 2,4-dimethylaniline were found to be mutagenic in TA100. One bacterial reverse mutation study showed positive results on 2,4-dimethylanilines with rat or hamster S9 mix. In *in vitro* chromosome aberration studies (TG 473), 2,4-dimethylaniline showed chromosomal aberrations. In *in vivo* gene mutation assays with MutaTM mice (nasal tissue, bone marrow and liver), 2,5- and 2,6-dimethylanilines increased mutation frequency of lacZ and cII genes in the nasal tissue, and 2,5-dimethylaniline also increased mutation frequency of lacZ gene in the bone marrow. On the other hand, 3,5-dimethylaniline showed a negative result in the *in vivo* gene mutation assay. In *in vivo* micronucleus assays, all six isomers of dimethylanilines was non-clastogenic in the bone marrow and 2,5-, 2,6- or 3,5-dimethylaniline in the peripheral blood in mice. 2,6-Dimethylaniline did not affect DNA repair in a DNA repair host-mediated assay or an unscheduled DNA synthesis assay *in vivo*. In SCG

(Comet) assays *in vivo*, all six isomers of dimethylanilines induced DNA damage in the bone marrow (only for 3,4- and 3,5-dimethylanilines), lung, kidney or liver in mice.

Conclusion: Muta 2 based on positive comet assay *in vivo*, positive bacterial reverse mutation assay and positive *in vitro* chromosomal aberration study for 2,4-dimethylaniline although micronucleus assay was negative. Supporting data comes from structural analogs 2,5- and 2,6-dimethylaniline which are positive in *in vivo* gene mutation assay with MutaTM mice.

Carcinogenicity

Key information: A carcinogenicity study demonstrated that 2,4-dimethylaniline induced pulmonary tumors in female mice. In addition, 2,4-dimethylaniline is mutagenic.

Conclusion: Classification not possible due to lack of detail.

It is possible or even likely that 2,4-dimethylaniline is carcinogenic based on pulmonary tumors in female mice. However, all information on tumor incidence, rate, historical controls, mechanism of action etc that would substantiate this statement and allow assignment in a carcinogenicity sub-category is missing. Supporting evidence for carcinogenicity comes from the analogs 2,6-dimethylaniline where increased tumor incidence in nasal adenocarcinomas and carcinomas, papillary adenomas and rhabdomyosarcomas, subcutaneous fibromas and fibrosarcomas were observed after treatment, and 2,5-dimethylaniline which lead to increased subcutaneous fibromas and fibrosarcomas and vascular tumors after administration.

Reproductive toxicity

Key information: Information on reproductive toxicity in animals is only available for 2,6-dimethylaniline. No data for 2,4-dimethylaniline

Conclusion: No classification is possible due to lack of data.

Reproductive tox data are available for the structural analog 2,6-dimethylaniline (OECD 407) where reproductive toxicity effects were seen at doses with maternal toxicity. Although the metabolic profile is similar for those two isomers, 2,4-dimethylaniline exhibits systemic toxicity at doses lower than those for 2,6-dimethylaniline. It is not known how or whether this will affect the reproductive toxicity of 2,4-dimethylaniline.

Aspiration hazard

Key study: None available

Conclusion: No classification possible due to lack of data

Environment

Key information: All category members are not expected to undergo hydrolysis in the environment due to the lack of hydrolysable functional groups. Inherent biodegradation test [OECD TG 302C] for 2,4-dimethylaniline showed 0% biodegradation after two weeks. Ready biodegradation tests [OECD TG 301C] with the analogs 2,3-dimethylaniline, 2,5-dimethylaniline, 2,6-dimethylaniline and 3,5-dimethylaniline resulted in 3, 1, 0 and 3 % biodegradation after 28 days, respectively => Not rapidly degradable.

2,4-Dimethylaniline is not expected to bioaccumulate in the aquatic environment based on a measured bioconcentration factor of <10. These results show a low potential for bioaccumulation of dimethylanilines for aquatic organisms. => Not bioaccumulative

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Acute fish: No reliable studies were identified. The predicted 96-hour LC₅₀, based on read across from 3,5-dimethylaniline = 33.9 mg/L. The predicted 96-hour LC₅₀, based on ECOSAR (v 1.11) = 37.2 mg/L

Acute invertebrate [*Daphnia magna*]: 48 h EC₅₀ = 9.9 mg/L [DIN38412, static]

Acute algae: No reliable studies were identified. The predicted 72-hour EC₅₀, based on read across from 3,4-dimethylaniline = 8.59 mg/L; The predicted 96-hour EC₅₀, based on ECOSAR (v 1.11) = 37.0mg/L

Chronic fish: No studies available and not read across candidates available.

Chronic invertebrate: No reliable studies were identified. The predicted 21 d NOEC, based on read across from 3,4-dimethylaniline = 0.0095 mg/L

Chronic algae: No reliable studies were identified. The predicted 72 h NOEC, based on read across from 2,5-dimethylaniline = 2.03 mg/L

Conclusion aquatic acute: Aquatic Acute 2 based on lowest L(E)C₅₀ of 9.9 mg/l in invertebrates.

Conclusion aquatic chronic: Based on the data for the substance self, the classification is Aquatic Chronic 2 based on surrogate chronic toxicity of 9.9 mg/l in invertebrates in combination with not rapid degradability due to the absence of aquatic chronic toxicity data. Using available chronic aquatic toxicity data for the available analogs, the classification should be Aquatic Chronic 1 based on not rapid degradability and a NOEC in invertebrates of 0.0095, 0.096, 0.03 and 0.1 mg/L in invertebrates using read-across from 3,4-, 2,5-, 3,5-, and 2,3-dimethylaniline although 2,6-dimethylaniline had a NOEC in invertebrates of 2.23 mg/L.

Detailed justification for the suitability of the read-across is missing and the justification given in the beginning of the SIAP is poor which lowers the confidence in the read-across results for chronic aquatic toxicity.

CH classification proposal for 2,4-Dimethylaniline (CAS 95-68-1)

General Comment

It would be helpful to clearly indicate which is the key study and which are supporting studies and to find a table with the data that is important for classification at the end of the human health part of the SIAP.

Acute Toxicity

Oral

Cat 3

based on LD₅₀ value in mice 250 mg/kg bw

Classification based on the information in the SIAP is possible.

Inhalation

Not classified

No valid data available which allows classification.

Irritation

Skin

Not classified

Classification is not possible since relevant data (Scoring points) is missing. This data is also not found in the SIAR and the IUCLID file.

Eye

Not classified

Classification is not possible since relevant data (Scoring points) is missing. This data is also not found in the SIAR and the IUCLID file.

Sensitization

Not classified

No data available for classification

Germ cell mutagenicity

Cat 2

based on the mutagenicity *in vitro* (OECD 471, 473) and *in vivo* (SCG (Comet) Assay

Carcinogenicity

Cat 2

based on the induction of pulmonary tumors in female mice and the *in vivo* genotoxic activity

Reproductive toxicity

Not classified

Data is conclusive. However, it is not sufficient for classification.

STOT SE

Not classified

No data available and therefore classification is not possible.

STOT RE

Not classified

Although adverse effects were observed at low concentrations (NOAEL of 2 mg/kg bw/day due to effects on the blood, liver and kidneys), details on the severity of these effects for classification into Cat. 1 are missing. Further investigations are needed to determine the relevance of the observed adverse effects.

Therefore, the data is not sufficient for classification into Cat.1.

Environmental Hazards

Hazard class		Classification	hazard statement	Rational for the classification
11	Hazardous to the aquatic environment (acute)	Category 2	H401 Toxic to aquatic life	Classification is based primarily on acute toxicity to the most sensitive species <i>Daphnia magna</i> (48h-EC50 9.9 mg/L).
11	Hazardous to the aquatic environment (chronic)	Category 2	H411 Toxic to aquatic life with long lasting effects	Classification is based on acute toxicity data for <i>Daphnia magna</i> (see above) and the fact, that the substance is not readily biodegradable. The conclusion on the absence of rapid biodegradation is based on read-across of ready-biodegradation-tests results for four structural analoges 2,3-dimethylaniline, 2,5-dimethylaniline, 2,6-dimethylaniline and 3,5-dimethylaniline.

Key studies used for classification:**Water solubility**

Calculation by WSKOW (version 1.41a) with melting point of -14.3 °C and log Kow of 1.68

Result (calculated): 6.1 g/L at 25 °C

Reference: OECD SIDS dossier for the Dimethylaniline Category, submitted by Japan for CoCAM-3

Partition coefficient octanol-water

OECD Guideline 107 (Partition Coefficient (n-octanol / water), Shake Flask Method)

Result: log Kow 2.02 at 23°C, pH=8

Reference: ECHA, REACH registration dossier for CAS 2,4 xylydine, robust study summary based on study report (Exp Key Partition coefficient.001, report date: 2008-03-13); http://apps.echa.europa.eu/registered/data/dossiers/DISS-9d86c937-6ea4-4d7f-e044-00144f67d249/AGGR-0a0f02a1-e7cd-4788-8e70-b1796e6e6bd0_DISS-9d86c937-6ea4-4d7f-e044-00144f67d249.html#AGGR-0a0f02a1-e7cd-4788-8e70-b1796e6e6bd0

Biodegradation

Similar to OECD TG 302 B (Inherent biodegradability: Zahn-Wellens/EMPA Test); test duration: 16d; deviations from standard test conditons: only municipal wastewater sludge is used and the concentrations of the test substances and sludge were lower; reliability: 2 (valid with restrictions)

Result (measured): DOC removal: 14% (3h), 21% (3d), 74% (14d)

Conclusion: 2,4-Dimethylaniline is inherently biodegradable

Reference: ECHA, REACH registration dossier for CAS 2,4 xylidine, robust study summary based on study report (Exp Key Biodegradation in water: screening tests.001, report date: 1981-08-12); http://apps.echa.europa.eu/registered/data/dossiers/DISS-9d86c937-6ea4-4d7f-e044-00144f67d249/AGGR-5e6726ce-44c9-4c54-8bf6-1ab812d5a6a9_DISS-9d86c937-6ea4-4d7f-e044-00144f67d249.html#AGGR-5e6726ce-44c9-4c54-8bf6-1ab812d5a6a9

Remark: No ready-biodegradation test is available for 2,4-Dimethylaniline. However, ready biodegradation tests [OECD TG 301C] for 2,3-dimethylaniline, 2,5-dimethylaniline, 2,6-dimethylaniline and 3,5-dimethylaniline resulted in 3, 1, 0 and 3 % biodegradation after 28 days, respectively. Inherent biodegradation tests [OECD TG 302C] for 2,4-dimethylaniline and 3,4-dimethylaniline resulted in 0 and 7 % biodegradation after two weeks, respectively.

Bioaccumulation

Test species: *Cyprinus carpio*

OECD TG 305 (Bioconcentration: Flow-through Fish Test); test (uptake) duration: 6 weeks; test concentrations: 0.1 mg/L and 1.0 mg/L (nominal), 0.0874-0.0958 and 0.859-0.909 (measured, 1d-6wk); reliability: 2 (valid with restrictions)

Results: BCF 4.1-4.3 (0.89 mg/L), BCF <10 (0.092 mg/L)

Conclusion: BCF <10 (low potential for bioconcentration)

Reference: Chemicals Evaluation and Research Institute, Japan (1977): Study of bio-concentration of 2,4-dimethylaniline.

Acute (short-term) aquatic toxicity

Fish

Test substance: CAS 108-69-0 3,5-Dimethylaniline

Test species: *Oryzias latipes*

OECD TG 203, performed under GLP, exposure: 96h, semi-static conditions, concentration: ; reliability: 1 (reliable without restriction)

Result: 96h-LC50 33.9 mg/L (measured)

Reference: EA (Environmental Agency), Japan (1998b) Final report of Fish (*Oryzias latipes*), Prolonged Toxicity Test: 14-Day Study of 3,5-Dimethylaniline, Study No. 7B804G, conducted by Mitsubishi Chemical Safety Institute Ltd.

Remark: This study was used for read-across for acute toxicity for fish because it is the lowest measured data for a structurally close analogue.

Invertebrates

Test species: *Daphnia magna*

DIN 38412, Part II (Daphnia short-time test), exposure: 48h, static conditions; concentration: The concentration steps of the test solution were selected so as to give 3-4 EC values in a range between EC0 and EC100, of which at least one value was below and one above EC50.; reliability: 2 (valid with restrictions)

Result: 48h-EC50 9.9 mg/L (95%CL: 5.6-17 mg/L)

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Reference: Kuhn, R., M. Pattard, K.D. Pernak, and A. Winter (1989): Results of the Harmful Effects of Selected Water Pollutants (Anilines, Phenols, Aliphatic Compounds) to *Daphnia magna*. *Water Res.* 23(4):495-499.

Algae

Test species: *Desmodesmus subspicatus*

OECD TG 201 (Alga, Growth Inhibition Test), performed under GLP, exposure: 72h, static conditions, concentrations: 6.25, 12.5, 25, 50, 100 mg/L; reliability: 1 (reliable without restriction)

Results: 72h-ErC50 28.6 (27.5 – 29.9) mg/L (growth rate, nominal); 72h-EyC50 15.5 (14.4 – 16.8) (yield, nominal)

Reference: ECHA, REACH registration dossier for CAS 2,4 xylidine, robust study summary based on study report (report date: 2010-07-04); http://apps.echa.europa.eu/registered/data/dossiers/DISS-9d86c937-6ea4-4d7f-e044-00144f67d249/AGGR-fa6d9cb4-9fbd-4011-8a1f-75bd60576a15_DISS-9d86c937-6ea4-4d7f-e044-00144f67d249.html#AGGR-fa6d9cb4-9fbd-4011-8a1f-75bd60576a15

Chronic (long-term) aquatic toxicity

No reliable data is available for 2,4-Dimethylaniline

IT classification proposal for 2,4-Dimethylaniline (CAS 95-68-1)

	Asp. Tox.	Acute Tox.	Skin Irrit.	Eye Irrit.	Skin sensitiz.	STOT SE	STOT RE	Muta.	Carc.	Repro.	Aqua. Acute	Aquatic Chronic
Proposed draft classification ITALY	No Data	Acute Tox 3, H301; Acute Tox 4, H332	No Classified	Eye Irrit 2B, H320	No data	INCONCLUSIVE	STOT RE 2, H373	Muta. 2, H314	INCONCLUSIVE	No data	Acute Aquatic 2, H401	Chronic Aquatic 1, H410

	<u>Mammalian toxicity endpoints</u>					<u>Rationale/Comments</u>
	Acute toxicity Oral					
	Organism	Methods	Data	Reference	GHS classification	
CAS: 95-68-1 Chemical name: 2,4-Dimethylaniline	CF-1 mouse Male Sprague-Dawley rat Male	Method: T03-03:Single oral dose toxicity was determined in which the LD50 and its 95 % confidence limits were estimated by the moving average	LD ₅₀ = 250 mg/kg/bw LD ₅₀ = 470 mg/kg bw	Vernot et al. (1977) in the SIAR	Acute Tox 3, H301	Mouse LD ₅₀ = 250 mg/kg/bw was chosen as it is lower than rat LD50 even if rat is the preferred species for oral acute toxicity tests. According to the rat value the resulting classification should have been Acute Tox 4, H302

	technique.				
Acute toxicity Inhalation					
Organism	Methods	Data	Reference	GHS classification	
Wistar rat male/female	No specified	LC ₅₀ = 1.53 mg/l (approx. 306 ppm) 4 hour	HRC 1988a (not published) in BUA161 report 1994 (IUCLID Data Set)	Acute Tox 4, H332	This data not included in SIDS Initial Assessment Profile and SIAR. Derived from IUCLID
Acute toxicity Dermal					
Organism	Methods	Data	Reference	GHS classification	
rabbit	No data	LD50 = 3,300 mg/kg (DFGOT vol.19 (1998)).	GHS Classification Guidance for the Japanese Government (Sep 5, 2008 version)	Acute Tox 5, H313	This data not included in SIDS Initial Assessment Profile, SIAR and IUCLID. As for CLP also the Japanese system doesn't take the category 5.
Corrosive/irritant skin					
Organism	Methods	Data	Reference	GHS classification	
New Zealand White rabbit	OECD Guideline 404 (Acute Dermal Irritation / Corrosion)	White New Zealand rabbits caused slight erythema in 2/3 animals 24 hours after removing the patch following semi-occlusive application of undiluted chemicals for 4 hours, but effects were reversible.	Hofmann and Weigand 1986 in the SIAR	Not Classified	

		Not irritant			
Corrosive/irritant eyes					
Organism	Methods	Data	Reference	GHS classification	
white New Zealand rabbits	OECD Guideline 405 (Acute Eye Irritation / Corrosion)	In two test of eye irritation white New Zealand rabbits caused swelling and redness of the conjunctiva, iritis and clouding of the cornea after instillation All irritation symptoms were reversible 7 days after application	Hofmann and Weigand 1986	Eye Irrit 2B, H320	The BUA report says that : “ according to guideline 83/467/EEC the product was classified as an irritant” As scores are not reported we couldn't check the adequacy of the resulting classification as Eye Irrit 2B .
Sensitizer Skin and respiratory tract					
Organism	Methods	Data	Reference	GHS classification	
No data	No data	No data	No data	No data	
Gene mutation					
Organism	Methods	Data	Reference	GHS classification	
TA100 <i>S. typhimurium</i> OECD TG 471	<i>S. typhimurium</i> OECD TG 471	Positive in with S9 mix	NITE (2002)	Muta. 2, H314	
Chinese Hamster Lung cells (CHL/IU) with and without rat liver S9	Chromosome aberration test OECD TG 473	Clastogenic, with S9 mix	NITE (2002)		

	Comet assay	Mouse (ddY) Males	Positive (lung, kidney, liver)	Hayashi et al., 2000: unpublished data		
	Single Cell Gel Electrophoresis (SCG) (Comet) Assay	Mouse (B6C3F1) Males	Positive (liver)	Przybojewska (1999)		
In vivo negative, but a possibility that this isomer shows in vivo genotoxic potential cannot be ruled out because it induced DNA damage in various tissues <i>in vivo</i> (SIAR)						
Carcinogenicity						
	Organism	Methods	Data	Reference	GHS classification	
	Sprague- Dawley rat male	No specified	After 2 years, a total of 39% of the rats developed subcutaneous fibromas and fibrosarcomas and hepatomas.	Russfield et al., 1973, cited in BUA 161	Inconclusive	<ul style="list-style-type: none"> • Adopting the read across with 2,6 DMA for which the EU harmonized classification is available (carc cat 2), Carc. 2, H351 could be applied to all the members of the category. • classified into the Category 2 (DFGOT vol.19 (1998)) in Germany DFG. • group 3 ("Not classified")according to IARC Suppl.7 (1987
Reproductive/Developmental toxicity						
	Organism	Methods	Data	Reference	GHS classification	
	No data	No data	No data	No data	No data	
Single dose toxicity						
	Organism	Methods	Data	Reference	Classificazione GHS	
	No data	No data	For animals, there is a description of "Hyperpnea was seen." in 4 hours inhalation exposure test employing rats	GHS Classification Guidance for the Japanese Government (Sep 5, 2008	INCONCLUSIVE	The Japanese database is based on the BUA report and DFGOT vol.19 . We went back to BUA report and DFGOT where in our opinion data don't justify the classification as STOT SE 1 (methemoglobinemia is questionable)

			<p>(DFGOT vol.19 (1998)), and there is a description of "Methemoglobin was produced about 1 hour and a half after administration" in the oral administration test where the lethal dose in rats were calculated (DFGOT vol.19 (1998)). All these symptoms were seen within the range of the guidance value of Category 1., caused methemoglobinemia on humans." is described in DFGOT vol.19 (1998). As mentioned above, it was classified into Category 1 (blood systems). In addition, in ICSC (2007), "The high concentration exposure may cause lowering of consciousness. The high concentration exposure may produce methemoglobin.</p>	<p>version)</p>		
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			These effects may occur backwardly. Although there is a description of "Medical follow up is needed, the details of doses are unknown.			
Repeated dose toxicity						
Organism	Methods	Data	Reference	GHS classification		
Crj: CD(SD) rats	OECD 407	50 mg/kg bw/day in females Effects: decreased hemoglobin	NITE, 2002	STOT RE 2, H373		
Osborne-Mendel rats	No specified	<i>Supporting study</i> 375 ppm (18.75 mg/kg bw/day) oral (6 moths) Effects on the kidneys and liver	Lindstrom et al, 1963			
Aspiration Toxicity						
Organism	Methods	Data	Reference	GHS classification		
No data	No data	No data	No data	No data		
Environmental toxicity endpoints						
Acute toxicity						
Organism	Methods	Data	Reference	GHS classification		
Fish	RA con 3,5 DMA	EC50 [mg/L]96h= 33,9	RA			
	ECOSAR	EC50 [mg/L]96h= 37,2	ECOSAR			
Daphnia	DIN38412	EC50 [mg/L]= 9,9	Kühn et al.,	Acute Aquatic 2,	The classification is based on the lowest	

	Static		1989	H401	experimental value . In the notified classification acute 2 is not present as it is not taken by CLP	
Alghe	RA con 3,4 DMA	EC50 [mg/L]= 8,59	RA			
	ECOSAR	EC50 [mg/L] = 37	ECOSAR			
Chronic toxicity						
Organism	Methods	Data	Reference	GHS classification		
Fish	No data	No data	No data	No data		
Daphnia	RA con 3,4 DMA	NOEC [mg/L] = 0,0095	RA	Chronic Aquatic 1, H410	M= 10	According to GHS
Alghe	RA con 3,4 DMA	NOEC [mg/L] = 2	RA	<i>(Chronic Aquatic 2, H411)</i>		The CLP classifications notified to the ECHA Inventory were made before the adoption of the II ATP to CLP, so that they are based on LC50 and not on NOEC.

