Committee for Risk Assessment

RAC

Annex 1

Background document

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

n-hexane

EC Number: 203-777-6
CAS Number: 110-54-3

CLH-O-0000007203-83-01/F

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

Adopted

1 December 2022

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

n-Hexane

EC Number: **203-777-6**

CAS Number: **110-54-3**

Index Number: **601-037-00-0**

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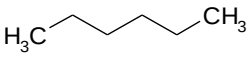
Version number: 3.0 **Date: December 2021**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Hexane
Other names (usual name, trade name, abbreviation)	
ISO common name (if available and appropriate)	
EC number (if available and appropriate)	203-777-6
EC name (if available and appropriate)	*n*-hexane
CAS number (if available)	110-54-3
Molecular formula	C_6H_{14}
Structural formula	
SMILES notation (if available)	CCCCCC
Molecular weight or molecular weight range	86.18 g/mol

1.2 Composition of the substance

Table 2: Test substances (non-confidential information) (this table is optional)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 3: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	**Hazard statement Code(s)**	**Pictogram, Signal Word Code(s)**	**Hazard statement Code(s)**	**Suppl. Hazard statement Code(s)**		
Current Annex VI entry	601-037-00-0	*n*-hexane	203-777-6	110-54-3	Flam. Liq. 2 Asp. Tox. 1 Skin Irrit. 2 STOT SE 3 Repr. 2 STOT RE 2 * Aquatic Chronic 2	H225 H304 H315 H336 H361f*** H373** H411	GHS07 GHS02 GHS09 GHS08 Danger	H225 H304 H315 H336 H361f*** H373** H411		STOT RE 2; H373: C ≥ 5%	
Dossier submitters proposal					**modify** STOT RE 1	**modify** H372 (nervous system)		**modify** H372 (nervous system)		**delete** STOT RE 2; H373: C ≥ 5%	
Resulting Annex VI entry if agreed by RAC and COM					Flam. Liq. 2 Asp. Tox. 1 Skin Irrit. 2 STOT SE 3 Repr. 2 Aquatic Chronic 2 STOT RE 1	H225 H304 H315 H336 H361f*** H411 H372 (nervous system)	GHS07 GHS02 GHS09 GHS08 Danger	H225 H304 H315 H336 H361f*** H411 H372 (nervous system)			

Table 4: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)		
Oxidising gases		
Gases under pressure		
Flammable liquids		
Flammable solids		
Self-reactive substances		
Pyrophoric liquids		
Pyrophoric solids		
Self-heating substances		
Substances which in contact with water emit flammable gases		
Oxidising liquids		
Oxidising solids		
Organic peroxides		
Corrosive to metals		
Acute toxicity via oral route		
Acute toxicity via dermal route		
Acute toxicity via inhalation route		
Skin corrosion/irritation		
Serious eye damage/eye irritation		
Respiratory sensitisation		
Skin sensitisation		
Germ cell mutagenicity		
Carcinogenicity		
Reproductive toxicity		
Specific target organ toxicity-single exposure		
Specific target organ toxicity-repeated exposure	**Harmonised classification proposed**	Yes
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment		
Hazardous to the ozone layer		

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The current legal classification of n-hexane for repeated dose toxicity STOT RE 2*; H373 arises from translation of classifications listed in Annex I to Directive 67/548/EEC (Dangerous Substances Directive; DSD). For STOT repeated exposure, the classification according to the criteria in DSD does not correspond exactly to the classification in a hazard class and category under Regulation (EC) No 1272/2008 (CLP) as the guidance values that trigger classification are different. This means it is a minimum classification following Annex VI, 1.2.1 of the CLP Regulation: Minimum classification for a category is indicated by the reference * in the column 'Classification' in Table 3.1. As stated in CLP, this (minimum) classification shall be applied if the following condition is not fulfilled:

- The manufacturer or importer has access to data or other information as specified in Part 1 of Annex I that lead to classification in a more severe category compared to the minimum classification. Classification in the more severe category must then be applied.