Hazard class	Substance data / Note	Criteria of Category according to GHS	Conclusion/ Commentary
Explosives	No data available	There are no chemical groups associated with explosive properties present in the molecule	Not classified
Flammable gases	Substance is a liquid		Not classified
Aerosols	Substance is a liquid		Not classified
Oxidizing gases	Substance is a liquid		Not classified
Gases under pressure	Substance is a liquid		Not classified
Flammable liquids	Boiling point: 214°C Flash point: no data available		Not classified
Flammable solids	Substance is a liquid		Not classified
Self-reactive substances and mixtures	No data available	There are no chemical groups present in the molecule associated with explosive or self-reactive properties	Not classified
Pyrophoric liquids	No data available	Substance is known to be stable at room temperature for prolonged periods of time (days)	Not classified
Pyrophoric solids	Substance is a liquid		Not classified
Self-heating substances and mixtures	No data available		Not classified
Substances and mixtures which, in contact with water, emit flammable gases	Soluble (6069 mg/l); the chemical structure of the substance does not contain metals or metalloids	The chemical structure of the substance does not contain metals or metalloids	Not classified.
Oxidizing liquids	Study scientifically unjustified as the substance is incapable of reacting exothermically with combustible materials on the basis of its chemical structure	The substance does not contain oxygen, fluorine or chlorine	Not classified
Oxidizing solids	Substance is a liquid		Not classified
Organic peroxides	Substance is not organic peroxides		Not classified

Corrosive to metals	No data available		Not classified
Acute toxicity / oral	The acute oral LD50 in male rats was 470 (360 - 690) mg/kg; in male mice it was 250 (150 - 420) mg/kg. / Data from IUCLID, the reliability of the study: 4 (not assignable)		Not classified <i>The reliability of the study: 4 (not assignable)</i>
Acute toxicity / dermal	No data available		Not classified
Acute toxicity / inhalation	The 4-hour LC50 in the rat was calculated to be 1.53 mg/l (approx. 306 ppm) / Data from IUCLID, the reliability of the study: 4 (not assignable)		Not classified <i>The reliability of the study: 4 (not assignable)</i>
Skin corrosion/irritation	Weak irritant to skin		Not classified. <i>Classification is not possible due to lack of details on study</i>
Serious eye damage/ eye irritation	Irritant effects on the eyes of rabbits		Not classified. <i>Reliability of the study: 4 (not assignable)</i>
Respiratory or skin sensitization	No data available		Not classified
Germ cell mutagenicity	Positive result (mouse, <i>in vivo</i> , <i>in vitro</i>)	Positive evidence obtained from <i>in vivo</i> somatic cell genotoxicity tests which are supported by positive results from <i>in vitro</i> mutagenicity assays	Category 2
Carcinogenicity	Substance induced pulmonary tumours in female mice. It can be predicted that all members of the category may be carcinogenic due to their <i>in vivo</i> genotoxic activity	The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1	Category 2
Reproductive toxicity	No data available		Not classified
Specific target organ toxicity – Single exposure	No data available		Not classified
Specific target organ toxicity	NOAEL (rat, oral, 28d) = 2 mg/kg	Category 1: oral, rat, 28d	Category 1

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- Repeated exposure	bw/day Substance can have an adverse effect on the blood, liver and kidneys	$C \leq 30 \text{ mg/kg bw/d}$	
Aspiration hazard	No data available		Not classified
Hazardous to the aquatic environment/ acute hazard	Fish: No reliable studies were identified. The predicted 96-hour LC_{50} , based on read across from 3,5-dimethylaniline = 33.9 mg/L; The predicted 96-hour LC_{50} , based on ECOSAR (v 1.11) = 37.2 mg/L Invertebrate [Daphnia magna]: 48 h EC_{50} = 9.9 mg/L [DIN38412, static] Algae: No reliable studies were identified. The predicted 72-hour EC_{50} , based on read across from 3,4-dimethylaniline = 8.59 mg/L; The predicted 96-hour EC_{50} , based on ECOSAR (v 1.11) = 37.0mg/L	Category Acute 2: 48 h EC_{50} (for crustacea) > 1 but $\leq 10 \text{ mg/l}$	Category Acute 2
Hazardous to the aquatic environment/ long-term hazard	Not readily degradable. BCF <10 Invertebrate: No reliable studies were identified. The predicted 21 d NOEC, based on read across from 3,4-dimethylaniline = 0.0095 mg/L Algae: No reliable studies were identified. The predicted 72 h NOEC, based on read across from 2,5-dimethylaniline = 2.03 mg/L	Category Chronic 2: $1.00 < L(E)C_{50} \leq 10.0$ and lack of rapid degradability and/or $BCF \geq 500$ or, if absent $\log K_{ow} \geq 4$	Category Chronic 2
Hazardous to the ozone layer	Substance not listed in Annexes to the Montreal Protocol	Substance not listed in Annexes to the Montreal Protocol	Not classified

BIAC (JCIA) classification proposal for 2,4-Dimethylaniline (CAS 95-68-1)

Proposed draft classification	Asp. Tox.	Acute Tox.	Skin Irrit.	Eye Irrit.	Skin sentiz.	STOT SE	STOT RE	Muta.	Carc.	Repro.	Aqua. Acute	Aquatic Chronic
	NC	Acute tox. 4 (o)(i)	NC	eye Irrit. 2	NC	STOT SE 1 (b)	STOT RE 1 (b)(l)	NC	NC	NC	Aqua. Acute 2	Aqua. Chro. 2
	Data lacking	oral: LD50 (rat) 470 mg/kg bw (DFG-MAK)	Data sufficient	based on previous conclusion (DFG-MAK); but scores not reported	No data	adverse effects observed at low concentrations (250 mg/kg bw: effects on blood)	28d 20mg/kg rats effects on kidney and blood	data not sufficient . +ve comet assay, +/-ve bacterial reverse mutation assay, +/-ve chromosomal aberration	data not sufficient: but possible/likely based on pulmonary tumors in female mice	No data	Daphnia magna 48 h EC50 = 9.9 mg/L	Daph acute (EC50 9.9 mg/L), read across not RB

Note:

(i): inhalation (o): oral (d): dermal (b): blood system (l): liver

NC = not classified

JP Classification for Environment: proposal for 2,4-Dimethylaniline (CAS 95-68-1)

	2,4-dimethylaniline
Aquatic Acute Classification	Aq Ac 2 Daphnia 48h EC50 = 9.9 mg/L
Aquatic Chronic Classification	Aq Ch 1(read-across) Not readily biodegradable Daphnia 21d NOEC 0.0095 mg/L (3,4-dimethylaniline)

Classifications for Nonane (CAS. 111-84-2)*Notified classifications for Nonane (CAS. 111-84-2)*

Notification s	Asp. Tox.	Acute Tox.	Skin Irrit.	Eye Irrit.	Skin sentiz .	STOT SE	STOT RE	Muta .	Carc .	Repro .	Aqua. Acute	Aqua. Chronic
ECHACHEM 1				Eye Irrit. 2								
ECHACHEM 2	Asp. Tox. 1		Skin Irrit. 2			STOT SE 3						Aqua. Chro. 1
ECHACHEM 3	Asp. Tox. 1											
ECHACHEM 4	Asp. Tox. 1	Acute Tox. 4 (i)	Skin Irrit. 2	Eye Irrit. 2		STOT SE 3						
ECHACHEM 5	Asp. Tox. 1	Acute Tox. 3 (i)										Aqua. Chro. 4
ECHACHEM 6	Asp. Tox. 1	Acute Tox. 4 (i)		Eye Irrit. 2		STOT SE 3						Aqua. Chro. 1
ECHACHEM 7	Asp. Tox. 1	Acute Tox. 4 (i)	Skin Irrit. 2	Eye Irrit. 2		STOT SE 3						
ECHACHEM 8	No data											
ECHACHEM 9	Asp. Tox. 1		Skin Irrit. 2			STOT SE 3					Aqua. Acute 1	Aqua. Chro. 1
ECHACHEM 10	Asp. Tox. 1	Acute Tox. 4 (i)	Skin Irrit. 2	Eye Irrit. 2		STOT SE 3						Aqua. Chro. 4
ECHACHEM 11	Asp. Tox. 1		Skin Irrit. 2			STOT SE 3*						Aqua. Chro. 1
ECHACHEM 12	Asp. Tox. 1	Acute Tox. 4 (i)	Skin Irrit. 2	Eye Irrit. 2		STOT SE 3						
ECHACHEM 13	Asp. Tox. 1	Acute Tox. 4 (i)		Eye Irrit. 2		STOT SE 3						
ECHACHEM 14	Asp. Tox. 1	Acute Tox. 4 (i)				STOT SE 3						Aqua. Chro. 4
ECHACHEM 15	Asp. Tox. 1		Skin Irrit. 2	Eye Irrit. 2		STOT SE 3*						
GHS-J	Asp. Tox. 1	Acute Tox. 4 (i)	Skin Irrit. 2	Eye irrit. 2A 2B		STOT SE 3*						

Notifications	Asp. Tox.	Acute Tox.	Skin Irrit.	Eye Irrit.	Skin sentiz.	STOT SE	STOT RE	Muta.	Carc.	Repro.	Aqua. Acute	Aqua. Chronic
HSNO CCID		6.1D (i) 6.1E (o)	6.3B	6.4								9.1A (fish, crustacean, algal)

Note:

(i): inhalation (o): oral (d): dermal

RIVM-NL classification proposal for Nonane (CAS. 111-84-2)

Notifications	Asp. Tox.	Acute Tox.	Skin Irrit.	Eye Irrit.	Skin sentiz.	STOT SE	STOT RE	Muta.	Carc.	Repro.	Aqua. Acute	Aqua. Chronic
Proposed draft classification - RIVM	NCP	NCN (i) NCN* (o, d)	NCP	NCP	NCP	STOT-SE 3	NCN*	NCN*	NCP	NCN*	Aquatic Acute 1 M-factor 1	Aquatic Chronic 1 M-factor 1

Note: (i): inhalation (o): oral (d):dermal

NCP: No classification possible, see further remarks in the justification on the reason why no classification could be made

NCN: No classification needed. Based on available information the substance does not need classification.

*: based on read-across

Background information on the substance

Nonane is characterized as a linear molecule (n-paraffin or normal paraffin) with a carbon number of C9 and is a category member of the C9-C14 Aliphatic [\leq 2% aromatic] hydrocarbon solvent category. Individual category member substances are comprised of aliphatic hydrocarbon molecules with carbon numbers between C₉ and C₁₄; approximately 80% of the aliphatic constituents for a given substance fall within the C₉-C₁₄ carbon range and <100 ppmV benzene. The distinguishing characteristics of this category are the limitation on carbon range (C9-C14) and the limitation of aromatic constituents to < 2% (in most cases the levels of aromatics are well below 2%) of the total hydrocarbons present.

Key studies and Justification for proposed draft conclusion**Acute toxicity**

Key information: Acute inhalation study conducted according to OECD TG 403 is available. The following LC50 value for nonane was determined 23775 mg/m³ (vapour). Acute dermal information is only available for structural analogues. Acute single application, 14-day dermal toxicity was conducted to according to, or similar to OECD TG 402 in rabbits or rats on category members. The dermal LD50 values resulted

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greater than the limit doses of 2.0 g/kg. Acute 14-day, single dose, oral gavage, toxicity studies were conducted similar to OECD TG 401 in rats for category members and constituents. C9 aliphatic constituents were not covered; however as the higher molecular weight constituents are not acutely toxic by oral administration, it is expected that the C9 aliphatic constituents would be similarly non-toxic. The LD50 results of the oral studies in rats ranged from 5.0 to 15.8 g/kg.

Conclusion: No classification for inhalation acute toxicity. Based on read across, classification is also not necessary for acute dermal or oral toxicity. Nonane: LC50 inhalation = 23775 mg/m³ = 23.78 mg/L

Read-across: LD50 dermal ≥ 2.0 g/kg

Read-across: LD50 oral = > 5.0 g/kg to 15.8 g/kg

Corrosion/irritation skin

Key information: The isoparaffinic, normal paraffinic, and mixed aliphatic category members produced minimal to slight skin irritation when tested in rabbits and are also not normally irritating to human skin but can produce irritant responses if evaporation is inhibited and to humans. It should also be noted that prolonged or repeated exposure to hydrocarbon solvents can lead to severe irritant dermatitis due to defatting of the skin.

Conclusion: Classification is not possible due to lack of details on scoring, observation time, number of rabbits etc.

Corrosion/irritation eye

Key information: The member of the category produced minimal to slight eye irritation when tested in rabbits.

Conclusion: Classification is not possible due to lack of details on scoring, observation time, number of rabbits etc.

Sensitisation (skin & respiratory)

Key information: The category members do not cause skin sensitization (no further data included).

Conclusion: Classification not possible due to lack of data

STOT-SE

Key information: According to the SIAP, members of the C9-C14 (< 2% aromatics as well as members of constituents of these solvents have been tested for acute central nervous system. Aliphatic hydrocarbons with carbon numbers up to approximately C10 can produce acute, reversible effects to the central nervous system. Based on available information hydrocarbon solvents with carbon numbers greater than C10 do not produce acute CNS effects at the maximally attainable vapor concentrations. Rats were exposed to n-alkanes ranging from C₉-C₁₃ for 8 hour periods. In the study of nonane, Sprague-Dawley rats were exposed to concentrations ranging from 2414 to 5280 ppm. The authors reported mortality among the rats and calculated that the LC₅₀ value for nonane was 4467 ppm (23,775 mg/m³). The authors also reported that there was

evidence of acute CNS effects and that both the time to onset and severity of effects was related to the vapor concentrations to which the rats were exposed. In studies of n-decane, n-undecane, n-dodecane and n-tridecane the rats were exposed for 8 hours to the maximally attainable vapor concentrations. There were no deaths and no evidence of CNS effects. The authors also measured blood/air and brain/air ratios for these substances and reported that these ratios declined with increasing carbon number above C₁₀.

Human effects: There are a number of reports dealing with the potential for hydrocarbon solvent exposure to cause chronic neurological effects in humans. A review of the epidemiological literature regarding exposure to hydrocarbon solvents, focusing on white spirit, a C₉-C₁₁ aliphatic hydrocarbon solvent containing approximately 15-20% aromatics and described by the CAS RN of 8052-41-3, 64742-82-1, and 64742-88-7 has been conducted. Similar reviews have been conducted by the International Programme on Chemical Safety (IPCS) and Scientific Committee on Occupational Exposure Limit (SCOEL). The IPCS and SCOEL evaluations were also re-evaluated by the ECHA Committee for Risk Assessment (RAC). These evaluations include retrospective epidemiological studies involving painters with long-term exposure to white spirit. Confounding factors in these studies include co-exposure to other solvents and a lack of measured exposure data. Epidemiological studies reported an increased incidence of complaints of memory impairment, fatigue, impaired concentration, irritability, dizziness, headache, anxiety and apathy. Several studies that included neuropsychological tests demonstrated impairment in some of these tests; primarily in the short-term visual memory test and in the symbol-digit test. In some studies, life-time exposure to high concentrations of white spirit was correlated with an increase incidence of effect. Using a weight of evidence approach, the RAC concluded that chronic exposure to these white spirits cause adverse central nervous system (CNS) effects that can progress in severity. These CNS effects can include deficits in psychomotor, perception, memory parameters, and disturbances in mood. With respect to the C₉-C₁₄ aliphatic hydrocarbon (<2% aromatics) category, it is not known whether the effects attributed to white spirit were due to the aliphatic or aromatic constituents or a combination thereof.

Conclusion: STOT-SE category 3, based on narcotic effects (the information provided sufficient information to make a conclusion)

Human experience: There are well documented see above (key information) that CNS effects (memory impairment, fatigue, impaired concentration, irritability, dizziness, headache, anxiety and apathy) are observed with respect to exposure to C₉-C₁₄ aliphatic hydrocarbon (<2% aromatics) category members.

Animal data: LC₅₀ value for nonane was 4467 ppm (23,775 mg/m³), authors also reported that there was evidence of acute CNS effects and that both the time to onset and severity of effects was related to the vapor concentrations to which the rats were exposed.

STOT-RE

Key information: There were six repeated *inhalation* toxicity studies in rats, 2 with mixed aliphatic solvents and 4 with isoparaffinic solvents. The full range of the constituents was covered by the samples tested. In summary, excluding liver weight changes as being adaptive in response and the kidney change in male rats as being alpha 2u-globulin mediated and not relevant to humans, the overall NOECs were the highest concentrations tested in most of the studies (1390 mg/m³ – 10,400 mg/m³). The lowest NOEC value obtained was 1390 mg/m³ (vapour??) based on severe reductions in on body weight gain in females.

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Seven repeated *oral* toxicity studies (five OECD TG 403 and two OECD TG 422) in rats have been conducted, one C10-C13 aliphatic solvent, one C11-C14 aliphatic solvent, two isoparaffinic solvents (C10-C12, C12), two normal paraffinic (C10, C11) and a study of the analogue substance tetramethylcyclohexane (rats and beagle dogs). In aggregate these seven studies covered all constituents for the C9-C14 hydrocarbon (< 2% aromatics) therefor covering C9 constituent. There were some reports of kidney changes in male rats in the studies of the lower molecular weight paraffinic and mixed aliphatic solvents. The kidney effects were only in male rats and the histological findings were consistent with an alpha 2u-globulin mediated response. There were also some reports of reduced weight gain. This was attributed due to the use of corn oil as a diluent in some studies. Assuming liver weight increases to be adaptive rather than adverse and kidney changes in male rats to be not relevant to humans, a NOAEL of 1000 mg/kg/day was obtained.

A 28 day *dermal* administration study in rabbits was conducted with C12-C14 normal paraffins. The protocol was equivalent to OECD 410. The repeated dermal treatment under occlusive patch conditions caused severe dermal irritation in the highest treatment group (2000 mg/kg/day), leading to early termination. There was no evidence of systemic effects in the animals from the highest dose group, because of the early terminations of some animals. Conclusions based on this experimental group were considered unreliable. Accordingly, the overall NOAEL for hydrocarbons C12-C14 n-paraffins (<2% aromatic) was judged to be 500 mg/kg/day.

Conclusion: Based on read-across, no classification needed.

In summary, the members of the C9-C14 aliphatic [<2% aromatics] hydrocarbon solvent category did not appear to produce significant systemic effect. This is assuming that the studies were conducted following standard guidelines and the data presented in the SIAP is judged adequate. The principal findings in the repeated dose studies were increased liver weights in males and females and kidney changes in male rat. There were no pathological changes, levels of liver enzymes markers were not elevated, and the weight differences were reversed when rats were held without treatment for 28 days. The kidney changes were found only in male rats and were consistent with effects mediated by alpha 2u-globulin, an effect that has been determined not to be relevant to humans.

Mutagenicity

Key information: Category members (C₉-C₁₄ aliphatic, < 2% aromatic hydrocarbons) fluids are not mutagenic using *in vitro* genotoxicity assays. In bacterial tests, C₉-C₁₄ aliphatic, < 2% aromatic hydrocarbons fluids were not mutagenic in Salmonella strains tested in the presence or absence of metabolic activation. C₉-C₁₄ aliphatic, <2% aromatic hydrocarbon fluids did not induce mutations in an *in vitro* mammalian cell gene mutation assay. In sister chromatid exchange and in chromosomal aberration studies conducted under *in vitro* conditions, C₉-C₁₄ aliphatic, < 2% aromatic hydrocarbons fluids did not produce effects. C₉-C₁₄ aliphatic, < 2% aromatic hydrocarbons fluids were not genotoxic when tested by gavage in an *in vivo* mouse bone marrow micronucleus assay, when tested by inhalation in a mouse micronucleus test, and when tested in dominant lethal studies utilizing an inhalation route of exposure.

Conclusion: Based on read-across, no classification need.

Members of the C₉-C₁₄ Aliphatic [≤ 2% Aromatic] Hydrocarbon Solvents Category have shown no mutagenic activity in a number of *in vitro* bacterial, mammalian cell mutagenicity tests and were not active when tested in *in vitro* tests for chromosome aberration and sister chromatid exchange. These substances were not genotoxic when tested under *in vivo* conditions in bone marrow assays for chromosome damage and in dominant lethal tests.

Carcinogenicity

Key information: A carcinogenicity study Male and Female F-344 rats and B6C3F1 mice were exposed for two years to vapors of Stoddard solvent IIC (CAS RN 64742-88-7) is available.

Summary rats: The NTP concluded that under the conditions of these 2-year inhalation studies, there was some evidence of carcinogenic activity of Stoddard solvent IIC in male F344/N rats based on increased incidences of adrenal medulla neoplasms (pheochromocytoma). There was no evidence of carcinogenic activity of Stoddard solvent IIC in female F344/N rats exposed to 550, 1100, or 2200 mg/m³.

Summary mice: The NTP further concluded there was no evidence of carcinogenic activity of Stoddard solvent IIC in male B6C3F1 mice exposed to 550, 1100, or 2200 mg/m³. There was equivocal evidence of carcinogenic activity of Stoddard solvent IIC in female B6C3F1 mice based on increased incidences of hepatocellular adenoma. The NTP considered that the increased liver tumor incidence in female mice was equivocal based on a statistical analysis which indicated that this increase could be explained by the increased body weights of the mice in that group. The NTP also appeared to put little weight on the renal tumors in male rats as other data were obtained during this study to show that levels of α 2u-globulin were increased providing support for the view that these tumors, although treatment-related, are not relevant to humans.

Conclusion: Classification not possible due to lack of detail.

The SIAP provides a good overview of the studies mentioned above however we consider that additional factors necessary in order to be able classify the nonane for carcinogenicity are not present. Such as historical controls, the incidence of malignant pheochromocytomas, incidence in females etc.

Reproductive and developmental Toxicity

Key information: Overall there were no effects on either fertility or development in any of the studies presented.

Two repeated dose/reproductive toxicity screening tests on category members (C₁₀, C₁₁ normal paraffinic solvents) in which exposure was oral;

- A reproduction/developmental Toxicity Screening Test in Sprague-Dawley rats, similar to OECD Guideline 422, was conducted using Undecane (CAS RN: 1120-21-4). The administration was carried out by oral gavage in doses of 0, 100, 300 or 1000 mg/kg/day. There were no significant differences in body weight gain, Liver and adrenal weights were elevated in the high dose group, but there were no unusual histological findings. There were also no differences in mating frequency. No effects of undecane administration were observed on the sex cycle of females and copulation and conception of males and females. In addition, no effects of undecane administration were observed on the weights of reproductive organs (testis, epididymis and ovary) and there were no abnormalities noted in the dissection and histopathological examination. There were no histopathological findings in cases where animals failed to successfully mate; abnormal deliveries (2 in the 300 mg/kg/day group and 1 in the 1000 mg/kg/day group) were confirmed to be spontaneous and at frequencies similar to historical controls. Those cases observed in the present study were considered to be unrelated to undecane. There were no differences in the number of live pups born in the control, 100 mg/kg, or the 1000 mg/kg treated animals; there was a decrease in the 300 mg/kg group, but as no difference was noted in the 1000 mg/kg treated animals, this finding was determined by the authors to not be test material related. There was no difference in the number of pups alive on post-natal day 4. The body weights at post-natal day 4 of both males and females in the 1000 mg/kg group were slightly reduced (-5.6% and -4.1%), but were not significantly different from the

weights of offspring in the control group, and there were no notable clinical or pathological effects. The NOAEL for reproductive performance and developmental effects is considered to be 1000 mg/kg/day.

- A Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test similar to OECD Guideline 422 was conducted using decane, CAS RN: 124-18-5. Animals were dosed with 0, 25, 150, or 1000 mg/kg/day. In the assessment of developmental and reproductive effects there were no treatment-related effects at any dose level on any of the reproductive parameters evaluated in this study. These included measures of reproductive performance (mating, conception, gestation length, and litter size), offspring survival (gestation and postnatal survival indices, percent pre- and post-implantation loss). There were no treatment-related effects at any dose level on any of the developmental parameters evaluated in this study including external abnormalities of pups, number of live and still births, mortality, sex determination, and weights of pups. Based on these data, the no-observable-adverse-effect level (NOAEL) for developmental toxicity was 1000 mg/kg/day and the NOAEL for reproductive dose and reproductive toxicity was 1000 mg/kg/day.

Two developmental toxicity studies on category members (C₉-C₁₁ mixed aliphatic solvent, C₁₀-C₁₂ isoalkanes) in which exposure was by inhalation;

- A Prenatal Developmental Toxicity Study equivalent or similar to OECD Guideline 414 with Hydrocarbons, C₉-C₁₁, isoalkanes, cyclics, < 2% aromatics (CAS RN 64742-48-9). The test material was administered to pregnant female rats by inhalation exposure to vapor concentrations of 0, 300 or 900 ppm (5220 mg/m³). Pregnancy rate, mortality, body weight gain and gross postmortem observations were unaffected by treatment. Hydrocarbons, C₉-C₁₁, normal paraffins, isoalkanes, cyclics, < 2% aromatics treatment at either dose level had no effect on reproductive endpoints, fetal size, sex distribution, ossification variation, or fetal examination endpoints. Thus, there was no evidence of maternal or fetal toxicity at either exposure level of Hydrocarbons, C₉-C₁₁, normal, isoalkanes, cyclics, < 2% aromatics tested. Based on these results, both the maternal and developmental NOAELs were greater than or equal to 900 ppm (5220 mg/m³).
- Hydrocarbons, C₁₀-C₁₂, isoalkanes, < 2% aromatics (CAS RN 90622-57-4) was administered to pregnant female Sprague-Dawley rats by inhalation exposure to vapor concentrations of 0, 300 or 900 ppm. Hydrocarbons, C₁₀-C₁₂, isoalkanes, < 2% aromatics treatment at either dose level had no effect on reproductive endpoints, fetal size, sex distribution, ossification variation, or fetal examination endpoints. Pregnancy rate, mortality, body weight gain and gross postmortem observations were unaffected by treatment. Thus, there was no evidence of maternal or fetal toxicity at either exposure level of the chemical tested. Based on these results, both the maternal and developmental NOAELs were greater than or equal to 900 ppm (5220 mg/m³).

Supporting information:

Repeated dose/reproductive toxicity screening test on a C₉-C₁₆ analogue substance in which exposure was dermal; Reproductive toxicity test on a C₉-C₁₆ analogue substance in which the exposure was oral, and a classical developmental toxicity study on a C₉-C₁₆ analogue substance. The studies for reproductive and/or development that have conducted with the these analogues show no effects on either fertility or development in any of these studies.

The two analogue substances are hydrodesulfurized kerosine (CAS RN 64742-81-0, analogue) and jet fuel (JP-8, analogue). These two analogue substances are petroleum fuels, described as containing primarily C₉-C₁₆ aliphatic constituents with aromatic contents limited to a maximum of 25% (although the samples used in these tests contained about 20% aromatics. The data provided by tests of category and analogue substances subsumed the carbon numbers and molecular types found in the C₉-C₁₄ aliphatic (<25 aromatic) category.

Additionally, there have been 7 repeated exposure studies in which the reproductive organs were examined and found to have not been affected by test material administration.

Conclusion: Based on read-across no need for classification.

Environment

Acute Aquatic Toxicity

Key information: Specific substance toxicity LC50 or EC50s values for nonane for the trophic levels are not available in the SIAP. Therefore, there is not sufficient information to classify nonane, for acute (short-term) aquatic hazard. According to the SIAP, measured acute toxicity data are available for fish, invertebrates and freshwater algae but these are reported to be in the range between 0.01 to 0.2 mg/L for paraffinic hydrocarbons with a carbon of 10 and below. In light of this information one classify nonane based on read-across. This is assuming that the read-across is adequately justified. Nonane can be classified as Aquatic Acute 1 since the 0.01 – 0.2 mg/L is below the cut-off of 1mg/L. Using the lowest L(E)C50 value of 0.01 mg/L yields an M-factor of 1.

Conclusion: Based on read-across, Aquatic acute 1: M = 1

Chronic Aquatic Toxicity

Key information: There is information on one trophic level Daphnia. In absence of chronic toxicity for fish and algae, the classification is based on the chronic and toxicity data on Daphnia and surrogate chronic data in fish and algae.

The n-paraffin constituents belonging to the C₉-C₁₄ Aliphatic [\leq 2% aromatic] hydrocarbon solvent category have the potential to biodegrade rapidly (80 to 100% in 28 days). Based on read-across nonane is expected to be readily biodegradable. The NOEC value of 0.005 mg/L for *Daphnia magna*, is below \leq 0.01 mg/L limit. Using table 4.1.2 of the GHS guidance, nonane is classified as Chronic category 1 with and M factor of 1.

Conclusion: Based on surrogate data: nonane is classified as chronic category 1 with and M factor of 1.

CH classification proposal for Nonane (CAS. 111-84-2)

General Comment

It would be helpful to clearly indicate which is the key study and which are supporting studies and to find a table with the data that is important for classification at the end of the human health part of the SIAP.

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Classification

Based on the available data Nonane needs not to be classified for acute toxicity (dermal, oral and inhalation), skin sensitization, germ cell mutagenicity, reproductive toxicity and STOT RE. However, the classification is based on read-across for most endpoints. Therefore, it could be that the classification will change as soon as data for Nonane is available.

The classification for irritation is not possible since data (Scoring points) is missing. Data for carcinogenicity is conclusive. However, it is not sufficient for classification. No data is available for the endpoint STOT SE and therefore classification is not possible.

Aspiration Toxicity

Cat 1

(based on the physical and chemical properties, particularly the low viscosity of 1.008 mm²/s a at 20 °C)

Classification based on the information in the SIAP is not possible. However, the relevant information can be found in the IUCLID file.

Environmental Hazards

Hazard class		Classification	hazard statement	Rational for the classification
11	Hazardous to the aquatic environment (acute)	Category 1	H400 Very toxic to aquatic life	Classification is based on the calculated acute toxicity LC50 value of 0.368 mg/L for fish (ECOSAR) and the measured acute toxicity value 48h EC50 0.2 mg/L for Daphnids.
11	Hazardous to the aquatic environment (chronic)	Classification not possible	---	Due to the lack of chronic aquatic toxicity data and in consideration of the predicted rapid biodegradation (BIOWIN model) and the estimated moderate potential for bioconcentration (BCFBAF model) a classification for chronic aquatic toxicity is not possible/not warranted.

Key studies used for classification:

Water solubility

Result (measured): 0.22 mg/L at 25°C

Reference: Riddick, JA et al. (1986); data source: SRC PhysProp Database; <http://www.syrres.com/what-we-do/databaseforms.aspx?id=386>

Partition coefficient octanol-water

Result (measured): Log Pow 5.65

Reference: Lide DR (2005): Handbook of chemistry and physics. 86 th ed., CRC Press

Biodegradation

No reliable test data is available for nonane.

QSAR-Method: BIOWIN model

Result (estimated): Ready biodegradability prediction: YES; Ultimate biodegradation timeframe (BIOWIN 3): DAYS-WEEKS

Reference: BIOWIN v.4.10 (EPISUITE v.4.00), US EPA

Remark: Biowin5 and 6 also predict ready biodegradability, but for degradation in the OECD301C test only; using data from the Chemicals Evaluation and Research Institute Japan (CERIJ) database.

Bioaccumulation

No reliable test data is available for nonane.

QSAR-Method: BCFBAF model

Result (estimated): Log BCF = 2.021 (BCF = 104.9 L/kg wet-wt)

Reference: BIOWIN v.4.10 (EPISUITE v.4.00), US EPA

Acute (short-term) aquatic toxicity**Fish**

QSAR Method: Aquatic Toxicity Predictions Obtained Using the Petrotox computer model (v 3.04) for Hydrocarbons, 2010, CONCAWE, Brussels, Belgium

Result (estimated): Freshwater fish 96-h LL50 (Lethal Loading Rate) value is 1.125 mg/L based on mortality

Reference: ECHA, registration dossier for nonane, QSAR Key Short-term toxicity to fish.001;

http://apps.echa.europa.eu/registered/data/dossiers/DISS-9d8abd6f-cedb-01d9-e044-00144f67d249/AGGR-48ba6862-fe75-4d82-974e-dd9c76ca0264_DISS-9d8abd6f-cedb-01d9-e044-00144f67d249.html#AGGR-48ba6862-fe75-4d82-974e-dd9c76ca0264

QSAR Method: ECOSAR model for neutral organics

Result (estimated): 96h LC50 fish 0.368 mg/L

Reference: ECOSAR v.1.00 (EPISUITE v.4.00), US EPA

Remark: ECOSAR v1.00 SAR Limitations: Maximum LogKow: 5.0 (Fish 96-hr LC50; Daphnid LC50, Mysid LC50)

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Invertebrates

Test substance: n-nonane

Test species: *Daphnia magna*

Test method: 48h static freshwater-test at 20°C, test concentrations: 0, 0.32, 1.0, 3.2, 5.6, 10 mg/L (nominal); 0, 0.04, 0.04, 0.5, 2.1, 2.2 (initial measured); reliability: 2 (valid with restrictions)

Result (measured): 48h EC50 0.2 mg/L

Reference: The evaluation of the hazards of harmful substances carried by ships. Joint group of experts on the scientific aspects of marine pollution - GESAMP - reports and studies no. 17, 1982. Report date: 1987-05-07; Data source: ECHA, registration dossier for nonane, Exp Key Short-term toxicity to aquatic invertebrates.001; http://apps.echa.europa.eu/registered/data/dossiers/DISS-9d8abd6f-cedb-01d9-e044-00144f67d249/AGGR-f34e06f7-110c-4891-a8f5-7f228e416309_DISS-9d8abd6f-cedb-01d9-e044-00144f67d249.html#AGGR-f34e06f7-110c-4891-a8f5-7f228e416309

Algae

QSAR Method: Aquatic Toxicity Predictions Obtained Using the Petrotox computer model (v 3.04) for Hydrocarbons, 2010, CONCAWE, Brussels, Belgium

Result (estimated): freshwater algae 72-h EL50 (Effect Loading Rate) value is 1.098 mg/L based on biomass

Reference: ECHA, registration dossier for nonane, QSAR Key Toxicity to aquatic algae and cyanobacteria.001; http://apps.echa.europa.eu/registered/data/dossiers/DISS-9d8abd6f-cedb-01d9-e044-00144f67d249/AGGR-e67cb827-46d9-4e0c-8e8d-90cbd13f8209_DISS-9d8abd6f-cedb-01d9-e044-00144f67d249.html#AGGR-e67cb827-46d9-4e0c-8e8d-90cbd13f8209

Chronic (long-term) aquatic toxicity

No reliable data is available for nonane

IT classification proposal for Nonane (CAS. 111-84-2)

Proposed draft classification Italy	Asp. Tox.	Acute Tox.	Skin Irrit.	Eye Irrit.	Skin sentiz.	STOT SE	STOT RE	Muta.	Carc.	Repro.	Aqua. Acute	Aqua. Chronic
	Asp Tox 1, H304	No data (o and d) No classified (i)	Skin Irrit 2, H315	Inconclusive	No classified (read across)	No data	No classified	No Classified	Inconclusive	No classified (read across)	Acute Aquatic 1, H400 M factor =1	Acute Chronic 1, H410 M factor =1

CAS: 111-84-2 Chemical name: Nonane	<u>Mammalian toxicity endpoints</u>					
	Acute toxicity Oral					
	Organism	Methods	Data	Reference	GHS classification	Rationale/Comments
	No data	No data	No data	No data	No data	
	Acute toxicity Inhalation					
	Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments
	rat Harlan-Wistar male	OECD Guideline 403 (Acute Inhalation Toxicity)	LC50 – 4h = 23760 mg/m ³ (23,8 mg/L)	Carpenter, C.P. et al., 1978	No classified	This data not included in SIDS Initial Assessment Profile, SIAR and IUCLID. Data by registration dossier from ECHA Chem
	Acute toxicity Dermal					
	Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments
	No data	No data	No data	No data	No data	
	Corrosive/irritant skin					
	Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments
rat hairless CDHrBi	No specified	Nonane induced erythema within	Babu, R.J. et al., 2004	Skin Irrit 2, H315	not included in SIDS Initial Assessment Profile, SIAR and	

			1 hr and showed moderate irritation (erythema score 2.2 ± 0.4) at 24 hrs.			IUCLID. Data by registration dossier from ECHA Chem
Corrosive/irritant eyes						
Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments	
		early lacrimation slight irritation of eyes	Carpenter, C.P. et al., 1978	Inconclusive	Scores lacking. not included in SIDS Initial Assessment Profile, SIAR and IUCLID. Data by registration dossier from ECHA Chem	
Sensitizer Skin and respiratory tract						
Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments	
Guinea-Pig	Magnusson and Kligman Guinea-Pig Maximization test	Skin sensitization studies do not show skin sensitization in guinea pigs for C9-C14 aliphatic, < 2% aromatic hydrocarbons fluids. C9-C14 aliphatic, < 2% aromatic hydrocarbons fluids are not skin sensitizers or photosensitizers in humans.	ExxonMobil (1988 b,c; 1962; 1991c)	No classified (read across)	C9-C14 aliphatic, < 2% aromatic hydrocarbons fluids are not skin sensitizers. Skin sensitization studies on nonane are not available..	
Gene mutation						
Organism	Methods	Data	Reference	GHS	Rationale/Comments	

					Classification	
		Bacterial reverse mutation assay (e.g. Ames test) OECD Guideline 471	under the conditions of this assay, there was no evidence of mutagenic activity, with metabolic activation and without metabolic activation	Zeiger, E. et al. (1992)	No Classified	Nonane is not mutagenic in Ames test (Data by registration dossier from ECHA Chem). Supportive information: C9-C14 aliphatic, < 2% aromatic hydrocarbons fluids are not mutagenic using in vitro or in vivo genotoxicity assays.
		Bacterial reverse mutation assay (OECD TG 471)	<i>in vitro</i> or <i>in vivo</i> genotoxicity: C9-C14 aliphatic, < 2% aromatic hydrocarbons fluids are not mutagenic	Cepsa Química (1985) Shell (1999) Chevron Philips (1982) Shell (1998a)		
		In vitro Mammalian Chromosome Aberration Test (OECD TG 473)	bacterial tests: C9-C14 aliphatic, < 2% aromatic hydrocarbons	Shell (1998b)		
		In vitro Mammalian Cell Gene Mutation Test (OECD TG 476)	fluids were not mutagenic in Salmonella strains tested in the presence or absence of metabolic activation.	Chevron Philips Chemical Company, L.P (1982) INEOS OLIGOMERS (1996)		
		Genetic Toxicology: In Vitro Sister Chromatid Exchange Assay in Mammalian Cells (OECD TG 479)	C9 - C14 aliphatic, <2% aromatic hydrocarbon fluids were negative in vitro mammalian cell gene mutation	Chevron Philips (1983)		

		Micronucleus Assay in Mouse Bone Marrow (OECD TG 474)	assay. In sister chromatid exchange and in chromosomal aberration studies, C9-C14 aliphatic, < 2% aromatic hydrocarbons fluids did not produce an effect. C9-C14 aliphatic, < 2% aromatic hydrocarbons fluids were also non-mutagenic when tested in an in vivo mouse bone marrow micronucleus assay and when tested in dominant lethal studies utilizing an inhalation route of exposure.	ExxonMobil (1991f)		
		Genetic Toxicology: Rodent Dominant Lethal Test (OECD TG 478)		ExxonMobil (1978c; d)		
Carcinogenicity						
	Organism	Methods	data	Reference	GHS Classification	Rationale/Comments
			No data available for Nonane. The weight of evidence is derived from study records reported for the	SIAR	Inconclusiv e	C9-C14 Aliphatic Hydrocarbon Solvents [< 2% Aromatics]. C9-C14 Aliphatic Hydrocarbon Solvents [< 2% Aromatics] are unlikely to be human carcinogens, but it is not possible to exclude that nonane is a carcinogen.

			C ₉ -C ₁₄ Aliphatic Hydrocarbon Solvents [\leq 2% Aromatics]. C ₉ -C ₁₄ Aliphatic Hydrocarbon Solvents [\leq 2% Aromatics] are unlikely to be human carcinogens.			
Reproductive/Developmental toxicity						
Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments	
		No data available for the substance Nonane. The available data on potential reproductive and developmental effects of members of the C ₉ -C ₁₄ Aliphatic [\leq 2% Aromatic] Hydrocarbon Solvents Category are limited to animal studies. These studies suggest that these solvents are not expected to be reproductive or developmental	SIAR	No classified (read across)	C ₉ -C ₁₄ Aliphatic [\leq 2% Aromatic] Hydrocarbon Solvents Category are not expected to be reproductive or developmental toxicant.	

			toxicants.			
Single dose toxicity						
Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments	
No data	No data	No data	No data	No data		
Repeated dose toxicity						
Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments	
mouse C57BL male	OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	NOAEL oral: gavage = 0.1 g/kg all lesions except the proliferative and inflammatory lesions in the non-glandular forestomach (species-specific target organ).	study report , 2003 (unpublished)	No classified	Effect of specific target organ is not relevant to humans because it is species specific	
rat Harlan-Wistar male	equivalent or similar to Guideline OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day)	NOAEC male inhalation: vapour = 8400 mg/m ³ air (analytical) Serum glutamic pyruvic transaminase value for blood taken was statistically significantly greater than that of controls. However, the increases were not observed after 8 or 13 weeks suggesting a transient effect.	Carpenter, C.P. et al.,1978	No classified		
Aspiration Toxicity						
Organism	Methods	Data	Reference	GHS	Rationale/Comments	

				Classification	
	other guideline: EN ISO 3104	1.008 mm ² /s (static) Temp. 20 °C	Haltermann Products, Site Speyer, Branch of Dow Olefinverbund GmbH, 2010 (unpublished)	Asp Tox 1, H304	Nonane products may be an aspiration hazard based on its viscosities
<u>Environmental toxicity endpoints</u>					
Acute toxicity					
Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments
Fish	QSAR	96 h LL ₅₀ =1.125 mg/L	Aquatic Toxicity Predictions Obtained Using the Petrotox Model for Hydrocarbons (CONCAWE, Brussels, Belgium 2010)		
Aquatic Invertebrates	other guideline: As described in: The evaluation of the hazards of harmful substances carried by ships. Joint group of experts on the scientific aspects of marine pollution - GESAMP - reports and studies no. 17, 1982.	48 h EC ₅₀ =0.2 mg/L	Aquatic toxicity of compounds that may be carried by ships (Marpol 1973, Annex II). A Progress report for 1986. For: Ministry of Housing, Physical Planning and Environment (Adema, D.M.M. and van den Bos Bakker, G.H.)	Acute Aquatic 1, H400 M factor =1	

	Aquatic Plants	QSAR	The estimated freshwater algae 72-h EL ₅₀ = 1.098 mg/L based on biomass.	Aquatic Toxicity Predictions Obtained Using the Petrotox Model for Hydrocarbons (CONCAWE, Brussels, Belgium 2010)		
Chronic toxicity						
	Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments
	Fish	QSAR	NOELR 28d = 0.252 mg/L	Aquatic Toxicity Predictions Obtained Using the Petrotox Model for Hydrocarbons (CONCAWE, Brussels, Belgium 2010)		
	Aquatic Invertebrates	/	NOEC = 0.005 mg/l	(ExxonMobil, 2012a)	Acute Chronic 1, H410 M factor =1	
	Aquatic Plants	QSAR	The estimated freshwater algae 72-h NOELR (No Observed Effect Loading Rate) = 0.246 mg/L based on biomass.	Aquatic Toxicity Predictions Obtained Using the Petrotox Model for Hydrocarbons (CONCAWE, Brussels, Belgium 2010)		

Russian Federation classification proposal for Nonane (CAS. 111-84-2)