In the substance evaluation process on n-hexane the eMSCA concluded that sufficient information on the neurotoxicity of n-hexane in humans is available to justify classification as STOT RE 1. N-Hexane produces significant functional changes in the peripheral nervous system of humans following repeated exposure through inhalation. Available human data demonstrated that the incidence of peripheral neuropathy can be attributed reliably to prolonged occupational exposure to n-hexane. Short term exposure to n-hexane vapour results in vertigo, headache, nausea, and vomiting, while prolonged exposures have been unequivocally associated with sensorimotor peripheral polyneuropathies in occupationally exposed individuals and glue-sniffing addicts. Following the rules set down in Annex VI and the data available, n-hexane appears to fulfil the criteria for classification as STOT RE 1; H372.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Reason for a need for action at Community level:

- Change the existing entry in Annex VI due to changes in the criteria
- Disagreement by DS with current self-classification.

The entry in the C&L inventory for n-hexane currently comprises 63 aggregated entries resulting from almost 2600 notifications. Only 19 notifications state a classification as STOT RE 1 with hazard statement H372.

The existing information on n-hexane is sufficient to conclude that n-hexane produces significant functional changes in the peripheral nervous system of humans following repeated exposure through inhalation. Available human data demonstrated that the incidence of peripheral neuropathy can reliably be attributed to prolonged occupational exposure to n-hexane. The classification of n-hexane as STOT RE 2; H373 shall be considered as a minimum classification. The availability of sufficient information on the neurotoxicity of n-hexane in humans indicates that a classification as STOT RE 1; H372 may be appropriate.

According to the Guidance to CLP (Chapter 3.9.5: Re-classification of substances and mixtures classified for STOT RE according to DSD and DPD) "…Substances … classified with … R48/20 (for vapour) … shall be classified as STOT-RE Category 1 because less adverse effects and higher guidance values are required for classification according to CLP compared to DSD". In order to change a harmonised classification action is needed at community level. Furthermore, n-hexane is produced and imported in high tonnages in Europe. The use of the substance, which is a solvent with a vapour pressure of approx. 200-300 hPa at ambient temperature, is wide spread indicating significant occupational exposure.

5 IDENTIFIED USES

5.1 Manufacture

According to information provided by ECHA (public registration data, December 2016), n-hexane is manufactured and used within the total tonnage band of 1,000-10,000 tonnes per annum.

The manufacturing process of the substance is described by the registrant(s) using the following Process Categories (PROCs):

PROC 1: Use in closed process, no likelihood of exposure
PROC 2: Use in closed, continuous process with occasional controlled exposure
PROC 3: Use in closed batch process (synthesis or formulation)
PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises
PROC 8a: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at non-dedicated facilities
PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities
PROC 15: Use as laboratory reagent

According to literature sources, "commercial hexanes are manufactured by two-tower distillation of a suitable charge stock, e.g., straight-run gasoline that have been distilled from crude oil or natural gas liquids that have been stripped from natural gas" (Kirk-Othmer 2005). Usually the fractions are characterized by their distillation temperature/initial boiling point and are offered in different purity grades. Commercial hexanes may contain benzene which cannot be eliminated by fractionation due to its ability to form an azeotropic mixture with n-hexane. Aromatic-free hexane can be obtained from the BTX (benzene, toluene, xylenes) raffinate which "remains after removal of aromatics from catalytic reformats" (Kirk-Othmer 2005). Highly pure n-hexane is produced using molecular sieves.

5.2 Identified uses

According to Mears and Eastman (Kirk-Othmer 2005), the largest volume applications for hexane are the uses as fuel and for extraction of oil from seeds, for example from soybeans or peanuts. Other than that, hexane is used as solvent and reaction medium for "manufacture of polyolefins, synthetic rubbers, and some pharmaceuticals". In the Occupational Disease Report published by DGUV the use of n-hexane as solvent in lacquer, resins, glues (especially fast-drying glues) and adhesives is mentioned [BK1317]. Most of the applications in industrial and professional settings cover the use of n-hexane in preparations or mixtures.

A detailed list of products supplied to industrial and professional users by the Federal Office of Public Health (FOPH), Switzerland, assigned the largest number of products to the sector "sealants and glues" while the second largest number can be found in the sector "solvents, paint remover, degreaser, thinner". The content of n-hexane in these products exceeds 50 % by weight in some cases.