Hazard class	Substance data / Note	Criteria of Category according to GHS	Conclusion/ <i>Commentary</i>
Explosives	No data available	There are no chemical groups associated with explosive properties present in the molecule	Not classified
Flammable gases	Substance is a liquid		Not classified
Aerosols	Substance is a liquid		Not classified
Oxidizing gases	Substance is a liquid		Not classified
Gases under pressure	Substance is a liquid		Not classified
Flammable liquids	Flash point = 38 °C at 1 atm / This study is reported in the IUCLID 5 DataSet but it cannot be found in the SIAP	Category 3: Flash point $\geq 23^{\circ}\text{C}$ and $\leq 60^{\circ}\text{C}$	Category 3 <i>Based on IUCLID's data</i>
Flammable solids	Substance is a liquid		Not classified
Self-reactive substances and mixtures	No data available	There are no chemical groups present in the molecule associated with explosive or self-reactive properties	Not classified
Pyrophoric liquids	No data available	Substance is known to be stable at room temperature for prolonged periods of time (days)	Not classified
Pyrophoric solids	Substance is a liquid		Not classified
Self-heating substances and mixtures	No data available		Not classified
Substances and mixtures which, in contact with water, emit flammable gases	Slightly soluble (0,2 mg/l at 25°C), the chemical structure of the substance does not contain	The chemical structure of the substance does not contain metals or metalloids	Not classified.

	metals or metalloids		
Oxidizing liquids	Study scientifically unjustified as the substance is incapable of reacting exothermically with combustible materials on the basis of its chemical structure	The substance does not contain oxygen, fluorine or chlorine	Not classified
Oxidizing solids	Substance is a liquid		Not classified
Organic peroxides	Substance is not organic peroxides		Not classified
Corrosive to metals	No data available		Not classified
Acute toxicity / oral	LC ₅₀ > 5000 mg/kg / data for C9-C14 aliphatic, < 2% aromatic hydrocarbon	2000 < Category 5 ≤ 5000	Not classified <i>Based on read-across data</i>
Acute toxicity / dermal	The dermal LD50 results, greater than the limit doses of 2.0 g/kg, indicate that C9-C14 Aliphatic Hydrocarbon Solvents (≤ 2% Aromatics) were not acutely toxic by dermal administration	2000 < Category 5 ≤ 5000	Category 5. <i>Based on read-across data. It's not proven that the value > 5000 mg/kg</i>
Acute toxicity / inhalation	LC ₅₀ = 23775 mg/m ³ = 23.775 mg/l The inhalation LC ₅₀ data indicated that the C9-C14 Aliphatic Hydrocarbon Solvents (≤ 2% Aromatics) were not acutely toxic by inhalation	Category 5 is substances which are of relatively low acute toxicity but which, under certain circumstances, may pose a hazard to vulnerable population	Not classified. <i>Data classification for acute inhalation toxicity is not enough, it's possible to establish 5 category as for potentially dangerous substance</i>
Skin corrosion/irritation	Similarly, the isoparaffinic, normal paraffinic, and mixed aliphatic category members produced minimal to slight skin irritation when tested in rabbits		Not classified. <i>Classification is not possible due to lack of details on study</i>

	and are also not normally irritating to human skin but can produce irritant responses if evaporation is inhibited or prevented.		
Serious eye damage/ eye irritation	The members of the C9-C14 aliphatic (<2% aromatics) category produced minimal to slight eye irritation when tested in rabbits		Not classified. <i>Classification is not possible due to lack of details on study</i>
Respiratory or skin sensitization	Category members do not cause skin sensitization		Not classified <i>Based on read-across data. Classification is not possible due to lack of details on study</i>
Germ cell mutagenicity	Members of the C ₉ -C ₁₄ Aliphatic [\leq 2% Aromatic] Hydrocarbon Solvents Category have shown no mutagenic activity in a number of <i>in vitro</i> bacterial, mammalian cell mutagenicity tests and were not active when tested in <i>in vitro</i> tests for chromosome aberration and sister chromatic exchange		Not classified <i>Based on read-across data</i>
Carcinogenicity	Male and Female F-344 rats and B6C3F1 mice were exposed for two years to vapors of Stoddard solvent IIC (CAS RN 64742-88-7). The NTP concluded that under the conditions of these 2-year inhalation studies, there was some evidence of carcinogenic activity of Stoddard solvent IIC in male F344/N rats based on increased incidences of		Not classified <i>Based on read-across data. Classification is not possible due to lack of details on study</i>

	adrenal medulla neoplasms (pheochromocytoma). There was no evidence of carcinogenic activity of Stoddard solvent IIC in female F344/N rats exposed to 550, 1100, or 2200 mg/m ³ ...		
Reproductive toxicity	NOAEL was determined to be \geq 1000 mg/kg bw/day. Based on this study and the lack of systemic toxicity, C9-C14 aliphatic, <2% aromatic hydrocarbon fluids, are not expected to be reproductive toxicants		Not classified. <i>Based on available read-across data, the classification criteria are not met</i>
Specific target organ toxicity – Single exposure	Rats were exposed to n-alkanes ranging from C9-C13 for 8 hour periods. In the study of nonane, Sprague-Dawley rats were exposed to concentrations ranging from 2414 to 5280 ppm. The authors reported mortality among the rats and calculated that the LC50 value for nonane was 4467 ppm (23,775 mg/m ³). The authors also reported that there was evidence of acute CNS effects and that both the time to onset and severity of effects was related to the vapor concentrations to which the rats were exposed.	Category 3: Central nervous system depression including narcotic effects in humans such as drowsiness, narcosis, reduced alertness, loss of reflexes, lack of coordination, and vertigo are included.	Category 3. <i>Based on narcotic effects</i>
Specific target organ toxicity –	The members of the C9-C14		Not classified

Repeated exposure	Aliphatic [$< 2\%$ aromatics] Hydrocarbon Solvents Category did not appear to produce significant systemic toxicity		<i>Based on available read-across data, the classification criteria are not met</i>
Aspiration hazard	Kinematic viscosity = 1.008 mm ² /s at 20°C	Substance is hydrocarbon and has a kinematic viscosity ≤ 20.5 mm ² /s at 40°C	Category 1. <i>Kinematic viscosity decreases with increase of the temperature</i>
Hazardous to the aquatic environment/ acute hazard	EC ₅₀ (48h, Daphnia magna) = 0,2 mg/l / This study is reported in the IUCLID 5 DataSet but it cannot be found in the SIAP	Category Acute 1: EC50 (48h for crustacean) ≤ 1 mg/l	Category 1 <i>Based on IUCLID's data</i>
Hazardous to the aquatic environment/ long-term hazard	NOEC (Daphnia magna) = 0.005 mg/l, readily degradable	Category Chronic 1: NOEC or EC _x ≤ 0.01	Category 1
Hazardous to the ozone layer	Substance not listed in Annexes to the Montreal Protocol	Substance not listed in Annexes to the Montreal Protocol	Not classified

Proposed draft classification	As p. To x.	Acute Tox.	Skin Irrit.	Eye Irrit.	S k i n s e n t i z .	STOT SE	STOT RE	Muta.	Carc.	Repro .	Aqua. Acute	Aqua. Chronic
	As p. To x. 1	Acute Tox. 4 (i)	Skin Irrit. 2	Eye Irrit. 2	NC	STOT SE 3* **	NC	NC	NC	NC	NC	NC
	Ph ys- ch em dat a	Inhalation: LC50 (rat) 3200 ppm (ACGIH)	based on previous conclusion (HSDB); but scores not reported	based on previous conclusion (HSDB); but scores not reported	d at a n o t s u f f i c i e n t : l a c k o f s t u d y d e t a i l	category member CNS effects (memory impairment, fatigue, impaired concentration, irritability, dizziness, headache, anxiety and apathy) and respiratory tract irritation observed(HSDB)	data sufficient (read across)	data sufficient (read across)	data not sufficient: lack of study detail	data sufficient (read across)	Data not sufficient	

Note:

*: may cause respiratory irritation

** : may cause drowsiness or dizziness

(i): inhalation (o): oral

JP Classification for Environment: proposal for Nonane (CAS. 111-84-2)

	Nonane
Aquatic Acute Classification	Aq Ac 1(read-across) L(E)C50 values of 0.01 to 0.2 mg/L for paraffinic hydrocarbon ≤ C10
Aquatic Chronic Classification	Aq Ch 1 Not readily biodegradable Daphnia 21d NOEC = 0.005 mg/L

Classifications for 2NA EDTA(CAS. 139-33-3)

Notified classification for Disodium EDTA (CAS. 139-33-3)

Notification s	Asp. Tox.	Acute Tox.	Skin Irrit.	Eye Irrit.	Skin sentiz .	STOT SE	STOT RE	Muta .	Carc.	Repro .	Aqua. Acute	Aquatic Chronic
ECHA CHEM 1		Acute Tox. 4 (o)		eye Irrit. 2								
ECHA CHEM 2		Acute Tox. 4 (o)	Skin Irrit. 2									
ECHA CHEM 3		Acute Tox. 4 (o)	Skin Irrit. 2	eye Irrit. 2		STOT SE 3						
ECHA CHEM 4			Skin Irrit. 2	eye Irrit. 2								
ECHA CHEM 5		Acute Tox. 4 (o)	Skin Irrit. 2	eye Irrit. 2		STOT SE 3						
ECHA CHEM 6		Acute Tox. 4 (i)										
ECHA CHEM 7		Acute Tox. 4 (o)	Skin Irrit. 2	eye Irrit. 2		STOT SE 3						
ECHA CHEM 8				eye Irrit. 2								
ECHA CHEM 9		Acute Tox. 4 (o)		eye Irrit. 2								
ECHA CHEM 10		Acute Tox. 4 (o)		eye Irrit. 2					Carc. 2			
ECHA CHEM 11		Acute Tox. 4 (o)										
ECHA CHEM		Acute Tox. 4	Skin Irrit.	eye Irrit.		STOT SE						

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Notification s	Asp. Tox.	Acute Tox.	Skin Irrit.	Eye Irrit.	Skin sentiz	STOT SE	STOT RE	Muta	Carc.	Repro	Aqua. Acute	Aquatic Chronic
12		(o)	2	2		3						
HSNO CCID 1		6.1E (o)	6.3B	6.4A								
HSNO CCID 2			6.3B	6.4A								
HSNO CCID 3		6.1E (o)	6.3B	6.4A								

Note:

(i): inhalation (o): oral (d): dermal

RIVM-NL classification proposal for Disodium EDTA (CAS. 139-33-3)

	Asp. Tox.	Acute Tox.	Skin Irrit.	Eye Irrit.	Skin sentiz.	STOT SE	STOT RE	Muta.	Carc.	Repro.	Aqua. Acute	Aquatic Chronic
Proposed draft classification - RIVM	NCN	NCP	NCP	NCP	NCN	NCP	NCN	NCN	NCN	Reprotox cat.2	Category 2	NCPa or Aquatic Chronicb 2

Note:

(i): inhalation (o): oral. NCP: No classification possible, see further remarks in the justification why no classification could be made. NCN: No classification needed. Based on available information the substance does not need classification. a) Classification not possible due to insufficient data on chronic aquatic toxicity. b) Classification using the surrogate system (adequate acute toxicity data in combination with environmental fate and transport data): aquatic acute tox from the structural analogue, **Na4EDTA**, with EC50 of 1.01 mg/l and not rapid degradability.

Key studies and Justification for proposed draft conclusion

Acute toxicity:

Inhalation: according to SIAP “Mortality was observed at 1103 mg/m³ (**1.103 mg/l**) following a single 6-h exposure” No LC50 value is given in SIAP. Following the worst-case scenario, this value may be assumed to be LC50. In addition, for acute toxicity LC50 is defined as a four hour value meaning that the six hour value also has to be translated into a four hour value. **Taking into account all uncertainties, it has to be concluded that information provided in SIAP is not detailed and clear enough to reach harmonized classification of the chemical assessed. Information missing: clear LC50-value.**

Dermal: according to SIAP structural analogues showed “LD₅₀ values ranging from >1800 to >2000 mg/kg bw”. Based on the available data a classification in category 5 cannot be excluded. **Taking into account all uncertainties, it has to be concluded that information provided in**

SIAP is not detailed and clear enough to reach harmonized classification of the chemical assessed. Information missing: clear LD50-value and lack of data allowing proper judgement for Category 5 (testing up to 5000 mg/kg bw).

Oral: according to SIAP “oral LD₅₀ values for Na₂EDTA were > 2000 mg/kg bw. Based on the available data a classification in category 5 cannot be excluded. **Taking into account all uncertainties, it has to be concluded that information provided in SIAP is not detailed and clear enough to reach harmonized classification of the chemical assessed. Information missing: clear LD50-value and lack of data allowing proper judgement for Category 5 (testing up to 5000 mg/kg bw).**

Skin and eye irritation: according to SIAP “The aminocarboxylic acid-based chelants are not irritating to moderately irritating to the intact skin, and slightly to moderately irritating to the eyes in rabbits. The irritancy potential is related to the pH of the individual salt. Thus, more acidic members of the category such as **disodium EDTA** have inherently greater irritancy potential.” **However, it has to be concluded that information provided in SIAP is not detailed and clear enough to reach harmonized classification of the chemical assessed. Information missing: scorings from the irritation tests.**

Skin sensitization: according to SIAP “The aminocarboxylic acid-based chelants are not skin sensitizers based on studies in mice and guinea pigs. Therefore no classification is needed for skin sensitization.

Mutagenicity: according to SIAP “Available data from *in vitro* [**Na₂EDTA**] and *in vivo* [**Na₂EDTA**] testing of representative chelant category members indicate that these materials generally do not induce gene mutations or chromosomal aberrations *in vitro* or *in vivo*. Although there have been some positive findings reported *in vitro* and *in vivo* for some category members, these positive effects have been generally attributed to the threshold mechanisms of pH changes and the chelation of critical nutrient metals such as zinc.” Therefore no classification is needed for mutagenicity.

Carcinogenicity: according to SIAP no evidence of carcinogenicity was found for the structural analogue, Na₃EDTA trihydrate. Therefore no classification is needed for carcinogenicity.

Reproductive toxicity:

Reproductive toxicity: according to SIAP “The weight of evidence from a two-generation reproductive toxicity study in rats shows that dietary ingestion of 1% **Na₂EDTA** (approx. 920 mg/kg bw/day) had no effect on reproduction; however, no litters were produced at 5% (approx. 4600 mg/kg bw/day); the NOAEL for reproductive toxicity was 920 mg/kg bw/day.” From the repeated dose description: “In a 13-week repeated-dose toxicity study, rats (both sexes) fed Na₂EDTA (0, 1, 5, 10%) showed mortality at the highest dose. In addition, there was decreased food consumption (emaciation at 10%) and diarrhea at doses of 5% (approximately 4206 mg/kg bw/day) and above. Taking into account that no litters were produced at dose which showed only diarrhea as adverse effect, classification as Reproductive toxicant category 2 is proposed.

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Developmental toxicity: according to SIAP “Studies on developmental toxicity showed a specific fetotoxic and teratogenic potential of EDTA, **Na₂EDTA** and CaNa₂EDTA; a LOAEL of 1000 mg/kg bw/day was determined. Increased proportions/litter and significantly lower fetal body weights are indicative for an impaired fetal development. The pattern of malformations comprised cleft palate, severe brain deformities, eye defects, micro- or agnathia, syndactyly, clubbed legs and tail anomalies. These effects were exhibited in studies using maternally toxic dose levels..” As developmental effects are due to the specific mechanism of Zn depletion, and not due to maternal toxicity, **Na₂EDTA** should be labeled as developmental toxicant. However, there are not enough information to decide if it should be Category 1B or Category 2.

Specific target organ toxicity following repeated exposure: no relevant effects were found in SIAP for **Na₂EDTA** or structural analogues at dose levels relevant for classification. Therefore no classification is needed for this endpoint.

Aquatic environment, acute toxicity: according to SIAP LC/EC50 for **Na₂EDTA** is 320 – 860 mg/l, and is not readily biodegradable. However, for algae there is only data from 24 DAYS study (or is this typo?). Therefore we should use the structural analogue, **Na₄EDTA**, with EC50 of 1.01 mg/l. Therefore, classification as Aquatic Acute Category 2 is proposed for acute aquatic toxicity.

Aquatic environment, chronic toxicity: data included in SIAP is not sufficient for classification (NOEC (algae) for **Na₂EDTA** is FOR 24 DAYS STUDY. and data from structural analogues is also insufficient). Therefore classification for chronic aquatic toxicity is not possible. However, using the surrogate system (aquatic acute tox from the structural analogue, **Na₄EDTA**, with EC50 of 1.01 mg/l and not rapid degradability) Na₂EDTA should be classified as Aquatic Chronic 2.

CH classification proposal for Disodium EDTA (CAS. 139-33-3)

General Comment

It would be helpful to clearly indicate which is the key study and which are supporting studies and to find a table with the data that is important for classification at the end of the human health part of the SIAP.

Acute Toxicity

Oral

Cat. 5

based on LD₅₀ value in rats 2000-3980 mg/kg bw

Classification based on the information in the SIAP is not possible. The most relevant data for classification was found in the IUCLID file.

Inhalation

Cat. 4

based on LC₅₀ value in rats >1103 mg/m³ ~ 1.103 mg/l for 6h which was extrapolated to 4h

Classification based on the information in the SIAP is only partly possible. The most relevant data for classification was found in the IUCLID file.

Dermal

Not classified

No data available

Irritation

Skin

Not classified

Classification based on the information in the SIAP is not possible since relevant data (Scoring points) is missing. The most relevant data for classification was found in the IUCLID file.

Eye

Not classified

Classification based on the information in the SIAP is not possible since relevant data (Scoring points) is missing. The most relevant data for classification was found in the IUCLID file.

Sensitization

Not classified

Classification based on the information in the SIAP is not possible since relevant data (Scoring points) is missing. The most relevant data for classification was found in the IUCLID file.

Germ cell mutagenicity

Not classified

based on no mutagenicity *in vitro* (Ames test, Chromosome aberration test) and *in vivo* (OECD 474)

Classification based on the information in the SIAP is only partly possible. The most relevant data for classification was found in the IUCLID file.

Carcinogenicity

Not classified

based on no evidence of carcinogenicity in a two-year study in mice and rats with Na₃EDTA

Reproductive toxicity

Not classified

Data is conclusive. However, it is not sufficient for classification.

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STOT SE

Oral

Not classified

based on LD₅₀ value in rats 2000-3980 mg/kg bw and the clinical signs that were only observed at higher doses

Classification based on the information in the SIAP is only partly possible. The most relevant data for classification was found in the IUCLID file.

STOT RE

Oral

Not classified

based on NOAEL of 692 mg/kg bw/day and clinical observations that were only found in the higher doses

Classification based on the information in the SIAP is possible. However, additional information that supports the classification was found in the IUCLID file.

Environmental Hazards

Hazard class		Classification	hazard statement	Rational for the classification
11	Hazardous to the aquatic environment (acute)	Category 3	H402 Harmful to aquatic life	Classification is based on read-across of acute toxicity to the most sensitive fish species <i>Lepomis macrochirus</i> for the close structural analogue Na ₄ EDTA (96h LC ₅₀ 41 mg H ₄ EDTA/L).
11	Hazardous to the aquatic environment (chronic)	Category 3	H412 Harmful to aquatic life with long lasting effects	Chronic NOEC values for fish and daphnia for the structural analogue are above 1 mg/L. Therefore classification for chronic aquatic toxicity is based on acute toxicity to the most sensitive fish species <i>Lepomis macrochirus</i> for the close structural analogue Na ₄ EDTA (96h LC ₅₀ 41 mg H ₄ EDTA/L) and the lack of rapid biodegradation of disodium EDTA.

Key studies used for classification:**Water solubility**

Test method: No information on method

Result (measured): 108 g/L at 20°C, pH=5.3

Reference: O'Neil, Maryadele J. et al. (2006); Source of data: The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals (14th Edition - 1st Electronic Update) ; ECHA, registration dossier for disodium dihydrogen ethylenediaminetetraacetate, Exp Key Water solubility.001; http://apps.echa.europa.eu/registered/data/dossiers/DISS-97dbed15-c225-0814-e044-00144f67d031/AGGR-e7e9ccce-a2ee-411d-ac3c-9cd1e06f2e7a_DISS-97dbed15-c225-0814-e044-00144f67d031.html#AGGR-e7e9ccce-a2ee-411d-ac3c-9cd1e06f2e7a

Partition coefficient octanol-water

Test method: Shake flask method, no further details specified

Result: log Pow -4.3 at 25°C, pH=4.5

Reference: ECHA, registration dossier for disodium dihydrogen ethylenediaminetetraacetate, Exp Key Partition coefficient.002; http://apps.echa.europa.eu/registered/data/dossiers/DISS-97dbed15-c225-0814-e044-00144f67d031/AGGR-22df2ac3-f8a9-4cee-8d38-2523b6f1aedd_DISS-97dbed15-c225-0814-e044-00144f67d031.html#AGGR-22df2ac3-f8a9-4cee-8d38-2523b6f1aedd

Biodegradation

Test substance: CAS 6381-92-6 Ethylenediaminetetraacetic acid, disodium salt, dehydrate

Test method: OECD TG 301 B (Ready Biodegradability: CO₂ Evolution Test); initial conc. 61.9 mg/L; test duration: 28d; reliability: 2 (valid with restrictions)

Result: 0-10% in 28d

Conclusion: not readily biodegradable

Reference: BASF AG (2000). Pruefung der biologischen Abbaubarkeit von NA-EDTA im CO₂-Entwicklungstest, unpublished data, Department of Product Safety, Report No. 99/0668/22/2

Bioaccumulation

Test substance: Na₄EDTA (radiolabelled)

Test method: No guideline method. 28d flow-through bioconcentration test at 21°C and pH 7.4, test concentrations: 0.76 and 0.08 mg/L. The study was conducted according to the methods described by Branson DR, Blau GE, Alexander HC, and Neely WB (1975). Transactions of the American Fisheries Society, Vol. 104, No. 4: 785-792; reliability 2 (valid with restrictions)

Test species: *Lepomis macrochirus*

Result: BCF ca. 1.8 at 0.08 mg/L, BCF ca. 1.1 at 0.76 mg/L

Conclusion: The test substance is not considered to have a potential for bioaccumulation.

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Reference: Bishop WE, Maki AW (1980): A critical comparison of two bioconcentration test methods. Proc. 3rd. Ann. Symp. on Aquatic Toxicity, Eaton JG, Parrish PR, Bionomics, and Hendricks AC, ASTM STP 707: 61-77; data source: ECHA, registration dossier for disodium dihydrogen ethylenediaminetetraacetate, Read across Subs Key Bioaccumulation: aquatic / sediment.001; http://apps.echa.europa.eu/registered/data/dossiers/DISS-97dbed15-c225-0814-e044-00144f67d031/AGGR-fd2408d8-5869-43cc-9270-be241af1b2f8_DISS-97dbed15-c225-0814-e044-00144f67d031.html#AGGR-fd2408d8-5869-43cc-9270-be241af1b2f8
Remark: These test results for the structurally similar analogue Na₄EDTA is used for read-across to the target substance Na₂EDTA.

Acute (short-term) aquatic toxicity

Fish

Test substance: tetrasodium EDTA

Test species: *Lepomis macrochirus*

Test method: 96h acute test static waters of different hardness (test method details reported);

Results: 41 mg H₄EDTA/L in very soft water (10-13 mg/l CaCO₃); 159 mg H₄EDTA/L in medium hard water (103 mg/l CaCO₃); 532 mg H₄EDTA/L in hard water (280-320 mg/l CaCO₃)

Reference: Batchelder TL, Alexander HC and McCarty WM (1980). Acute fish toxicity of the Versenne family of chelating agents. Bull. Environm. Toxicol. 24, 543-549; data source: ECHA, registration dossier for disodium dihydrogen ethylenediaminetetraacetate, Read across Subs Key Short-term toxicity to fish.002; http://apps.echa.europa.eu/registered/data/dossiers/DISS-97dbed15-c225-0814-e044-00144f67d031/AGGR-a872a113-841a-4971-8666-953a87e601cd_DISS-97dbed15-c225-0814-e044-00144f67d031.html#AGGR-a872a113-841a-4971-8666-953a87e601cd

Remark: read-across of acute toxicity for fish from the structurally similar analogue Na₄EDTA to the target substance Na₂EDTA

Invertebrates

Test substance: disodium EDTA

Test species: *Daphnia magna*

Test method: DIN 38412, part 11; 48h static; concentrations: 58, 100, 180, 320 and 580 mg/L; reliability 2 (valid with restrictions)

Result: 48h EC50 140 mg/L

Reference: ECHA, registration dossier for disodium dihydrogen ethylenediaminetetraacetate, Exp Key Short-term toxicity to aquatic invertebrates.001, report date: 1989-09-26; http://apps.echa.europa.eu/registered/data/dossiers/DISS-97dbed15-c225-0814-e044-00144f67d031/AGGR-ffbed38a-6565-4d59-b1a4-74eacdfa1b0_DISS-97dbed15-c225-0814-e044-00144f67d031.html#AGGR-ffbed38a-6565-4d59-b1a4-74eacdfa1b0

Algae

No reliable test data available for disodium EDTA.

Chronic (long-term) aquatic toxicity**Fish**

Test substance: CaNa₂EDTA

Test method: OECD TG 210 (Fish, Early-Life Stage Toxicity Test), 35d flow-through test according GLP, test concentrations: 0.0, 1.1, 3.3, 7.7, 16.4 and 35.1 mg/L (EDTA-anion: 0.0, 0.8, 2.3, 5.4, 11.5 and 24.6 mg/L); reliability: 1 (valid without restrictions)

Test species: *Danio rerio*

Result: NOAEC \geq 25.7 mg H₄EDTA/L; Over the whole study period (day 0 - 35) no statistically significant decreases in survival were observed in any of the concentration groups in comparison to the control group.

Reference: ECHA, registration dossier for disodium dihydrogen ethylenediaminetetraacetate, Read across Subs Key Long-term toxicity to fish.001, report date: 2001-02-27; http://apps.echa.europa.eu/registered/data/dossiers/DISS-97dbed15-c225-0814-e044-00144f67d031/AGGR-642759ad-6b54-413a-8fae-cb9ec193908a_DISS-97dbed15-c225-0814-e044-00144f67d031.html#AGGR-642759ad-6b54-413a-8fae-cb9ec193908a

Remarks: CaNa₂EDTA is considered as a very close structural analogue to the target substance H₂Na₂EDTA. Therefore the data is used for read-across.

Invertebrates

Test substance: disodium EDTA

Test method: EEC Guideline XI/681/86, Draft 4: "Prolonged toxicity study with *Daphnia magna*: Effects on reproduction", 21d semi-static according GLP, Test concentrations (nominal): 100, 50.0, 25.0, 12.5, 6.25, 3.13 and 1.56 mg/L; reliability: 1 (valid without restrictions)

Test species: *Daphnia magna*

Result: 21d NOEC (reproduction) 25 mg/L (nominal)

Reference: ECHA, registration dossier for disodium dihydrogen ethylenediaminetetraacetate, Exp Key Long-term toxicity to aquatic invertebrates.001, report date: 1998-10-25; http://apps.echa.europa.eu/registered/data/dossiers/DISS-97dbed15-c225-0814-e044-00144f67d031/AGGR-dadc3c4d-1c3d-4856-8c31-cc3bd4f325d7_DISS-97dbed15-c225-0814-e044-00144f67d031.html#AGGR-dadc3c4d-1c3d-4856-8c31-cc3bd4f325d7

	Asp. Tox.	Acute Tox.	Skin Irrit.	Eye Irrit.	Skin sentiz.	STOT SE	STOT RE	Muta.	Carc.	Repro.	Aqua. Acute	Aquatic Chronic
Proposed draft classification ITALY	No data	Acute Tox 4, H302 (o)	Not Classified	Not Classified	Not Classified	STOT SE 3, H335	Not Classified	Not Classified	Not Classified	Not Classified	Not Classified	Not Classified

CAS: 139-33-3 Chemical name: Disodium EDTA	<u>Mammalian toxicity endpoints</u>					
	Acute toxicity Oral					
	Organism	Methods	Data	Reference	GHS classification	Rationale/Comments
	Rat (Wistar)	OECD Guideline 401	> 1780 LC ₅₀ < 2000 mg/kg bw	BASF AG (1983).	Acute Tox 4, H302	This study is reported in the IUCLID DataSet but it cannot be found in the SIAR
	Acute toxicity Inhalation					
	Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments
	Rat (Wistar)	OECD Guideline 412	6/20 deaths at 1000 mg/m ³ (one day expos. only); exposure related congestion, edema, hemorrhage and inflammation after 5-days; fully reversible after 14 days; no NOAEC could be assigned	BASF SE, 2010	Not Classified	
	Acute toxicity Dermal					
	Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments
	No Data	No Data	No Data	No Data	No Data	No animal data are available as reported in SIAP table 4.

						Taking into account the poor dermal absorption (Foremann, 1954)
Corrosive/irritant skin						
Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments	
Rabbit Vienna white strain	Comparable to OECD 404 guideline study (1992) with acceptable restrictions	well defined erythema was observable in animal 2, which was fully reversible within 72 h. 1- 15 min exposure also did not cause edema or erythema Not irritating under the conditions of the study	BASF AG. (1973)	Not Classified	No classification according to GHS criteria as the mean value is 1,3 for erythema in 1/2 animal.	
Corrosive/irritant eyes						
Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments	
Rabbit Vienna white	equivalent or similar to OECD Guideline 405 (Acute Eye Irritation / Corrosion)	Both animals showed some redness of the conjunctivae (score 1), 24 h after application of the TS Non irritating	BASF AG 1973	Not Classified		
Rabbit	No specified	In both study (Na2EDTA	Olson 1962a; Pinkerton and		Only 1 animal used in both studies.	

			dehydrate) result slight irritant	Schwebel, 1976a		
Sensitizer Skin and respiratory tract						
Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments	
Guinea pig, strain Hartley, female	Guinea pig maximization test OECD 406	The authors concluded that although erythema was observed after the first challenge in 3/10 animals (the criterion for a positive test), it was not due to delayed hypersensitivity since it was mild and transient in nature and was observed only in 1/10 animals after the second challenge. Not sensitizing	Manciaux, 2000	Not Classified		
Gene mutation						
Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments	
	Ames	negative	DeFlora, 1981 Dunkel <i>et al.</i> , 1999	Not classified	Various negative findings in <i>in vitro</i> and <i>in vivo</i> tests The positive result obtained in micronucleus test (Zordan <i>et al.</i> , 1990) with extremely high dose is not confirmed in the SIAR.	
	Mouse lymphoma, L5178Y/TK+/-	negative	Dunkel <i>et al.</i> , 1999 Whittaker <i>et al.</i> 2001			
	Syrian Hamster Ovary Cell Transformation	negative	LeBoeuf <i>et al.</i> , 1990			

	Assay				
	CHL Fibroblasts/CA	Weak positive	Ashby and Ishidate, 1986		
	MN (mouse bone marrow)	Negative, (5)	BASF AG 2000		
	Cytogenicity (mouse germ cell)	Negative/Positive?	Zordan <i>et al.</i> , 1990		
	Dom. Lethal (mouse)	Negative	Muraldihara and Narasimhamurthy, 1991		
	MN (mouse bone marrow)	Negative (CAS 6381-92-6 tested, dihydrate of 139-33-3)	Russo and Levis, 1992		
Carcinogenicity					
Organism	Methods	data	Reference	GHS Classification	Rationale/Comments
Fischer 344 rats B6C3F1 mice	standard carcinogenicity study	An oral two-year study with Na ₃ EDTA trihydrate in mice and rats indicated no evidence of carcinogenicity	NCI-CG-TR-11, 1977 in RAR-EU	Not classified	No data available for Na ₂ EDTA
Reproductive/Developmental toxicity					
Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments
Wistar albino rats	No indicated	The weight of evidence from a two-generation reproductive toxicity study in rats shows that dietary ingestion of 1% Na ₂ EDTA (approx. 920	Yang, 1952	Not classified	Members of the aminocarboxylic acid-based chelants category would not be expected to exhibit reproductive and developmental effects in the absence of a metal deficiency which is not expected under normal nutrition.

			mg/kg bw/day) had no effect on reproduction; however, no litters were produced at 5% (approx. 4600 mg/kg bw/day); the NOAEL for reproductive toxicity was 920 mg/kg bw/day.			
	Sprague-Dawley rats	No indicated	Developmental study: LOEL = 1,000 mg/kg bw/day (dietary exposure) Effects: implantation sites having dead, resorbed or malformed fetuses, increased incidence in malformed live pups and a decreased mean fetal body weight. These effects are observed in the presence of maternal toxicity and are related to plasma zinc concentrations.	Swenerton and Hurley, 1971		
Single dose toxicity						
	Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments

	Wistar rat	No indication. Guideline study, following GLP	Conc. of 30, 300 or 1000 mg/m ³ ; 6/20 deaths at 1000 mg/m ³ (one day expos. only); exposure related congestion, edema, hemorrhage and inflammation after 5-days; fully reversible after 14 days; no NOAEC could be assigned	BASF SE, 2010	STOT SE 3,H335	Not classified as STOT SE 1 or 2
Repeated dose toxicity						
	Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments
	Holtzman rats	No specified	4206 . mg/kg bw Effect: Decreased body weights and food consumption; mortality, & diarrhea. There were no effects of treatment on hematocrit, hemoglobin, total and differential white cell counts, prothrombin time or serum calcium levels at 13 weeks.	Wynn et al. 1970	Not classified	
Aspiration Toxicity						
	Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments
	No data	No data	No data	No data	No data	
Environmental toxicity endpoints						

Acute toxicity						
Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments	
Fish	Directive 84/449/EEC, C.1 "Acute toxicity for fish"	LC ₅₀ = 320 mg/L	Balk, 1989	Not Classified		
Aquatic Invertebrates	DIN 38412 Part 11	EC ₅₀ = 140 mg/L	BASF AG. 1989	Not Classified	This study is reported in the IUCLID DataSet but it cannot be found in the SIAR. In the SIAR another study is reported which is missing in the IUCLID DataSet.	
Aquatic Plants	Study NA ₄ EDTA EU Method C.3	EC ₅₀ =2.77 mg/L	BASF AG (1995)	Not Classified	Toxicity is associated to the chelation of essential nutrients by the category members and it is not considered a relevant environmental hazard in view of the abundance of such nutrients in aquatic environments. Indirect effects like nutrient deficiency and eutrophication could only qualitatively be assessed; they are unlikely to occur in the environment although they cannot be excluded.	
Chronic toxicity						
Organism	Methods	Data	Reference	GHS Classification	Rationale/Comments	
Fish	No data	No data	No data	Not Classified	Adequate chronic toxicity data are not available. However acute toxicity fish and aquatic invertebrates LC50 and EC50 are greater than 100 mg/L so that no classification seems to be appropriate	
Aquatic Invertebrates	EEC Guideline XI/681/86	NOEC= 25 mg/L	BASF AC. (1998)	Not Classified		
Aquatic Plants	Study on NA ₄ EDTA EU	NOEC=0.39 mg/L	BASF AG (1995)	Not Classified	Toxicity is associated to the chelation of essential nutrients by the category	

		Method C.3				members and is not considered a relevant environmental hazard in view of the abundance of such nutrients in aquatic environments
	There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account. (RAR NA ₄ EDTA)					

Hazard class	Substance data / Note	Criteria of Category according to GHS	Conclusion/ Commentary
Explosives	No data available	There are no chemical groups associated with explosive properties present in the molecule	Not classified
Flammable gases	Substance is a solid		Not classified
Aerosols	Substance is a solid		Not classified
Oxidizing gases	Substance is a solid		Not classified
Gases under pressure	Substance is a solid		Not classified
Flammable liquids	Substance is a solid		Not classified
Flammable solids	No data available		Not classified
Self-reactive substances and mixtures	No data available	There are no chemical groups present in the molecule associated with explosive or self-reactive properties	Not classified
Pyrophoric liquids	Substance is a solid		Not classified
Pyrophoric solids	No data available / Study scientifically unjustified	Substance is known to be stable at room temperature for prolonged periods of time (days)	Not classified
Self-heating substances and mixtures	No data available		Not classified
Substances and mixtures which, in contact with water, emit flammable gases	Substance has a high water solubility (105 g/l at 20°C)		Not classified
Oxidizing liquids	Substance is a solid		Not classified
Oxidizing solids	No data available	Substance contains oxygen, which is chemically bonded	Not classified

		only to carbon	
Organic peroxides	Substance is not organic peroxides		Not classified
Corrosive to metals	No data available		Not classified
Acute toxicity / oral	LC ₅₀ (rat) = 2000 – 3980 mg/kg bw	300 < Category 4 ≤ 2000	Category 4
Acute toxicity / dermal	No data available on this substance		Not classified
Acute toxicity / inhalation	No deaths (1.13 mg/L, 7 hr)		Not classified. <i>The information is not enough for classification</i>
Skin corrosion/irritation	Slightly irritating. The aminocarboxylic acid-based chelants are not irritating or only slightly irritating to the intact skin. The irritancy potential is related to the pH of the individual salt. Thus, more acidic members of the category such as disodium EDTA have inherently greater irritancy potential.		Not classified. <i>Based on available data, the classification criteria are not met</i>
Serious eye damage/ eye irritation	Slightly irritating. The aminocarboxylic acid-based chelants are only slightly to moderately irritating to the eyes in rabbits. The irritancy potential is related to the pH of the individual salt. Thus, more acidic members of the category such as disodium EDTA have inherently greater irritancy potential.		Not classified. <i>Based on available data, the classification criteria are not met</i>

Respiratory or skin sensitization	The aminocarboxylic acid-based chelants are not skin sensitisers.		Not classified
Germ cell mutagenicity	Negative test results		Not classified
Carcinogenicity	No data available on this substance. Na ₃ EDTA trihydrate and CaNa ₂ EDTA were not carcinogenic in rats and mice, respectively. The amino carboxylic acid-based chelants category members are not expected to be carcinogens.		Not classified <i>Based on read-across data</i>
Reproductive toxicity	The weight of evidence from a two-generation reproductive toxicity study in rats shows that dietary ingestion of 1% Na ₂ EDTA (approx. 920 mg/kg bw/day) had no effect on reproduction; however, no litters were produced at 5% (approx. 4600 mg/kg bw/day); the NOAEL for reproductive toxicity was 920 mg/kg bw/day. Studies on developmental toxicity showed a specific fetotoxic and teratogenic potential of Na ₂ EDTA	Category 2: Substances for which there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development	Category 2
Specific target organ toxicity – Single exposure	Conc. of 30, 300 or 1000 mg/m ³ ; 6/20 deaths at 1000 mg/m ³ (one day expos. only, rat); exposure related congestion, edema, hemorrhage and inflammation after 5-days; fully reversible after		Not classified <i>The data is not enough for classification</i>

	14 days; no NOAEC could be assigned		
Specific target organ toxicity – Repeated exposure	NOAEL (rat, oral feed, 13 weeks) = 692 mg/kg bw	Category 2: oral, rat, 90d $10 < C \leq 100$ mg/kg bw/d	Not classified
Aspiration hazard	No data available		Not classified
Hazardous to the aquatic environment/ acute hazard	EC ₅₀ (Daphnia magna, 48h) = 140 mg/l	Category 3: $10 < EC_{50} \leq 100$	Not classified <i>Based on IUCLID's data</i>
Hazardous to the aquatic environment/ long-term hazard	NOEC (Daphnia magna STRAUS, 21d) = 25 mg/l	Category 3: $0.1 < NOEC \text{ or } EC_x \leq 1$	Not classified <i>Based on IUCLID's data</i>
Hazardous to the ozone layer	Substance not listed in Annexes to the Montreal Protocol	Substance not listed in Annexes to the Montreal Protocol	Not classified

BIAC (JCIA) classification proposal for Disodium EDTA (CAS. 139-33-3)

Proposed draft classification	Asp. Tox.	Acute Tox.	Skin Irrit.	Eye Irrit.	Skin sentiz.	STOT SE	STOT RE	Muta.	Carc.	Repro.	Aqua. Acute	Aquatic Chronic
	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
No data	data not sufficient: no LC50 in acute inhal study	Data sufficient	Data sufficient	Data sufficient	Data not sufficient	Data sufficient	Data sufficient	Data sufficient	Data sufficient (read across)	Data not sufficient (but conclusive)		Data sufficient (based in IUCLID data)

Note:

(i): inhalation (o): oral

NC = not classified

Comment: All of the hazardous classes indicating above are “Classification is not possible” or “Not classified”.

ENV/JM/MONO(2014)31/ANN

JP Classification for the Environment: proposal for Disodium EDTA (CAS. 139-33-3)

	EDTA(2Na)
Aquatic Acute Classification	Not classified Not sufficient; chelation not considered relevant for aquatic environment
Aquatic Chronic Classification	Not classified Not sufficient; chelation not considered relevant for aquatic environment

Classification for Titanium Dioxide (CAS. 13463-67-7)

Notified classification for Titanium Dioxide (CAS. 13463-67-7)

Notified classifications	Asp. Tox.	Acute Tox.	Skin Irrit.	Eye Irrit.	Skin sensitiz.	STOT SE	STOT RE	Muta.	Carc.	Repr. o.	Aqua. Acute	Aqua. Chro.	number of notified
ECHACHEM 1		Acute Tox. 4 (i)							Carc. 2				41
ECHACHEM 2				Eye Irrit. 2		STOT SE 3	STOT RE 1						36
ECHACHEM 3									Carc. 2				18
ECHACHEM 4			Skin Irrit. 2	Eye Irrit. 2		STOT SE 3							17
ECHACHEM 5						STOT SE 2			Carc. 2				10
ECHACHEM 6		Acute Tox. 4 (i)							Carc. 1B				9
ECHACHEM 7		Acute Tox. 4 (o)	Skin Irrit. 2	Eye Irrit. 2		STOT SE 3	STOT RE 1						8
ECHACHEM 8				Eye Irrit. 2		STOT SE 3	STOT RE 1					Aqua. Chro. 4	7
ECHACHEM 9				Eye Irrit. 2		STOT SE 3	STOT RE 1						5

ECHACHEM 10						STOT SE 3	STOT RE 1		Carc. 2			Aqua. Chro. 4	4
ECHACHEM 11				Eye Irrit. 2		STOT SE 3	STOT RE 1					Aqua. Chro. 4	4
ECHACHEM 12				Eye Irrit. 2		STOT SE 3	STOT RE 1						3
ECHACHEM 13		Acute Tox. 4 (o)(i)(d)											3
ECHACHEM 14						STOT SE 3							2
ECHACHEM 15			Skin Irrit. 2	Eye Irrit. 2		STOT SE 3			Carc. 2			Aqua. Chro. 4	2
ECHACHEM 16		Acute Tox. 4 (o)(i)											1
ECHACHEM 17				Eye Irrit. 2		STOT SE 3							1
ECHACHEM 18		Acute Tox. 4 (o)	Skin Irrit. 2										1
ECHACHEM 19				Eye Irrit. 2		STOT SE 3	STOT RE 1		Carc. 2			Aqua. Chro. 4	1
ECHACHEM 20				Eye Irrit. 2		STOT SE 3	STOT RE 1		Carc. 2			Aqua. Chro. 4	1
ECHACHEM 21				Eye Irrit. 2		STOT SE 3	STOT RE 1		Carc. 2			Aqua. Chro. 4	1
ECHACHEM 22						STOT SE 3							1
ECHACHEM 23							STOT RE 2		Carc. 2				1
ECHACHEM 24				Eye Irrit. 2		STOT SE 3	STOT RE 1		Carc. 2			Aqua. Chro. 4	1
ECHACHEM 25						STOT SE 3	STOT RE 1		Carc. 2			Aqua. Chro. 4	1
ECHACHEM 26				Eye Irrit. 2		STOT SE 3	STOT RE 1					Aqua. Chro. 4	1
ECHACHEM 27						STOT SE 3	STOT RE 1		Carc. 2			Aqua. Chro. 4	1
ECHACHEM 28				Eye Irrit. 2		STOT SE 3	STOT RE 1					Aqua. Chro. 4	1
GHS-J				Eye Irrit. 2B		STOT SE 3	STOT RE 1					Aqua. Chro. 4	-

ENV/JM/MONO(2014)31/ANN

BIAC (JCIA) classification proposal for Titanium Dioxide (CAS. 13463-67-7)

Proposed draft classification	Asp. Tox.	Acute Tox.	Skin Irrit.	Eye Irrit.	Skin sensitiz.	STOT SE	STOT RE	Muta.	Carc.	Repro.	Aqua. Acute	Aqua. Chro.
	NC	NC	NC	Eye Irrit. 2B	NC	NC	NC	NC	Carc. 2	NC	NC	NC
	No data	data not sufficient: no LC50 in acute inhal study	Data not sufficient: lack of study detail	based on previous conclusion (IUCLID); but scores not reported	data sufficient (based on IUCLID data)	Data not sufficient	Data not sufficient	Data sufficient	based on previous conclusion (IARC)	Data not sufficient		Data not sufficient

NC = not classified

CoCAM 5 – Phase Two of the Exercise***2-Vinylpyridine (2-VP) (CAS 100-69-6)****The Netherlands (RIVM)*

Note: Classification was proposed for skin corrosion even though this was not listed as an endpoint. However, the information was available and we felt it was proper to use in this case since the data available was useful for the classification of eye irritation.