The uses by workers in industrial settings are listed below:

- Formulation
- Distribution
- Formulation and (re)packing
- Use in coatings
- Use in cleaning agents
- Blowing agents
- Functional Fluids
- Polymer processing
- Mining Chemicals

The uses by professional workers are listed below:

- Use in coatings
- Use in cleaning agents
- Polymer processing
- Use in Laboratories
- Use as Functional Fluids
- Use in fuels

Identified consumer uses of n-hexane are listed below:

- Coatings which cover the following product categories

PC 1: Adhesives, sealants
PC 4: Anti-freeze and de-icing products
PC 8: Biocidal products (e.g. disinfectants, pest control)
PC 9a: Coatings and paints, thinners, paint removes
PC 9b: Fillers, putties, plasters, modelling clay
PC 9c: Finger paints
PC 15: Non-metal-surface treatment products
PC 18: Ink and toners
PC 23: Leather tanning, dye, finishing, impregnation and care products
PC 24: Lubricants, greases, release products
PC 31: Polishes and wax blends
PC 34: Textile dyes, finishing and impregnating products; including bleaches and other processing aids

- Other consumer uses

PC 28: Perfumes, fragrances
PC 39: Cosmetics, personal care products.

The SPIN database (2012) indicates a “very probable exposure” with a “wide range of applications”. n-Hexane was measured in scented toys (Glensvig, 2006) and electrical and electronic products (Mortensen, 2005).

6 DATA SOURCES

This CLH report is based on the registration dossiers as well as on reviews by a variety of international bodies/regulatory programs and original publications. Data available up to November 2016 have been assessed.

7 PHYSICOCHEMICAL PROPERTIES

Table 5: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Liquid	Lide D (Editor in Chief); 2008; Handbook of Chemistry and Physics, 89th edition; CRC Press, Boca Raton	
Melting/freezing point	-95.35°C	Lide D (Editor in Chief); 2008; Handbook of Chemistry and Physics, 89th edition; CRC Press, Boca Raton	
Boiling point	68.73 °C at 101.3 kPa	Lide D (Editor in Chief); 2008; Handbook of Chemistry and Physics, 89th edition; CRC Press, Boca Raton	
Relative density	0.6606 g/cm3 at 25 °C	Lide D (Editor in Chief); 2008; Handbook of Chemistry and Physics, 89th edition; CRC Press, Boca Raton	
Vapour pressure	10 kPa at 9.8°C 20 kPa at 25°C 30 kPa at ~ 35°C	Lide D (Editor in Chief); 2008; Handbook of Chemistry and Physics, 89th edition; CRC Press, Boca Raton	
Surface tension			The given information of the surface tension could not be validated. On the one site a waiver is given that based on the structure no surface activity is expected or predicted. On the other site the data of the supporting study, CRC Handbook 17.89 mN/m at 25°C, c = 1g/l, indicate surface tension. But no information on the primary source of this data or the methods used is available.
Water solubility	0.0098 g/l at 25 °C	Lide D (Editor in Chief); 2008; Handbook of	

Property	Value	Reference	Comment (e.g. measured or estimated)
		Chemistry and Physics, 89th edition; CRC Press, Boca Raton	
Partition coefficient n-octanol/water	4 at 20°C, pH = 7	Lide D (Editor in Chief); 2008; Handbook of Chemistry and Physics, 89th edition; CRC Press, Boca Raton	Shake-flask method, standard temperature and pressure assumed.
Flash point	< -20 °C	CHEMSAFE, 2012	Closed cup
Flammability-explosion limits in air:	*Lower:* 1,0 vol% *Upper:* 8,1 vol%	CHEMSAFE, 2012	
Flammability	Highly flammable due to flash point	BAM, 2013	Flammability upon ignition (solids, gases): Testing can be waived, substance is a liquid. Flammability in contact with water: The classification procedure needs not to be applied because the substance does not contain metals or metalloids. Pyrophoric properties: The classification procedure needs not to be applied because the substance is known to be stable into contact with air at room temperature for prolonged periods of time (days).
Explosive properties	No explosive properties	BAM, 2013	The classification procedure needs not to be applied because there are no chemical groups associated with explosive properties present in the molecule.
Self-ignition temperature	230 °C	CHEMSAFE, 2012	DIN 51 794
Oxidising properties	No oxidising properties	BAM, 2013	The classification procedure needs not to be applied because the organic substance does not contain oxygen or halogen atoms.
Granulometry			The granulometry study does not need to be conducted as the substance is marketed or used in a non solid or granular form.
Stability in organic solvents and identity of relevant degradation products			In accordance with column 1 of REACH Annex IX the stability in organic solvents study is not required as stability of the substance is not considered to be critical.