Acute Toxicity*Inhalation***Classification proposal:** Not possible**Key Data:**

Rationale: Lack of reliable data. Three experimental studies in animals (2 in rats and 1 in mouse) all scored unreliable (Klimisch score 4). Agree with assigned reliability mainly due to the lack of information regarding the experimental details for example, information on purity and concentration of substance used, type of exposure and descriptive details etc. Secondary information on humans is available however this is also considered limited for classification purposes.

Level of detail: SIAP, SIAR and dossier*Dermal***Classification proposal:** Cat 1**Key Data:** LD₅₀ of 640 mg/kg bw in rabbit and 160 mg/kg bw in guinea pigs**Rationale:** Lowest LD₅₀ was used**Level of detail:** SIAP*Oral***Classification proposal:** Cat 2**Key Data:** LD₅₀ in rats range between 50 mg/kg bw < LD₅₀ > 300 mg/kg bw (OECD 423)**Rationale:** Based on lower and upper limits of range**Level of detail:** SIAPIrritation*Eye***Classification proposal:** Cat 1**Key Data:** Data relating to animal studies that indicates 2-VP to be corrosive to the skin.

Reliable (with restriction) secondary source of experimental result (1994): skin necrosis was observed at all test sites 48h after application in rabbit (New Zealand White)

Rationale: Testing and evaluation strategy for serious eye damage and eye irritation, Step 1b (Figure 3.3.1, GHS Guidance)**Level of detail:** SIAR and dossier

Skin

Classification proposal: Not possible

Key Data: The substance is considered corrosive therefore most likely to be highly irritant.

Rationale: Lack of data, scoring data not provided

Level of detail: SIAP/SIAR/dossier

Skin Corrosion

Classification proposal: Cat 1B

Key Data: Reliable (with restriction) secondary source of experimental result (1994): skin necrosis was observed at all test sites 48h after application in rabbit (New Zealand White)

Other considerations: Study report (1983) GLP compliant: skin irritation was determined by a 24-h occluded skin irritation test in guinea pigs and was found to be severe.

Rationale: skin necrosis observed (in at least 1 of 3 tested animals) after exposure ≤ 1 hour (guidance Table 3.2.1).

Level of detail: SIAR and dossier

Sensitization

Respiratory Sensitization

Classification proposal: Not possible

Key Data:

Rationale: Lack of data

Level of detail: SIAP/SIAR/dossier

Skin Sensitization

Classification proposal: Cat 1

Key Data: Equivalent or similar study to OECD 429 in mouse (LLNA) indicated the substance to be positive for sensitizing, stimulation index > 3.

Rationale: For Cat 1 in animal studies, a stimulation index of three or more is considered a positive response in the LLNA

Other considerations: positive data from patch testing: in one case study the authors suspected cross-sensitization among pyridine derivatives and in second case study the author concluded that an employee became sensitized to 2-vinylpyridine despite wear full protective gear (patient developed severe dermatitis at the site of contact, secondary eczematization over the flexures and periungual areas and marked systemic upset); positive animal data from non-standard methods or reporting of minimal data; and structural alert for pyridine as a known sensitizers

Level of detail: SIAP, SIAR and Dossier

Germ Cell Mutagenicity

Classification proposal: Not possible

Key Data: - 2-VP induced gene mutations in a bacterial test *in E. coli* with exogenous metabolic activation.

- In four other studies (3-reliable with restrictions and 1 not reliable), mutagenicity to *Salmonella typhimurim* was observed in only one study in the presence of exogenous reductive metabolic activation.
- 2-VP induced chromosomal aberrations in CHL/IU cells with or without exogenous metabolic activation.
- In a non-bacterial *in vitro* UDS assay (rat hepatocytes cells), 2-vinylpyridine showed negative results at a low concentration (2.5 mmol). 2-VP is cytotoxic at low concentrations.

Rationale: Lack of data. Based on the data, 2-VP could be considered *in vitro* mutagenic. But the data is not sufficient to draw a conclusion for classification purposes based on Figure 3.5.1 of the GHS guidance.

- *In vivo* data for 2-VP is not available (germ or somatic) and structural similarity to known germ cell mutagens was not investigated.

Level of detail: SIAP, SIAR and dossier

Carcinogenicity

Classification proposal: Not possible

Key Data:

Rationale: No reliable carcinogenicity studies were identified. In a limited tumorigenicity study in which female A/J mice were treated with 2-VP by intraperitoneal injections for 7 weeks, no tumors were seen.

Level of detail: SIAR and SIAP

Reproductive Toxicity

Classification proposal: Cat 2

Key Data: Reproduction /development toxicity screening study available (OECD 421; GLP: rats); Parental general toxicities are considered to be 20 mg/kg bw/day and more; **NOAEL** for reproductive toxicity is 20 mg/kg bw/day. This is based on dystocia at 50 mg/kg bw/day. **LOAEL** for developmental toxicity is 20 mg/kg bw/day, the lowest dose. This is based on decreased body weights in pups in all treatment groups. NOAEL could not be determined.

Other considerations: In the repeated dose 92-day study, increased relative ovary weights were observed and 1 female rat (1/20) exhibited ovarian congestion at 180 mg/kg bw/day. However, it is unknown as to how these changes affected reproductive parameters in this study.

Rationale: Observed effect of dystocia at 50 mg/kg bw/day. Prolonged parturition is considered an effect on sexual function. The dystocia effect is clear however what is unknown is whether the effect is itself direct or secondary in the presence of maternal toxicity.

Level of detail: SIAP, SIAR and dossier

STOT-SE

Inhalation

Classification proposal: Not possible

Key Data:

Rationale: No data

Level of detail: SIAP/SIAR/dossier

Dermal

Classification proposal: Not possible, useful information not available

Key Data: Clinical signs, such as lethargy, prostration, labored breathing, aggressive behavior, and convulsions, were observed in animals treated with lethal doses (≥ 300 mg/kg bw). Severe irritations were observed.

Rationale: Studies do not provide useful information for STOT-SE evaluation

Level of detail: SIAP, SIAR and dossier

Oral

Classification proposal: No classification

Key Data: Toxicological effects on stomach (28-d and 92-d repeated dose studies in rats): **NOAELs** for local and systemic effects = 12.5 mg/kg bw/day. Conclusion: local gastric effects

Rationale: These effects are covered by oral LD₅₀ (LD₅₀ in rats range between 50 mg/kg bw < LD₅₀ > 300 mg/kg bw (OECD 423)).

2-VP is classified as Acute Toxicity Cat 2 (oral) therefore it is not necessary to classify as STOT-SE.

Level of detail: SIAP, SIAR and dossier

STOT-RE

Oral

Classification proposal: No classification

Key Data: 92-day repeated dose study in rats (EPA OPPTS 870.31000; GLP)

NOAELs for local and systemic effects = 12.5 mg/kg bw/day.

LOAELs of both local and systemic effects estimated to be 20 mg/kg bw/day (based on increased relative kidney weights and histopathological changes in the gastric epithelium).

Supporting information: 28-day repeated dose study in rats (Japanese guideline under GLP)

NOAEL of local and systemic effects = 12.5 mg/kg bw/day (based on toxicological effects on stomach, squamous hyperplasia and submucosal edema in the forestomach in both sexes receiving 50 and 200 mg/kg bw/day, along with thickening of the mucosa at higher doses. Submucosal edema and/or erosion in the glandular stomach in females receiving 50 or 200 mg/kg bw/day).

Rationale: Local gastric irritation (mainly protective effect) is normally an acute effect that is irreversible. Irritation/corrosion effects are more dependent on concentration rather than dose.

Level of detail: SIAR and dossier

Aspiration Toxicity

Classification proposal: Not possible

Key Data:

Rationale: Lack of data

Level of detail: SIAP/SIAR/dossier

Environment

Acute Aquatic Toxicity

Classification proposal: Acute Aquatic Cat 2

Key Data: Fish (*Oryzias latipes*) 96-h LC₅₀ = 6.5 mg/L (OECD 203; GLP);
Invertebrate (*Daphnia magna*) 48-h EC₅₀ = 9.5 mg/L (OECD 202; GLP)
and algae (*Pseudokirchneriella Subcapitata*) 72-h ErC50 = 62 mg/L
(OECD 201; GLP)

Rationale: Information is available for three trophic levels and for classification purposes the lowest aquatic toxicity value (Fish 96-h LC₅₀ = 6.5 mg/L) is used

Level of detail: SIAP, SIAR and dossier

Chronic Aquatic Toxicity

Classification proposal: Chronic Aquatic Cat 2

Key Data: Invertebrate (*Daphnia magna*) 21-d NOEC reproduction = 0.9 mg/L (OECD 211; GLP)
Algae (*Pseudokirchneriella Subcapitata*) 72-h NOErC = 27 mg/L (OECD 201; GLP)

Rationale: Chronic information is available for invertebrates and algae. The surrogate approach (according to Figure 4.1.1 of the GHS guideline environment section) was used for classification purposes.
21-d NOEC for Daphnia = 0.9 mg/L; 72-h NOErC for algae = 27 mg/L;
96-h LC50 fish = 6.5 mg/L.
2-VP is not readily biodegradable.

Level of detail: SIAP, SIAR and dossier

Switzerland (Human Health only)

2-Vinylpyridine (CAS 100-69-6)

Acute Toxicity

Inhalation

Classification proposal: Not classified

Key Data: none

Rationale: No valid data available which allows classification.

Level of detail: SIAP/SIAR/dossier

Dermal

Classification proposal: Cat 2

Key Data: LD₅₀ of 160 mg/kg bw in guinea pig

Rationale: LD₅₀ for rabbit (640 mg/kg bw) and guinea pig (160 mg/kg bw) were available. Since both studies have a reliability of two and both are listed without any further prioritisation, the lowest LD₅₀ was used for classification purposes.

Level of detail: SIAP

Oral

Classification proposal: Cat 3
Key Data: LD₅₀ >50 and <300 mg/kg bw in rats
Rationale: LD₅₀ >50 and <300 mg/kg bw leads to a classification into Cat 3.
Level of detail: SIAP

Irritation

Eye

Classification proposal: Serious Eye Damage Cat 1
Key Data: Same as for skin corrosion
Rationale: 2-vinylpyridine caused skin necrosis in all rabbits 48 hours after dosing and was therefore classified as skin corrosive.
Level of detail: SIAP/SIAR/dossier

Skin

Classification proposal: Corrosive Cat 1
Key Data: The application of 0.5 ml [480 mg] undiluted 2-vinylpyridine to the shaved intact skin of New Zealand white rabbits (5 males and 1 female) for 1 hour (skin wiped after 1 hour) caused skin necrosis in all rabbits 48 hours after dosing.
Rationale: 2-vinylpyridine caused skin necrosis in all rabbits 48 hours after dosing and was therefore classified as corrosive
Level of detail: SIAP/SIAR

Sensitization

Respiratory Sensitization

Classification proposal: Not classified
Key Data: none
Rationale: No valid data available which allows classification.
Level of detail: SIAP/SIAR/dossier

Skin Sensitization

Classification proposal: Cat 1
Key Data: Stimulation index >3 in mouse LLNA; strong positive reactions to 0.5% 2-vinylpyridine in human patch test and 80% response in GPMT
Rationale: All above mentioned test results lead to a classification into category 1.
Level of detail: SIAR/Dossier

Germ Cell Mutagenicity

Classification proposal: Not classified
Key Data: Positive in OECD TG 471, 472 and 473
No *in vivo* data identified
Rationale: Insufficient data. No information on structural analogs is available in the dossier.
Level of detail: SIAP/SIAR/dossier

Carcinogenicity

Classification proposal: Not classified

Key Data: none
 Rationale: No adequate carcinogenicity study was identified.
 Level of detail: SIAP/SIAR/dossier

Reproductive Toxicity

Classification proposal: Not classified
 Key Data: On the basis of stillbirth in pups at 50 mg/kg bw/day, NOAEL for reproductive toxicity was estimated to be 20 mg/kg bw/day, while parental general toxicity was observed at 20 mg/kg bw/day. On the basis of the decreased body weights in pups in all treatment groups, LOAEL for developmental toxicity was estimated to be 20 mg/kg bw/day in rats, the lowest dose tested.
 Rationale: Due to the fact that it is not clear whether the observed developmental effects are due to parental toxicity, a classification is not possible.
 Level of detail: SIAP/SIAR/dossier

STOT-SE

Inhalation

Classification proposal: Not classified
 Key Data: none
 Rationale: No valid data available which allows classification.
 Level of detail: SIAP/SIAR/dossier

Dermal

Classification proposal: Not classified
 Key Data: none
 Rationale: A classification is not possible since details on toxic effects were missing.
 Level of detail: SIAP/SIAR/dossier

Oral

Classification proposal: Not classified
 Key Data: Clinical signs of toxicity including excessive salivation, soft feces, reddening of legs and auricles, soiling of perioral and perianal regions, tachypnea, prostration, weakness, tremors, vasodilatation, and anorexia.
 Rationale: The substance is not classified since the observed effects do not support a classification.
 Level of detail: SIAP/SIAR/dossier

STOT-RE

Oral

Classification proposal: Not classified
 Key Data: NOAEL of 12.5 mg/kg bw/day and clinical signs of toxicity (decrease in body weight, increased kidney, liver, ovary, testis weights and decreased spleen weight)
 Rationale: The substance is not classified because all the observed clinical signs were either reversible or not substance related.

Level of detail: SIAP/SIAR/dossier
Aspiration Toxicity
Classification proposal: Not classified
Key Data: none
Rationale: No valid data available which allows classification.
Level of detail: SIAP/SIAR/dossier

Russian Federation

2-Vinylpyridine (CAS 100-69-6)

Human Health

Acute Toxicity

Inhalation

Classification proposal: Not classified
Key Data: LC₅₀(rat): > 160 — < 5500 ppm
Reliability: 2 (reliable with restrictions)
Rationale: Classification is not possible due to lack of details on study (unknown time)
Level of detail: dossier

Dermal

Classification proposal: Cat 2
Key Data: LD₅₀ was 640 mg/kg bw in rabbits and 0.16 mL/kg in guinea pigs
Rationale: For classification purposes the lowest LD₅₀ was used (LD₅₀ in guinea pigs: 0.16 ml/kg = 159.73 mg/kg at density 0.9983 g/cm³)
Level of detail: SIAP

Oral

Classification proposal: Cat 3
Key Data: LD₅₀ in female rats was 50 – 300 mg/kg bw and LD₅₀ in male and female rats was 336 (240–472) mg/kg bw
Rationale: For classification purposes the lowest LD₅₀ was used
Level of detail: SIAR

Irritation

Eye

Classification proposal: Cat 2A
Key Data: Strong eye irritation was observed in rabbits. Corneal and adnexal staining was observed in all animals. Prompt irrigation with distilled water was palliative
Rationale: Data is enough for classification purposes
Level of detail: SIAR

Skin

Classification proposal: Cat 1B

Key Data: Skin necrosis was observed at all test sites of New Zealand White rabbits (5 males and 1 female/dose) 48 h after application.
Duration of treatment / exposure: 1h
Reliability: 2 (reliable with restrictions)

Rationale: Data is enough for classification purposes

Level of detail: dossier

Sensitization

Respiratory Sensitization

Classification proposal: Not classified

Key Data: No information is available

Rationale:

Level of detail: SIAP/SIAR/dossier

Skin Sensitization

Classification proposal: Cat 1B

Key Data: In a standardized skin sensitization test performed on 10 guinea pigs, 2 animals showed no response, 3 showed a weak response, 4 showed a moderate response, and 1 showed a potent response.

Rationale: Data is enough for classification purposes

Level of detail: SIAR

Germ Cell Mutagenicity

Classification proposal: Cat 2

Key Data: In a bacterial reverse mutation assay (Ames test) with multiple strains of *Salmonella typhimurium* and *Escherichia coli* (OECD TG 471 and 472 and Guidelines for screening mutagenicity testing of chemicals, Japan), 2-vinylpyridine showed clear mutagenic responses in *Escherichia coli* with exogenous metabolic activation, although no mutagenicity to *Salmonella typhimurium* was observed with or without exogenous metabolic activation. In addition, an *in vitro* chromosomal aberration test (OECD TG 473) showed positive results both with and without metabolic activation.

Rationale: Positive result from *in vitro* experiment

Level of detail: SIAP/SIAR

Carcinogenicity

Classification proposal: Not classified

Key Data: 2-Vinylpyridine was administered at total doses of 200 µmol by i.p. injection to 25 female A/J mice per group. None of the animals developed significant number of lung adenomas or any other type of tumors. In contrast, the positive control (treated with the tobacco specific N-nitrosamine NNK) induced a high incidence of lung adenomas in A/J mice (24.0 ± 7.0/mice).

Rationale: On the basis of data from these bioassays, 2-vinylpyridine was considered to be non-tumorigenic in A/J mice. No information is available about carcinogenicity studies (inhalation/ dermal/ oral)

Level of detail: SIAP/SIAR/dossier

Reproductive Toxicity

Classification proposal: Cat 2

Key Data: Three female rats died during gestation and 4 female rats died during lactation at 125 mg/kg bw/day. Two female rats showed dystocia at 125 mg/kg bw/day and were euthanized. All newborn pups from 1 dam were stillborn at 50 mg/kg bw/day, which was estimated to be a toxicological effect. NOAEL for reproductive toxicity was estimated to be 20 mg/kg bw/day, while parental general toxicity was observed at 20 mg/kg bw/day (see the repeated dose section). In offsprings, body weights decreased in all dosing groups. LOAEL for developmental toxicity in rats was therefore estimated to be 20 mg/kg bw/day.

Rationale: Positive evidence from experiments in mammals

Level of detail: SIAR

STOT-SE

Inhalation

Classification proposal: Not classified

Key Data: No information is available

Rationale:

Level of detail: SIAP/SIAR/dossier

Dermal

Classification proposal: Not classified

Key Data: No information is available

Rationale:

Level of detail: SIAP/SIAR/dossier

Oral

Classification proposal: Not classified

Key Data: No information is available

Rationale:

Level of detail: SIAP/SIAR/dossier

STOT-RE

Oral

Classification proposal: Cat 1

Key Data: NOAEL (rat, 28d) = 12.5 mg/kg bw/day, toxicological effects observed in the stomach
LOAEL (rat, 92d) = 20 mg/kg bw/day, the increased relative kidney weights in males and histopathological changes in the gastric epithelium

Rationale: For classification purposes NOAEL was used

Level of detail: SIAR

Aspiration Toxicity

Classification proposal: Not classified

Key Data: No information is available

Rationale: The substance is not a hydrocarbon and there is no human evidence

Level of detail: SIAP/SIAR/dossier

Environment

Acute Aquatic Toxicity

Classification proposal: Cat 2

Key Data: Fish (*Oryzias latipes*, 96 h): LC₅₀ = 6.5 mg/L (nominal)
Daphnia (*Daphnia magna*, 48 h): EC₅₀ = 9.5 mg/L (measured)
Algae (*Pseudokirchneriella subcapitata*, 72 h): ErC₅₀ = 62 mg/L
(measured, growth rate)

Rationale: For classification purposes the lowest LC₅₀ was used

Level of detail: SIAP

Chronic Aquatic Toxicity

Classification proposal: Cat 2

Key Data: 2-vinilpyridine is considered to be not-readily biodegradable
Daphnia (*Daphnia magna*, 21 d): LOEC = 1.8 mg/L (measured)
Daphnia (*Daphnia magna*, 21 d): NOEC = 0.90 mg/L (measured)
Algae (*Pseudokirchneriella subcapitata*, 72 h) NOErC = 27 mg/L
(measured; growth rate)

Rationale: For classification purposes NOEC was used

Level of detail: SIAP

2-vinylpyridine CAS-no. 100-69-6				
HUMAN HEALTH	SIAP	SIAR	IUCLID	Comment
Acute Toxicity				
Oral	nad OECD TG 423 Tested dose: >50 and <300 mg/kg rats. LD50: >50 and <300 mg/kg bw. Clinical signs of toxicity.	Acute Cat 2 H300. OECD TG 423 Substance tested >50 and <300 mg/kg female rats No deaths at 50 mg/kg. Clinical signs of toxicity. On the basis of these results, the LD50 of 2-vinylpyridine in female rats was estimated to be 200 mg/kg bw . As this is just an estimation we prefer the lowest LD50-value.		Conclusive
Inhalation	nad	nad	nad	Inconclusive
Dermal	nad LD50 values reported: 640 mg/kg bw (rabbits) and 160 mg/kg (guinea pigs). No information on test methods, study design etc.	Acute Cat 2 H310 or Cat 3 H311. Key study: EPA OTS- guidance (GLP-compliance) LD50 in rabbit estimated to 640 mg/kg Supporting study: LD50 in guinea pig 160 mg/kg (Inconclusive, guideline not specified)	Acute Cat 2 H310 or Cat 3 H311. EPA OTS- guidance (GLP-compliance) LD50 in rabbit estimated to 640 mg/kg LD50 in guinea pig 160 mg/kg (Inconclusive)	Occlusion 24 hrs in both rat and guinea pig study, nowadays 4 hrs is used. It is not specified according to which guideline the study with guinea pigs is performed.
Skin irritation				
	nad Possible skin corrosion Cat 1; H318 but no information on method or score	Skin Corr Cat 1B H314 CFR 173.136-137 rabbit (undiluted) Exposure 1 hr skin necrosis after 48 hrs all animals.		Conclusive

Eye Irritation

	nad Possible Eye Damage Cat 1; H314 based on possible skin corrosion Cat 1; H318 but no information on method or score	nad Possible Eye Damage Cat 1; H318 Moderate to strong eye irritation in rabbits. Corneal and adnexal staining was observed in all animals but no information on scores or reversability is reported. Inconclusive.	nad	GLP compliance, reliability 2 with restrictions. Data not enough for classification but Skin Corr Cat 1B H314 Exposure 1 hr skin necrosis after 48 hrs all animals..
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Skin sensitisation

	Skin sens 1? LLNA (mice) similar to TG 429 sensitizing. Supporting studies guinea pig. Indications from two human case reports.	Skin sens 1 LLNA (mice) similar to TG 429 sensitizing. Supporting studies from a limited skins sensitization test performed on guinea pigs. Indications from two human case reports.	Skin sens 1 LLNA (mice) similar to TG 429 (reliability 2 with restrictions). sensitizing. Stimulation index > 3.0. Supporting studies guinea pig. Indications from two human case reports	Data too unspecific in SIAP. Further details on specifically the two human cases in SIAP, which made a classification possible, supported by further data in IUCLID on e.g. Stimulation index.
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Mutagenicity

	nad TG 471, 472 and 473) Positive <i>in vitro</i> studies, but no <i>in vivo</i> or QSAR data.	nad TG 471, 472 and 473) Positive <i>in vitro</i> studies, but no <i>in vivo</i> or QSAR data	nad TG 471, 472 and 473) Positive <i>in vitro</i> studies, but no <i>in vivo</i> or QSAR data	Data inconclusive.
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Carcinogenicity

	nad	nad	nad	Inconclusive. No reliable studies were identified.
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Reproductive Toxicity

	<p>nad *TG 421 (GLP) in rat Parental general toxicity observed at 20 mg/kg/d. At 125 mg/kg/d 9 of 12 females died due to prolonged parturition. Remaining 3 total litter loss. Significant decreased bw gain during gestation. Fertility index were not significantly affected. In 50 mg/kg/d one female was euthanized due to dystocia and one due to total litter loss. The dam with dystocia alle pups were still-born. Pup deaths suggests developmental effect. NOAEL 20 mg/kg/d for reproductive toxicity on the basis of dystocia. LOAEL for developmental toxicity estimated to 20 mg/kg/d is based on lower bw of pups in all treatment groups. Effects on fertility/developm. observed at doses where parental general toxicity occurs.</p>	<p>REP Cat. 2; H361 (?) *TG 421 (GLP) in rat Parental general toxicity observed at 20 mg/kg/d. At 125 mg/kg/d 9 of 12 females died due to prolonged parturition. Remaining 3 total litter loss. Significant decreased bw gain during gestation. Fertility index were not significantly affected. In 50 mg/kg/d one female was euthanized due to dystocia and one due to total litter loss. The dam with dystocia alle pups were still-born. Pup deaths suggests developmental effect. NOAEL 20 mg/kg/d for reproductive toxicity on the basis of dystocia. LOAEL for developmental toxicity estimated to 20 mg/kg/d is based on lower bw of pups in all treatment groups . *In a 92-day study (GLP, EPA OPPTS 870.31000) increased relative ovary weights in female rat (1/20) exhibited ovarian congestion at 180 mg/kg/d.</p>		<p>2 studies; 1 species. It may still be some doubt regarding a classification based on these data as you cannot fully exempt the fact the the repro effects may be the result of a secondary effects of maternal tox.</p>
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STOT SE

Oral	nad	nad	nad	
Inhalation	nad	nad	nad	
Dermal	nad	nad	nad	

STOT RE

Oral	<p>No classification</p> <p>'92-day study acc. to EPA OPPTS 870.31000 (90-day oral toxicity in rodents). Guideline under GLP compliance.</p> <p>*28-day study as key study. (MHLW Japan, 1997).</p> <p>*28 day oral study according to Japanese guidelines for repeated dose.</p> <p>Changes in organ weights and hyperkeratosis and acanthosis of the gastric epithelium (may be due to irritation) are seen. No toxicologically significant changes which have affected the function or morphology of a tissue/organ are seen. LOAEL for local and systemic effects 20 mg/kg/d. Overall NOAEL for local and systemic effects 12.5 mg/kg/d.</p>	<p>No classification</p> <p>*92-day study acc. to EPA OPPTS 870.31000 (90-day oral toxicity in rodents). Guideline under GLP compliance.</p> <p>*28-day study as key study. (MHLW Japan, 1997).</p> <p>*28 day oral study according to Japanese guidelines for repeated dose.</p> <p>Changes in organ weights and hyperkeratosis and acanthosis of the gastric epithelium (may be due to irritation) are seen. Hematology and clinical chemistry showed slight changes in atypical lymphocytes, polymorphonuclear leucocytes, number of lymphocytes, and ALT. LOAEL for local and systemic effects 20 mg/kg/d. Overall NOAEL for local and systemic effects 12.5 mg/kg/d.</p>		<p>Not classified. The local effects e.g. thickening of mucosa, squamous hyperplasia and submucosal edema seen in the stomach is most probably due to irritation/corrosion.</p>
Inhalation	nad	nad	nad	
Dermal	nad	nad	nad	

Aspiration toxicity

	nad	nad	nad	
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ENVIRONMENT

SIAP

SIAR

IUCLID

Comment

Aquatic acute	<p>Not applicable EC/LC50 values > 1 mg/l → criteria for classification not fulfilled for Aquatic acute 1</p>			Conclusive
Aquatic chronic	<p>Chronic 1; H410 or Chronic 2; H411 Not readily biodegradable (OECD 301). *Key studies: Lowest EC/LC50 in the range 1-10 mg/L (Fish, daphnia, OECD 202 & 203) *Chronic NOEC < 0,22 mg/l (daphnia, OECD 211; NOEC based on the total number of juveniles per parent animal at the start of the test) Note that the NOEC value of 0,90 mg/l (based on the total number of juveniles per parent animal at the start of the test) is not applicable as the adult mortality shows a dose dependant relationship and can not be regarded as accidental or inadvertant. -> Based on acute data a classification as Chronic 2, H411 is warranted. -> Based on chronic data, which should normally be given higher weight, it is not possible to conclude whether the classification should be Chronic 1 (H410) or Chronic 2</p>	Same conclusion as SIAP.		Not possible to determine whether Chronic 1 or Chronic 2 should apply

(H411)

ENV/JM/MONO(2014)31/ANN

nad = not adequate data

France

Substance: 2-vinylpyridine

<Acute toxicity - oral>

Classification proposal: Category 3

Key Data: Acute oral toxicity study (MHLW, 2006)

Rationale: No mortality at 50 mg/kg bw (threshold for classification between Cat 1-2); all rats died at 300 mg/kg bw (threshold for classification between Cat 2-3).

Level of detail: SIAP/SIAR

<Acute toxicity - dermal>

Classification proposal: Category 1 can be envisaged

Key Data: Acute dermal toxicity study in rabbits (Toxikon, 1992) and Skin irritation study in guinea pigs (Eastman Kodak, 1983)

Rationale: According to the acute dermal toxicity study in rabbits, a classification as Acute tox cat 2 is required ($200 < LD_{50} = 640$ mg/kg bw < 1000). According to the skin irritation study in guinea pigs, a classification as Acute tox cat 1 is required ($50 < LD_{50} = 0.16$ mL/kg = 160 mg/kg bw < 200 mg/kg w). The most conservative classification is proposed.

Level of detail: SIAP

<Acute toxicity - inhalation>

Classification proposal: no conclusion

Key Data: no reliable information available

Rationale: data not sufficient to propose a classification for this endpoint (a study with a reliability of 2 (Trochimowicz, 2001) was only cited in the Dossier, but does not permit to conclude on classification for this endpoint: lack of study details; LC_{50} between 0.7 mg/L (no death) and 24 mg/L (100% mortality)).

Level of detail: SIAP/SIAR/dossier

<Skin irritation>

Classification proposal: Category 1B can be envisaged

Key Data: Skin irritation study (Barr, 1994)

Rationale: No individual scores were available; however, a classification 1B can be envisaged based on the skin necrosis reported in the Dossier (observed at all test sites 48h after 1h application). This can also be supported by human data (severe skin burns with reddish-brown color lasted for several weeks).

Level of detail: SIAP/SIAR/dossier

<Eye irritation>

Classification proposal: no conclusion

Key Data: Eye irritation study (Eastman Kodak, 1983)

Rationale: No individual scores were available in order to conclude on this endpoint. Moderate to strong irritation reported in the Dossier. Data is not sufficient to propose a classification for this endpoint.

Level of detail: SIAP/SIAR/dossier

<Skin sensitisation>

Classification proposal: classification 1A can be envisaged

Key Data: Skin sensitization study

Rationale: A classification 1A or 1B may be envisaged based on the 80% positive response but the lack of details (protocol, individual data) does not permit to propose a firm classification for this endpoint.

Furthermore, case reports indicated sensitization in humans. The most conservative classification is proposed.

Level of detail: SIAP/SIAR/dossier

<Mutagenicity>

Classification proposal: none

Key Data: *In vitro* genotoxicity studies

Rationale: Conflicting results *in vitro*: positive results in *E. Coli* and in chromosomal aberration test; negative and positive results in *Salmonella*; UDS negative. No *in vivo* studies were available. There is no information on structure activity relationship to known germ cell mutagens. No classification is required.

Level of detail: SIAP/SIAR/dossier

<Carcinogenicity>

Classification proposal: no conclusion

Key Data: no reliable data

Rationale: Only one negative study in mice exposed by intraperitoneal injection: study not relevant for classification. No sufficient data is available for this endpoint in order to conclude on classification.

Level of detail: SIAP/SIAR/dossier

<Reproductive toxicity>

Classification proposal: Category 2

Key Data: Reproductive/developmental toxicity screening test

Rationale: All newborn pups from 1 dam were stillborn and all pups from 1 dam died during lactation at 50 mg/kg bw/day. At the highest dose, excessive maternal toxicity may explain the pup mortality.

Level of detail: SIAP/SIAR/dossier

<STOT SE>

Classification proposal: none

Key Data: Acute toxicity studies

Rationale: classification not required

Level of detail: SIAP/SIAR/dossier

<STOT RE>

Classification proposal: none

Key Data: Repeated toxicity studies

Rationale: classification not required (toxicity related to irritation of the gastric mucosa).

Level of detail: SIAP/SIAR/dossier

<Aspiration hazard>

Classification proposal: no conclusion

Key Data: -

Rationale: data not sufficient to conclude on a classification for this endpoint

Level of detail: SIAP/SIAR/dossier

Member Country (HH/ENV): FR (ENV)

Substance: 2-Vinylpyridine

<Acute aquatic toxicity>

Classification proposal: Category Acute 2

Key Data: Short term toxicity to fish of 2-vinylpyridine (MOE Japan, 2002)

Rationale: According to acute toxicity tests, fish is the most sensitive species to 2-vinylpyridine. As a consequence, the following toxicity threshold is taken into account for the classification:

LC50(96h) = 6.5 mg/L

Level of detail: SIAP

<Chronic aquatic toxicity>

Classification proposal: Category Chronic 2

Key Data:

- Short term toxicity to fish of 2-vinylpyridine (MOE Japan, 2002)
- Ready biodegradability test (CITI, 1991b)

Rationale: Chronic toxicity of 2-vinylpyridine has been assessed on aquatic invertebrates and algae. No data on chronic toxicity of 2-vinylpyridine to fish is available in the dossier. As fish is the most sensitive species to 2-vinylpyridine according to acute toxicity tests, chronic toxicity data has not been taken into account for the classification. Chronic classification of 2-vinylpyridine is based on acute toxicity to fish, considering the substance as not readily biodegradable. Nonetheless it should be noticed that a classification Category Chronic 1 could be applied based on the chronic data obtained with daphnia (NOEC = 0.90 mg/L). However, in this case, as no chronic data are available for the most sensitive species, no M factor can be chosen.

Level of detail: SIAP

Japan (Human Health only)

Substance: 2-Vinylpyridine

<Acute toxicity, oral>

Classification proposal: Category 3

Key Data: OECD TG 423 study (MHLW Japan, 2011)

Rationale: oral LD50 >50 and < 300 mg/kg bw in rats

Level of detail: SIAP/SIAR

<Acute toxicity, dermal>

Classification proposal: Category 2

Key Data: EPA OTS 798.1100, acute dermal toxicity test (Toxikon Co., 1992)

Rationale: dermal LD50 = 160 mg/kg bw in guinea pigs

Level of detail: SIAP/SIAR

<Skin Corrosion/Irritation>

Classification proposal: Classification not possible. It could be Category 1B if more detailed information was available for key data.

Key Data: Barr et al., 1994: unpublished data, cited in U.S. EPA HPVIS

Rationale: At 48 h after 1 h application, skin necrosis was observed in 6/6 rabbits, but no detailed information is available.

Level of detail: SIAP/SIAR/Dossier

<Serious eye damage/Eye Irritation>

Classification proposal: Classification not possible. It could be Category 1 or 2 if more detailed information was available for key data.

Key Data: Eastman Kodak Co., 1983 (eye irritation), Barr et al., 1994: unpublished data, cited in U.S. EPA HPVIS (skin corrosion)

Rationale: Eastman Kodak Co., 1983 showed strong or moderate eye irritation (could be Category 1 or 2), but detailed information such as reversibility or level of damage was not reported. If skin corrosion potential was confirmed, it could be Category 1.

Level of detail: SIAP/SIAR/Dossier

<Skin sensitization>

Classification proposal: Category 1 (subcategory cannot be classified)

Key Data: LLNA similar to TG429 (cited in ECHA web page), Human studies (Sasseville et al, 1996, Rajpar et al, 2006).

Rationale: positive result (stimulation index > 3) in LLNA (secondary data, but reliability=2); Due to lack of detailed information, subcategory cannot be classified. Patch test data in humans showed positive reaction (it was difficult to choose subcategory from the study results).

Level of detail: SIAP/SIAR/Dossier

<Germ cell mutagenicity>

Classification proposal: Category 2

Key Data: OECD TG 471 and 472, OECD TG 473

Rationale: positive results in in vitro mutagenicity tests.

Level of detail: SIAP

<Carcinogenicity >

Classification proposal: Classification not possible

Key Data:

Rationale: no adequate carcinogenicity studies.

Level of detail: SIAP/SIAR

<Reproductive toxicity >

Classification proposal: Classification not possible

Key Data: OECD TG 421 (MHLW, Japan)

Rationale: Although dystocia and decreased body weight in pups were observed, these changes were likely considered to be secondary effects. Further studies, such as OECD TG 415, or 416 may be needed for the purpose of the classification.

Level of detail: SIAP/SIAR

<Specific target organ toxicity, Acute toxicity >

Classification proposal: Category 3 (respiratory irritation)

Key Data: overall conclusion from all acute, repeated dose, and irritation studies

Rationale: Animal data indicate irritation potential of 2-vinylpyridine (tachypnea, edema, and thickened mucosa). Respiratory tract irritation was reported in humans.

Level of detail: SIAP/SIAR

<Specific target organ toxicity, Repeated dose toxicity >

Classification proposal: Category 2

Key Data: A 92-day study [Eastman Kodak Co., 1983]

Rationale: LOAEL(oral)=20 mg/kg bw/day in rats based on kidney and gastric effects.

Level of detail: SIAP/SIAR

2,4-Dimethylaniline (CAS 95-68-1)

The Netherlands (RIVM)

Human Health

Acute Toxicity

Oral

Classification proposal: Cat 4 (based on read-across from 2,6-dimethylaniline)
Key Data: The only toxicity study (Vernot et al 1977) available for 2,4-dimethylaniline is considered unreliable (Klimisch score 4). On the fact of, insufficient detail in the report published in the literature. The acute LD50 for 2,4-dimethylaniline was 470 mg/kg bw (male SD rat) and 25 mg/kg bw (male CF-1 mouse).
Two reliable studies for 2,6-dimethylaniline are available. The study performed according to OECD 423 under GLP was chosen as key study for classification purposes (MHLW Japan, 2005). The LD50 was between 300 and 2000 mg/kg bw in females.
Rationale: Based on lower and upper limits of range (Table 3.1.1 GHS guidance)
Level of detail: SIAP, SIAR and dossier

Carcinogenicity

Classification proposal: Category 1B or 2 (see rationale).
Key Data: There is no reliable carcinogenicity study for 2,4-dimethylaniline. On the other hand, there is a reliable study available for 2,6-dimethylaniline¹ in rats (CRL:COBS CD (SD) BR rats) (Montgomery CA et al, 2002). The following effects were reported:

- The induction of several types of tumors and increase in incidence were observed.
 - o Significant increases of carcinoademas or carcinomas of the nasal cavity in high dose (males 28/56 and females 24/56) and mid dose (female 1/56).
 - o Papillary adenomas high dose (males 10/56 and females 6/56) and mid dose (2/56 males)
 - o Rhabdomyosarcoma was observed in dosed rats of each sex.
 - o Increased incidences of subcutaneous fibromas and fibrosarcomas in male and female rats
 - o Increased incidence of neoplastic nodules of the liver in female. SIAR mentions that this could be related to the administration of the substance. But there is no

¹ The reference cited in the SIAR for this study is 'NTP, 1999' however, in the IUCLID dossier the study details are reported under the 'Montgomery CA et al, 2002' reference.

description or reasoning why this conclusion was reached.

- Hematological changes

Supporting studies

Study performed on 2,4-dimethylaniline and 2,5-dimethylaniline (Weisburger et al, 1978) in male Charles river rats and male and female HaM/ICR mice. The following effects were reported:

- 2,5-dimethylaniline led to an increase in subcutaneous fibromas and fibrosarcoma in male rats and increase of vascular tumors was elevated when compared to control
- 2,4-dimethylaniline led to significant increase of pulmonary tumors at the high dose in female mice but not in male mice and male rats.
- Same group of authors reported the result of a feeding study of 2,5-dimethylaniline in male SD rats. After 2 years, a total of 39% of the rats developed subcutaneous fibrosarcomas and hepatomas

Rationale:

Based on read-across from 2,6-dimethylaniline. Considering the information above, 2,6-dimethylaniline can be considered carcinogenic for male and females CR CD rats. The substance caused significant increases in incidences of adenomas and carcinomas of the nasal cavity. Rhabdomyosarcoma (a rare tumor (according to the SIAR)) was also observed in dosed rats and increased incidences of subcutaneous fibromas and fibrosarcomas in both sexes. Some carcinogenic indication of 2,4-dimethylaniline and 2,5-dimethylaniline is also reported. For example, the increase of similar tumors was reported: increase in subcutaneous fibromas and fibrosarcoma in male rats. In addition all dimethylanilines are considered to be mutagenic which is a possible mechanism for carcinogenicity.

Data on the incidence of tumors in the treatment group and control per dose are not reported for the above mentioned studies. This information is needed to confirm the findings of the above studies. For instance, the rarity of Rhabdomyosarcoma in treated animals in comparison to the control group. A better picture of the dose-response relationship is also desired in order to support the information. Such information would strengthen the evidence above and provide a clearer picture as to whether 2,4-dimethylaniline falls into a category 1B carcinogen or category 2.

Level of detail: SIAP, SIAR and dossier

STOT RE

Classification proposal: Cat 1 or 2 (Target organ systems blood,)

Key Data: Reliable sub-acute repeated dose study in rats (Crj:CD SD IGS) from 28-d studies following OECD 407 for 2,3-, 2,4-, 2,5-, 3,4- and 3,5- dimethylanilines and a screening study following OECD TG 422 for 2,6-dimethylaniline.

- The lowest **NOAEL** of 2 mg/kg bw/day was derived in 2,4-dimethylaniline due to effects on the blood, liver and kidneys at 10 mg/kg bw/day. The changes were usually fully recovered within a 2 week period except for the hemosiderin deposition and in some cases, the kidney necrosis. Nevertheless both of these effects are reduced in their severity during recovery (OECD TG 422 and 407).

Other reliable studies: included a NTP study (2-week, 13-week and 2-year) for 2,6-dimethylaniline, a non-GLP 28-day study for 2,4-, 2,5-, and 2,6-dimethylanilines, a 6-month feeding study for 2,4- and 2,6-dimethylanilins, and a 5 to 20-day study for 2,4- and 2,6-dimethylanilines in rats.

- The **LOAEL** of 2 mg/kg bw/day for 2,6-dimethylaniline in dogs was derived from a non-GLP 28-day study, in which dogs were dosed 2,4-, 2,5- or 2,6-dimethylanilines at 0, 2, 10 or 50 mg/kg bw/day, and fatty degeneration of the liver was observed at 2 mg/kg bw/day in the 2,6-dimethylaniline treatment. In contrast to rats, 2,6-dimethylaniline showed stronger toxicity comparing to 2,4- and 2,5-dimethylaniline in dogs.

Rationale:

The toxic effects of dimethylanilines of the studies mentioned above were closely comparable.

The lowest NOAEL of 2 mg/kg bw/day was derived in 2,4-dimethylaniline due to effects on the blood, liver and kidneys at 10 mg/kg bw/day. The value 10 mg/kg bw/day falls within the guidance value range; oral (rat) ≤ 10 mg/kg bw/d for a 90-day study. For a 28-day study it is 30 mg/kg bw/day.