Property	Value	Reference	Comment (e.g. measured or estimated)
Dissociation constant			In accordance with the General Rules for Adaptation of the Standard Testing Regime (Annexes VII-X) as stated in REACH Annex XI, this can be waived as the substance does not have any functional groups that dissociate and therefore testing does not appear scientifically necessary.
Viscosity	0.3 mPa.s at 25°C	Lide D (Editor in Chief); 2008; Handbook of Chemistry and Physics, 89th edition; CRC Press, Boca Raton	

8 EVALUATION OF PHYSICAL HAZARDS

No changes in the classification for the physical hazards are proposed in this dossier. Classification for flammability of *n*-hexane is already included in Annex VI of Regulation (EC) No 1272/2008.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 6: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Disposition and Pharmacokinetics, no guideline available, GLP Rat, Fischer 344 M/F number not specified 1) i.v. bolus 10 mg/kg (no vehicle) 2) nose-only inhalation, a) single (6 h) 900 and 9000 ppm, b) 8 d, 6 h/d 900 ppm 3) dermal 6 h at 1.1 and 11 mg/cm²	Absorption: 2 a) 900 ppm: 12- 14% 9000 ppm: 6-7% Excretion: 47 to 80 % exhaled 40-65 % as n-hexane 35-47 % as $^{14}CO_2$ 1) & 2a) 9000 ppm: 12-17% urine 2) 900 ppm: 25-39% urine 3) 1-3% in urine estimated half-life: 9.6 h not concentrated in any tissue 1), 2): 2.7-8.1 % in carcass 3) < 0.5 % in carcass	Commercial hexane (52 % n-hexane) Read-across based on grouping of substances (category approach). Radio labelled n-hexane and radio labelled methyl-cyclopentane	API 1990
Urinary Excretion of n-hexane metabolites, no guideline available, non-GLP Human, 41 shoe workers, sex not specified, in several work places in 5 shoe factories 32–500 mg/m³ n-hexane; mean concentration 183 mg/m³; median 160 mg/m³ 1 day	Mean concentrations of n-hexane metabolites in urine: 2,5-hexanedione, 5.4 mg/L, 2-hexanol, 0.19 mg/L Correlations between concentrations of n-hexane in air and urinary metabolites were best for total n-hexane metabolites (r=0.7858), followed by 2 -hexanol (r=0.6851) and 2,5 -hexanedione (r=0.6725).	Occupational exposure to hexane solvents that contained n-hexane. Metabolites of hexane were tested in the urine samples using acid extraction.	Perbellini et al. 1981

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification

9.1.1 Absorption

Absorption following oral and dermal exposure of n-hexane in humans and laboratory animals can be inferred from the presence of n-hexane and its metabolites in exhaled air, serum, and urine (ATSDR 1999, US EPA 2005, MAK 1997, Krasavage 1980 cf. chapter 5.6.1.1). Absorption of n-hexane into the human blood in relation to total respiratory uptake was about 17% (ATSDR 1999, US EPA 2005).

9.1.2 Distribution

In rats and humans n-hexane is widely distributed to the body tissues but not concentrated significantly by any of those tissues (API 1990, MAK 1997, ATSDR 1999). The various metabolites are distributed from the blood to various organs and tissues, including the peripheral nerve system (sciatic nerve), testes, liver, kidney, and brain (ATSDR 1999, US EPA 2005).

9.1.3 Metabolism

n-Hexane is extensively metabolized in the liver without qualitative differences between humans and test animals (US EPA 2005, MAK 1997, WHO 1991). The major metabolites in urine, predominantly in conjugated form, are considered to be 4,5-dihydroxy-2-hexanone for humans and 2- and 3-hexanol for rat, rabbit and monkey (MAK 1997). 2,5-Hexanedione is believed to be the major toxic metabolite in humans identified following acid hydrolysis of urine samples (Perbellini et al. 1981). The concentration of 2,5-hexanedione in human urine is 20-30 times higher than the concentration of 2-hexanol. In contrast, in urine of rats and guinea pigs the concentration of 2-hexanol is 3 times higher than the concentration of 2,5-hexanedione (MAK 1982).

9.1.4 Excretion

Exhaled breath and urine were the two primary routes for the excretion of n-hexane and its metabolites from rats and humans (API 1990, ATSDR 1999, US EPA 2005). A mean elimination half-life of 13 to 14 hours for urinary excretion of 2,5-hexanedione by humans and 7 hours by rats has been reported. The neurotoxic metabolite 2,5-hexanedione may therefore accumulate in the human body following repeated exposure to n-hexane (MAK 1997, WHO 1991).

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

The classification for acute toxicity was not part of the assessment.

10.2 Acute toxicity - dermal route

The classification for acute toxicity was not part of the assessment.

10.3 Acute toxicity - inhalation route

The classification for acute toxicity was not part of the assessment.

10.4 Skin corrosion/irritation

The classification for skin corrosion was not part of the assessment.

10.5 Serious eye damage/eye irritation

The classification for serious eye damage/eye irritation was not part of the assessment.

10.6 Respiratory sensitisation

The classification for respiratory sensitisation was not part of the assessment.

10.7 Skin sensitisation

The classification for skin sensitisation was not part of the assessment.

10.8 Germ cell mutagenicity

The classification for germ cell mutagenicity was not part of the assessment.

10.9 Carcinogenicity

The classification for carcinogenicity was not part of the assessment.

10.10 Reproductive toxicity

The classification for Reproductive toxicity was not part of the assessment.

10.11 Specific target organ toxicity-single exposure

The classification for specific target organ toxicity-single exposure was not part of the assessment.

10.12 Specific target organ toxicity-repeated exposure

Table 7: Summary table of animal studies on STOT RE.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Sub-chronic No guideline available Non-GLP Rat CD (SD) BR 5 m/group	*n*-Hexane (99%) Oral (gavage), once daily (5 days /week) 0, 568, 1135, 3973 (mg/kg bw/d) 90 d or 120 d (the highest dose group was observed over a 120-day period, the other groups over a 90-day period) Animals were sacrificed when they exhibited severe hindlimb weakness or paralysis, or after 90 or 120 days of	CLINICAL SIGNS AND MORTALITY Two rats in the 1135 mg/kg group and one in the 3973 mg/kg group died immediately after intubation. Only the 3973 mg/kg dose produced hindlimb paralysis in 90 days. BODY WEIGHT AND WEIGHT GAIN Body weight gain was reduced after 3 weeks of exposure at all dose levels. This reduction in body weight followed a reduction in food consumption. Significant and dose dependant weight reduction was seen in the 1135 and 3973 mg/kg groups. NEUROBEHAVIOUR Hindlimb paralysis was seen in the 3973 mg /kg dosed animals an average of 101.3 +/- 9. 4 days after start of exposure. HISTOPATHOLOGY: NON-NEOPLASTIC The 3973 mg/kg dose produced multifocal axonal swellings, adaxonal[1] myelin infolding, and paranodal myelin retraction. Atrophy of the germinal epithelium was also seen in the testes of animals at this dose level.	(Krasavage et al. 1980)