Then the question arise when is heamoytic anemia considered significant. In Europe we use 20% reduction in Hb or Hb plus MetHb. However, the Hb and MethHb values are not available making it difficult to assess the corresponding dose values. The substance would be at least Cat 2 because of the cyanosis at 50 mg/kg and probably Cat 1 because of the cyanotic dose of 50 mg/kg is close to 30 mg/kg for a 20-day study

Level of detail:

SIAP, SIAR and dossier

Environment

Chronic Aquatic Toxicity

Classification proposal: Aquatic Chronic 1

Key Data:

All category member substances are not readily biodegradable.

- 2,3-dimethylaniline, 2,5-dimethylaniline, 2,6-dimethylaniline and 3,5-dimethylaniline resulted in 3,1,0, and 3% biodegradation after 28 days, respectively. 2,4-dimethylaniline and 3,4-dimethylaniline resulted in 0 and 7% biodegradation after two weeks, respectively.

Bioaccumulation potential of dimethylanilines for aquatic potential is low.

- Measured partition coefficients between octanol and water (Log Kow) are in the range of 1.68-1.91.
- Measured BCF values for 2,4-dimethylaniline, 2,5-dimethylaniline, 2,6-dimethylaniline and 3,4-dimethylaniline are <10, <3.8, 2.4 and <10, respectively.
- Bioaccumulation potential for 2,3-dimethylaniline and 3,5-dimethylaniline are predicted to be low based on a BCF value of 7.6 and 7.5 (BIOWIN version 3.01) respectively.

Aquatic toxicity:

- Data on substance self: chronic data not available for all three trophic levels. Experimental data available for invertebrates (*Daphnia magna*) acute toxicity, 48-h EC₅₀ is 9.9 mg/L.
- 3,4-, 2,5-, 3,5-, 2,3-, 2,6-dimethylaniline 21-d NOECs for vertebrates: of 0.0095, 0.096, 0.03, 0.1 and 2.23 mg/L respectively
- 3,4-, 2,5-, 3,5-, 2,3-, 2,6-dimethylaniline 72-h NOECs for algae: 2.94, 2.0, 5.8, 4.32 and 32 mg/L respectively

Rationale:

There is limited data for the substance self (data for only 1 trophic). Adequate data for an acute study for invertebrates is available (*Daphnia magna*), 48-h EC₅₀ is 9.9 mg/L. Based on the substance self, the classification is Aquatic Chronic 2. This is based on the surrogate approach: surrogate chronic data for invertebrates in combination with not rapidly degradable due to the absence of chronic data.

Using available chronic toxicity data for all category members, the classification should be Aquatic Chronic 1. This is based on the NOECs for invertebrates (see above) and the substance is not rapidly degradable.

- Trend in the data: In acute aquatic toxicity fish studies LC50s were generally greater than 100 mg/L except 3,5-dimethylaniline. For daphnids EC50s were between 1.09 and 25 mg/L. The NOEC values for daphnids were generally less than 0.1 mg/L except for 2,6-dimethylaniline. One may expect to see the trend observed in the acute studies to carry over to the chronic studies where fish would not be expected to be the most sensitive species.

Based on this information the classification of Chronic 1 then could be considered as a 'worst case scenario'.

Level of detail:

SIAP, SIAR and dossier

Switzerland (Human Health only)

2,4-Dimethylaniline (CAS 95-68-1)

Acute Toxicity

Oral

Classification proposal: Cat 3

Key Data: LD₅₀ of 250 mg/kg bw in mice and 470 mg/kg bw in rats

Rationale: For classification purposes the lowest LD₅₀ was used.

Level of detail: SIAP

Carcinogenicity

Classification proposal: Cat 2

Key Data: Induction of pulmonary tumors in female mice in an *in vivo* carcinogenicity study and the *in vivo* genotoxic activity

Rationale:

Level of detail: SIAP

STOT RE

Classification proposal: Not classified

Key Data: NOAEL of 2 mg/kg bw/day due to effects on the blood, liver and kidneys

Rationale: Although adverse effects were observed at low concentrations (NOAEL of 2 mg/kg bw/day due to effects on the blood, liver and kidneys), details on the severity of these effects for classification into Cat. 1 are missing. Further investigations are needed to determine the relevance of the observed adverse effects.

Therefore, the data is not sufficient for classification into Cat. 1.

Level of detail: SIAP/SIAR/dossier

Russian Federation

2,4-Dimethylaniline (CAS 95-68-1)

Human Health

Acute Toxicity

Oral

Classification proposal: Not classified

Key Data: Data from SIAP: 2,4-dimethylaniline: LD₅₀ (in mg/kg bw): rats 470, mice 250

Data from dossier: The acute oral LD₅₀ for the test substance in male rats was 470 (360 - 690) mg/kg; in male mice it was 250 (150 - 420) mg/kg

Reliability: 4 (not assignable)
 Rationale: Due to lack of details of the research in SIAP it's better to specify the data in the dossier. The dossier provides us the same data, but gives more details about research including the reliability – 4. The data is unreliable, the classification is impossible.
 Level of detail: SIAP, dossier

Carcinogenicity

Classification proposal: Cat 2

Key Data: "In bacterial reverse mutation assays with multiple strain of S. typhimurium and E. coli (OECD TG 471), 4-dimethylaniline was found to be mutagenic in TA100" + "2,4-dimethylaniline induced pulmonary tumours in female mice" + read across data (2,6-dimethylaniline) + "It can be predicted that all members of the category may be carcinogenic due to their in vivo genotoxic activity".

Rationale: Studies has no details, but due to possibility of carcinogenic potential + according to 3.6.2.5.3 GHS: "it is recognized that genetic events are central in the overall process of cancer development. Therefore evidence of mutagenic activity in vivo may indicate that a substance has a potential for carcinogenic effects." It's possible to classify the substance as Cat 2.

Level of detail: SIAR

STOT RE

Classification proposal: Cat 1

Key Data: Effects on the kidney such as papillary necrosis, dilatation of renal tubules, and/or hyaline droplets were observed at 10 mg/kg bw/day in animals treated with 2,4-dimethylaniline.
 There were increased relative and/or absolute weights of the liver (enlarged liver) or hypertrophy at 10 mg/kg bw/day in animal treated with 2,4- dimethylaniline (OECD TG 422 and 407).
 2,4-Dimethylaniline showed stronger hepatic effects comparing to 2,5- and 2,6- dimethylanilines dosing (non-GLP 28-day study).
 The lowest NOAEL of 2 mg/kg bw/day was derived in 2,4- dimethylaniline due to effects on the blood, liver and kidneys at 10 mg/kg bw/day. The changes were usually fully recovered within a 2 week period except for the hemosiderin deposition and in some cases, the kidney necrosis. Nevertheless both of these effects are reduced in their severity during recovery (OECD TG 422 and 407).

Rationale: The dossier data confirms this information by study with reliability 1: "A GLP- and OECD 407 compliant 28 day study in Crj: CD(SD) rats using oral gavage at 2, 10 and 50 mg/kg bw/day was conducted (NITE, Japan, 2002). The NOAEL of this study was considered to be 2 mg/kg bw/day according to hematological effects and changes in the kidney and liver". Thus it is possible to apply the criteria of classification for 28days according to 3.9.2.9.5 and table 3.9.1 of GHS

Level of detail: SIAR, dossier

Environment

Chronic Aquatic Toxicity

Classification proposal: Cat 2

Key Data: Based on the read-across data – not biodegradable.
Invertebrate: No reliable studies were identified. The predicted 21 d NOEC, based on read across from 3,4-dimethylaniline = 0.0095 mg/L
Algae: No reliable studies were identified. The predicted 72 h NOEC, based on read across from 2,5-dimethylaniline = 2.03 mg/L
Invertebrate [Daphnia magna]: 48 h EC₅₀ = 9.9 mg/L [DIN38412, static]

Rationale: There are only read-across data for chronic aquatic toxicity. But it's possible to classify the substance upon data of acute aquatic toxicity and information on degradability.

Level of detail: SIAR

Denmark

2,4-Dimethylaniline CAS-no. 95-68-1				
HUMAN HEALTH	SIAP	SIAR	IUCLID	Comment
STOT RE				

	<p>*STOT RE 1 ; H372 or STOT RE 2 H373 *Key study 28-d study (TG 407 and 422), rat *NOAEL repeated dose 2 mg/kg bw/day due to effects in blood, liver and kidneys , mainly fully recover after 2 weeks except for hemosiderin deposition and in some cases kidney necrosis. The LOAEL of dimethylaniline category for repeated oral dose ranged between 10-60 mg/kg bw/day in rats. Target organ systems were the blood, spleen, liver and kidneys. Effects on the kidney such as papillary necrosis, dilatation of renal tubules, and/or hyaline droplets were observed at 10 mg/kg bw/day in animals treated with 2,4-dimethylaniline in the 28 d. study -> sufficient evidence for STOT RE 1 classification?</p>	<p>*STOT RE 1 ; H372 or STOT RE 2 H373 *Key study 28-d study (TG 407 and 422), rat Significant haematological changes 10 mg/kg bw/d. Hemosiderin depositions in liver, kidney and spleen (secondary effect to loss of erythrocytes). Increase in relative and absolute liver weights and centrilobular hypertrophy of hepatocytes at 10 mg/kg bw/d. In addition, supporting evidens from other reliable studies</p>	<p>*STOT RE 1 ; H372 or STOT RE 2 H373 *Key study 28-d study (TG 407 and 422), rat reliability 1</p>	<p>SIAP: Adverse effects were observed at low concentrations (NOAEL of 2 mg/kg bw/day due to effects on kidneys, and probably also liver and blood). Lack of details on the severity, quantities etc. of these effects. At least a classification STOT RE Cat. 2. SIAR: Provides further details supporting the classification based on the effects of kidney and further details on the effects on liver and blood. Still unclear if a classification STOT RE Cat. 1 or 2 is warranted. IUCLID: No further information clearly supporting Cat. 1 or 2. Application of NOAEL in table 3.9.1. does not provide further clarification as the key data orinates from 28-d study and the table is applicable for 90-d studies.</p>
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CARC

	<p>nad A carcinogenicity study demonstrated that 2,6-dimethylaniline induced pulmonary tumours in female mice. It can be predicted that all members of the category may be carcinogenic due to their in vivo genotoxic activity.</p>	<p>*possible Carc. 2 In the carcinogenicity study with 2,6-dimethylaniline a dose dependent increase in the incidences of carcinoadenomas or carcinomas of the nasal cavity (28/56 high dose males, 24/56 high dose females and 1/56 mid dose females) and of the papillary adenomas (10/56 high dose males, 2/56 mid dose males, and 6/56 high dose females) was observed.</p> <p>A supportive, not reliable study, feeding study of 2,4-dimethylaniline in male SD rats (abstract) demonstrated that after 2 years, a total of 39% of the rats developed subcutaneous fibromas and fibrosarcomas and hepatomas</p>		<p>SIAP: Induction of pulmonary tumours in female mice and the in vivo genotoxic activity, indicates carcinogenicity but data are lacking in order to classify in Category 1B or 2. SIAR: A clear conclusion on the carcinogenicity of dimethylanilines is not possible based on the available data</p>
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nad = not adequate data

ENVIRONMENT	SIAP	SIAR	IUCLID	Comment
Aquatic chronic				

ENV/JM/MONO(2014)31/ANN

	<p>*Aquatic chronic 1; H410 Not inherently biodegradable (OECD 302) nor readily biodegradable (OECD 301 - based on read-across to other dimethylanilines).</p> <p>Acute EC/LC50 (lowest value) in the range 1-10 mg/l. Chronic NOEC < 1,0 mg/l (based on read-across to other dimethylanilines.). The available short-term and long-term aquatic toxicity data are rather consistent and supports that read-across is justified.</p>			
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France

Substance: 2,4 dimethylaniline

<Acute toxicity - oral>

Classification proposal: Acute Tox 4

Key Data: Acute toxicity studies - Weight of evidence approach

Rationale: The available study on 2,4 dimethylaniline showed a LD₅₀ of 250 mg/kg bw (150-420 mg/kg bw) in mice and LD₅₀ of 470 mg/kg bw (320-690 mg/kg bw) in rats. However, this study can not be used as a key study for classification considering its reliability of 4. The LD₅₀ of the other considered dimethylaniline ranged from 300 to 2000 mg/kg bw. Based on a weight of evidence approach, a classification of Acute Tox 4 can be proposed for 2,4 dimethylaniline (300-2000 mg/kg bw).

Level of detail: SIAP/SIAR/dossier

<Carcinogenicity>

Classification proposal: Category 2

Key Data: Carcinogenicity studies – Weight of evidence approach

Rationale: No reliable study was available on 2,4 dimethylaniline (reliability of 3 and 4). However, a similar profile of carcinogenic effects was found across the dimethylaniline category. Based on a weight of evidence approach (structural similarity with 2,6 dimethylaniline for which a clear carcinogenicity effect was observed), a classification Carc cat 2 can be proposed for 2,4 dimethylaniline.

Level of detail: SIAP/SIAR

<STOT RE>

Classification proposal: no conclusion

Key Data: 28-day toxicity study

Rationale: Decreased hemoglobin in males at 10 mg/kg bw/day and in both sexes at 50 mg/kg bw/day but details on severity are lacking in order to conclude on a classification.

Level of detail: SIAP/SIAR

Member Country (HH/ENV): FR (ENV)

Substance: 2,4 dimethylaniline

<Aquatic acute toxicity>

Classification proposal: Category Acute 2

Key Data: Short term toxicity to aquatic invertebrate *Daphnia magna* of 3,4-Dimethylaniline

Rationale: Experimental data on acute aquatic toxicity for 3 trophic levels are available for 2,3-Dimethylaniline; 2,5-Dimethylaniline; 2,6-Dimethylaniline; 3,4-Dimethylaniline; 3,5-Dimethylaniline. In all case invertebrates are the most sensitive species. Considering the category approach, the lowest EC50(48h), *i.e.* EC50(48h) = 1.09 mg/L (3,4-dimethylaniline) is considered for classification of 2,4-Dimethylaniline. Same classification occurs based on data available for 2,4 dimethylaniline (EC50 = 9.9 mg/L for invertebrate and category approach indicate that invertebrate should be the most sensitive trophic level).

Level of detail: SIAP

<Aquatic Chronic toxicity>

Classification proposal: Category Chronic 1; M-factor = 10

Key Data: Long term toxicity to aquatic invertebrate *Daphnia magna* of 3,4-Dimethylaniline / All category member substances not readily biodegradable

Rationale: If only available experimental data dealing with 2,4-dimethylaniline are taken into account, then the chemical is classified as Category Chronic 2, considering that the substance is not rapidly biodegradable and that the lowest aquatic EC50 is between 1 and 10 mg/L.

However, other classification occurs considering the category approach. According to experimental data carried out on acute aquatic toxicity for 3 trophic levels on 2,3-Dimethylaniline; 2,5-Dimethylaniline; 2,6-Dimethylaniline; 3,4-Dimethylaniline; 3,5-Dimethylaniline, invertebrates are the most sensitive species. Chronic toxicity tests have been performed for 2,3-Dimethylaniline; 2,5-Dimethylaniline; 2,6-Dimethylaniline; 3,4-Dimethylaniline; 3,5-Dimethylaniline. Considering the category approach, the lowest NOEC, *i.e.* NOEC(21d) = 0.0095 mg/L (3,4-dimethylaniline) should be considered for classification of 2,4-Dimethylaniline and leads to the classification Category Chronic 1.

Level of detail: SIAP

Japan (Human Health only)

Substance: 2,4-dimethylaniline

<Acute toxicity, oral>

Classification proposal: Category 3

Key Data: Vernot *et al.* (1977)

Rationale: oral LD50=250 mg/kg bw in mice

Level of detail: SIAP/SIAR

<Carcinogenicity >

Classification proposal: Category 2

Key Data: Weisburger *et al.*, 1978 and other related data

Rationale: Weisburger *et al.*, 1978 showed pulmonary tumour in female mice, but this study itself was not sufficient for classification (description in Dossier was not detailed enough). From all available data (in vivo genotoxicity data, analogue substance data), carcinogenic potential of 2,4-dimethylaniline was suspected.

Level of detail: SIAP/SIAR/Dossier

<Specific target organ toxicity, Repeated dose toxicity >

Classification proposal: Category 1

Key Data: OECD 407 (NITE, 2002)

Rationale: Hematological effects and changes in the kidney and liver in rats at 10 mg/kg bw/day

Level of detail: SIAP/SIAR

Nonane (CAS 111-84-2)

The Netherlands

Irritation

Skin

Classification proposal: Not possible

Key Data: Animal data: No measured data for substance self therefore read-across approach is used. Isoparaffinic, normal paraffinic and mixed

aliphatic category members produced minimal to slight skin irritation when tested in rabbits. A total of 60 studies were examined and results, skin irritation all mean values of 24, 48 and 72-h for erythema and edema are provided in the IUCLID dossier.

Human data: Application of neat C9-C14 aliphatic, <2% aromatic hydrocarbons fluids under semi-occlusive conditions for 24 hours produced signs of dermal irritation in human volunteers in two studies. A third study that used a semi-occlusive path showed no dermal irritation to humans when neat C9-C14 aliphatic, <2% aromatic hydrocarbons fluids were applied for 24 hours. Application of the hydrocarbons fluids are not irritating to human skin when evaluated in a repeated patch test as a 50% w/w preparation as reported in two studies. In another study at the same concentration of the application of hydrocarbon fluids to the skin without or in conjunction with UV irradiation did not elicit an irritation response in any of the study participants. Normally the category members are not irritating to the human skin but can produce irritant responses if evaporation is inhibited or prevented.

Rationale: GHS does not use the average of all animals over the three time points but the average per animal. This information is not available in the IUCLID dossier. In addition, there are no clear criteria within the GHS for classification based on human data.

Level of detail: SIAP, SIAR and dossier

Switzerland

Nonane (CAS 111-84-2)

Irritation

Skin

Classification proposal: Not classified

Key Data: The members of the C9-C14 aliphatic (<2% aromatics) category produced minimal to slight eye irritation when tested in rabbits. Similarly, the isoparaffinic, normal paraffinic, and mixed aliphatic category members produced minimal to slight skin irritation when tested in rabbits and are also not normally irritating to human skin but can produce irritant responses if evaporation is inhibited or prevented. However, cycloparaffinic hydrocarbon fluids are considered to be dermal irritants to rabbits and to humans. It should also be noted that prolonged or repeated exposure to hydrocarbon solvents can lead to severe irritant dermatitis due to defatting of the skin.

Rationale: Classification not possible since data (scoring points) is missing.

Level of detail: SIAP/SIAR/dossier

Russian Federation

Nonane (CAS 111-84-2)

Irritation

Skin

Classification proposal: Not classified

Key Data: The members of the C9-C14 aliphatic (<2% aromatics) category produced minimal to slight eye irritation when tested in rabbits. Similarly, the isoparaffinic, normal paraffinic, and mixed aliphatic category members produced minimal to slight skin irritation when tested in rabbits and are also not normally irritating to human skin but can produce irritant responses if evaporation is inhibited or prevented. However, cycloparaffinic hydrocarbon fluids are considered to be dermal irritants to rabbits and to humans. It should also be noted that prolonged or repeated exposure to hydrocarbon solvents can lead to severe irritant dermatitis due to defatting of the skin. The C9-C14 aliphatic ($\leq 2\%$ aromatics) Category members do not cause skin sensitization.

Dossier: no adverse effect observed (not irritating).

Rationale: The lack of details of data, classification is not possible.

Level of detail: SIAR, dossier

Denmark

Nonane (C9 alkane) CAS-no. 111-84-2 (Read-across)				
HUMAN HEALTH				
Irritation				
	SIAP	SIAR	IUCLID	Comment
Skin	<p>*nad</p> <p>*OECD TG 404 (according to/similar) (rabbit)</p> <p>*Minimal to slight skin irritation</p> <p>Not normally irritating to human skin but may be irritating if evaporation is inhibited. Prolonged or repeated exposure to hydrocarbon solvents can lead to severe irritant dermatitis due to defatting of the skin.</p> <p>Conclusion: severe irritant dermatitis due to defatting with prolonged or repeated exposure</p>	<p>*nad</p> <p>*OECD TG 404 (according to/similar) (rabbit)</p> <p>*46/60 studies indicated irritation scores well-below the threshold for classification. Seven studies used occlusive dressing and did not report data useful for classification. Seven studies reported erythema scores for four of these studies were 2.0.</p> <p>According to sponsors, conclusion is: the isoparaffinic, normal paraffinic, and mixed aliphatic, C9-C14 Aliphatic Hydrocarbon Solvents (\leq 2% Aromatics) are not mild dermal irritants to rabbits.</p>	<p>*nad</p> <p>*no further information than provided in SIAR</p>	<p>SIAP: Classification is not possible due to lack of details on scoring, observation time, number of rabbits etc.</p> <p>SIAR: Read-across to C11-C14 n-alkanes (< 2% aromatics): Mildly irritating, scores below thresholds for classification. 7 studies and 4 were irritation score 2.0. Lack of details on scoring, observation time, number of rabbits etc. Indications that i should not be classified, but classification is not possible.</p> <p>IUCLID: Indications that i should not be classified, but classification is not possible.</p>

nad = not adequate data

France

Substance: nonane

<Skin irritation>

Classification proposal: no conclusion

Key Data: Skin irritation studies

Rationale: No data on nonane and contradictory data on C11-C14 n-alkanes, < 2% aromatics. No sufficient data to conclude on classification for this endpoint.

Level of detail: SIAP/SIAR

Japan

Substance: Nonane

<Skin irritation>

Classification proposal: Classification not possible

Key Data: OECD TG 428 (Babu et al., 2004), OECD TG 404 for similar substances (Cepca Química 2000a; Petrochem Carless Limited 1997)

Rationale: Two OECD TG 404 studies were conducted for Hydrocarbons, C11-C14, n-alkanes, < 2% aromatics, and one resulted mildly irritating and the other was not irritating. OECD TG 428 (in vitro study) indicated that the erythema scores increased with increase in the molecular weight of the hydrocarbon. It is difficult to read across from these available data.

Level of detail: SIAP/SIAR/Dossier