[1] DS: adaxonal refers to the innermost layer of the myelin sheath

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
	exposure.		
Sub-chronic limit test No guideline followed, non-GLP Rat, Wistar Male, 7 m/group	*n*-Hexane (99%), Whole body inhalation (vapour), daily (i.e. 7 days/ week), 12 h/day 0, 3000 ppm (10.56 mg/L) 16 weeks The motor nerve conduction velocity (MCV) and the distal latency were measured before the beginning of the exposure and after exposure for 4, 8, 12, and 16 weeks.	CLINICAL SIGNS AND MORTALITY Two animals died during the study. One animal died 1 day before the end of the exposure period, and one animal died three days before the end of the exposure period. BODY WEIGHT AND WEIGHT GAIN Body weight gain was significantly reduced at 4 weeks after start of exposure, and remained depressed for the rest of the experiment. NEUROBEHAVIOUR Unsteady gait was observed in one animal at 10 weeks exposure, and in 4 animals at 12 weeks. 2 animals at this time point also showed foot drop. At 16 weeks exposure, the five surviving rats had unsteady gait, and two had foot drop. All animals had muscular atrophy at this time point. HISTOPATHOLOGY: NON-NEOPLASTIC There were paranodal swellings in the myelinated fibers of the tibial nerve and dorsal trunk of the tail nerve. There were an excessive number of neurofilaments, vesicles, multivesicular bodies, mitochondria, myelin figures, and dense bodies in the paranodal axoplasm and no neurotubules. Denervated neuromuscular junctions in the muscles were observed. Muscle fibers were of irregular shape and size, and seemed to have an increased number of nuclei (probably indicating regenerative proliferation), and had disordered myofilaments, zig-zagging of the z-band, and invaginations of the plasma membrane. OTHER FINDINGS The motor nerve conduction velocity (MCV) was significantly less than controls by 4 weeks of exposure. MCVs could not be measured in 2 animals after 16 weeks due to nerve damage. Distal latencies (DL) were significantly prolonged after 4 weeks of exposure, and could not be measured in 2 animals at 16 weeks of exposure.	(Takeuchi et al. 1980)
Sub-chronic Equivalent or similar to OECD TG 413 (sub-chronic inhalation toxicity: 90-day), non-GLP Mouse B6C3F1 18 m + 18 f/	*n*-Hexane (99%) Whole body inhalation (vapour), for all doses: daily (5 d /week), 6 h/day An additional 1000 ppmV group was exposed for 22 h/d (5d/week)	CLINICAL SIGNS AND MORTALITY All animals survived to the end of the study. Clinical signs were limited to sneezing in the 35.2 mg/L/6h/d group. BODY WEIGHT AND WEIGHT GAIN Mean body weights of males in the 3.52 mg/L, 22 hr, exposure group and 35.2 mg/L/6h/d group were significantly reduced. The mean body weight of females in the 10,000 ppm group were also significantly reduced. HAEMATOLOGY Segmented neutrophils were significantly increased in male mice exposed to 35.2 mg/L/6h/d.	(Dunnick 1991)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
group (10 animals for core studies and 8 animals for neurobehavioral studies)	0, 500, 1000, 4000, 10000 ppmV (0, 1.76, 3.52, 14.08, 35.2 mg/L) 13 weeks	NEUROBEHAVIOUR Female mice in the 3.52 mg/L, 22 hr, exposure group and the 35.2 mg/L/6h/d group showed decreased locomotor activity. ORGAN WEIGHTS Liver, kidney, and heart weights were increased in the 3.52 mg/L, 22 hr, exposure group and the 35.2 mg/L/6h/d group. HISTOPATHOLOGY: NON-NEOPLASTIC Paranodal swellings in the tibial nerve were observed in the 35.2 mg/L/6h/d exposed males and females, and the 3.52 mg/L, 22 hr exposed females. Inflammation and regeneration of the respiratory epithelium and olfactory epithelium, and metaplasia was characterized by replacement of olfactory cells with a ciliated respiratory epithelium in mice exposed to 35.2 mg/L/6h/d. Olfactory lesions were generally limited to the olfactory epithelium in the dorsal meatus (anterior olfactory region); lesions of the olfactory turbinates in the posterior portion of the nasal cavity were less numerous and less severe. Similar lesions, but of less severity were also seen in females in the 14.08 mg/L/6h/d group and 3.52 mg/L, 22 hr, exposure group females. Females in the 3.52 and 1.76 mg/L/6h/d groups showed minimal olfactory epithelium changes. At lower exposure concentrations, the nasal lesions were almost always limited to the olfactory epithelium and rarely involved the respiratory epithelium. Males in the 3.52 mg/L, 22 hr exposure group, and 3.52 mg/L/6h/d group, had minimal lesions. Males in the 14.08 mg/L/6h/d group and 1.76 mg/L/6h/d group did not show nasal lesions.	
Chronic inhalation study Non-guideline, GLP Rat Sprague-Dawley 19 m/group	Pure *n*-hexane or mixed hexanes Inhalation (vapour), dynamic whole body, 22 h/d for 7 d/week 0, 125, 250, 500, 1500 ppmV (0, 0.44, 0.88, 1.76, 5.28 mg/L), positive control: *n*-hexane 6 months	No NOAEC identified Necrosis of liver, degenerative and regenerative renal changes for all dose groups 1.76 mg/L *n*-hexane pure: axonal degeneration, myelin vacuolation, muscle atrophy Positive control (*n*-hexane): abnormal gait Supporting study: type of hexane administered in groups not defined; study not available, (source: substance evaluation report for n-hexane (2013), no further details available)	(Ulrich 1983a)
Chronic inhalation study	Pure *n*-hexane or mixed hexanes	1.76 mg/L mixed hexanes & positive control: abnormal gait, average bw ↓	(Ulrich 1983b)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Non-guideline, GLP Rat Sprague-Dawley 20/group, sex not specified	Inhalation (vapour), dynamic whole body, 22 h/d for 7 d/week 0, 500, 1000 ppmV (1.76, 3.52 mg/L) 24 weeks	Supporting study: type of hexane administered in groups not defined; study not available, (source: substance evaluation report for n-hexane (2013), no further details available)	
Sub-chronic Non-guideline, non-GLP Rat Wistar 8 m/group	*n*-Hexane (>99%) Inhalation (vapour), daily, 12 h/day 0, 500, 1200, 3000 ppmV (1.76, 4.22, 10.56 mg/L) 16 weeks	NEUROLOGICAL EFFECTS Increasing concentrations of n-hexane exposure resulted in dose-dependent reduction of motor nerve conduction velocity (MCV). During the period of weeks 8-16, MCV in 4.22 and 10.56 mg/L groups was significantly reduced when compared with the control level. OTHER FINDINGS Nervous system-specific proteins in tail nerve tissues. The most salient observation was the decrease in beta-S-100 protein in all of the three exposure levels. The amount of beta-S-100 protein was significantly decreased to about 75% of the control level. Beta-S-100 is a nervous system-specific protein and a marker for neurological damages, diseases and neurotoxicity. HISTOPATHOLOGY: NON-NEOPLASTIC The peripheral nerve in 10.56 mg/L exposed rat was severely impaired after 16 weeks exposure. Remarkable paranodal swellings and demyelination as well as remyelination in the myelinated nerve fibers were observed. Similar changes were also seen in 4.22 mg/L-exposed rats but to a lesser degree. LOAEL: 1.76 mg/L/12h/d (decreased nervous system specific proteins in rat tail nerves, beta-S-100 protein); 4.22 mg/L/12h/d (reduced MCV)	(Huang et al. 1989)

The unit ppmV is converted to mg/L according to the Guidance on the Application of the CLP Criteria: mg/l = ppmV x MW (86.18 g·mol^{-1}) x 1/24,450 (conversion factor: 0.00352).

Table 8: Summary table of human data on STOT RE.

More detailed information and numerical data demonstrating the correlation between the incidences and magnitude of adverse neurological effects and *n*-hexane exposure as well as details on co-exposure to other toxic substances are presented in the confidential Annex I.

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
Cohort study	Cleaning solvents	Occupational exposure study in 16 factories, period not given, prolonged	15 workers with polyneuropathy and 2	(Wang et al.

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
(retro-spective)	containing *n*-hexane at concentrations ranging from 10–65% *n*-hexane air concentrations up to 190 ppmV (0.67 mg/L)	exposure due to overtime work; 59 press proofing workers employed for at least 2 months (57 Male / 2 Female workers, mean age 25.8 years with a standard deviation of 10.2 years); referent neurological data were collected from 150 healthy individuals (50 persons from three age groups, 10–35, 36–50, and 51–80 years, sex not stated); two exposure measures using a Sibata type personal air sampler on two different workers were taken in 14 of the 16 factories; no other organic chemicals known to cause polyneuropathy were present in significant amounts	asymptomatic workers with abnormal Motor Nerve Conduction Velocities (MCVs). All but one of these workers were employed in factories that used solvents with *n*-hexane concentrations >50%. Associations between frequency of polyneuropathy and abnormal MCV and *n*-hexane concentration in the cleaning solvents and between the frequency of polyneuropathy and *n*-hexane air concentrations >100 ppmV (>0.35 mg/L). Significant reduction in the MCV among workers exposed to air concentrations ≤ 23 ppmV (≤ 0.08 mg/L)	1986)
Case control study (pro-spective)	Glue or solvent that contained over 50% *n*-hexane Air concentrations were not measured 1 urine sample per study subject at end of weekly shift	Occupational exposure in 4 small shoe factories without protective equipment for about 7 h/d; 40 workers randomly chosen; age 16 - 58 years (mean 31.3); exposure time 1 to 28 years (mean: 12.4); reference values were obtained from 41 unexposed individuals; the threshold value of 7.5 mg/L of the *n*-hexane metabolite 2,5 -hexanedione was derived from the observation that the majority of electroneuromyography (ENM) effects was seen above this value.	Mild or nonspecific symptoms of polyneuropathy. Significant correlation between the *n*-hexane metabolite 2,5 -hexanedione concentration in urine and the ENM scores (decreased conduction velocities). The threshold value postulated by the authors of 7.5 mg/L of 2,5 -hexanedione in urine was closely related to the incidence of abnormalities. However, also 3 workers with lower concentrations of 2,5-hexanedione (3.0, 3.3, and 4.5 mg/L) displayed ENM changes.	(Governa et al. 1987)
Cohort study (retro-spective)	Hydrocarbon mixture containing *n*-hexane, cyclohexane, methyl ethyl ketone, and ethyl acetate; *n*-hexane concentration of 108 breathing zone samples: 0.24 mg/L	Occupational exposure in shoe factory (long term inhalation exposure); 95 shoe factory workers, 24 male / 71 female, age: 16-58 years (mean 29.6), exposure time 1-25 years (mean 9.1); comparison to 52 unexposed workers from the same factory. Gender, age, and employment time were similar in the exposed and referent groups. n-hexane concentration was quantified in 108 breathing zone samples. Co-exposure to cyclohexane and methyl ethyl ketone may have enhanced the neurotoxicity.	Neurological symptoms occurred more frequently among the exposed than the unexposed workers. Increases in the frequency of self-reported sleepiness, dizziness, weakness in the limbs, paresthesia (burning or tingling sensation in limbs), and hypoesthesia (partial loss of sensation and/or diminished sensibility). Increased motor nerve action potential (MAP) duration and decreased MCV in the	(Mutti et al. 1982)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
	(69 ppmV) in the mildly exposed group and 0.47 mg/L (134 ppmV) in the highly exposed group		median and ulnar nerves related to hydrocarbon exposure.	
Cohort study (retro-spective)	Air monitoring revealed that cyclohexane, pentane, toluene and ethyl acetate were present in the workshops' atmospheres; time-weighted average (TWA) exposure of workers to *n*-hexane: 0.08 mg/L (24 ppmV)	Occupational exposure in shoe making workshops (long term inhalation exposure); 27 asymptomatic male workers from 6 shoemaking workshops were studied and compared with a group of 20 age- and sex-matched normal controls with no history of exposure to any neurotoxic agent. They underwent physical examinations as well as conventional needle electromyographic examinations and sensory and motor nerve conduction studies of upper and lower extremities. The TWA exposure to *n*-hexane and urinary concentration of free 2,5-hexanedione were also determined. 84 air samples were collected from the breathing zone of 12 selected workers (7 samples per worker). Co-exposure to cyclohexane may have enhanced the neurotoxic effects.	The amplitudes of sensory nerve action potential (SAP) for median and sural nerves were significantly lower in exposed subjects than in unexposed normal controls. Additionally, a significant correlation was found between these decreases and the urinary concentration of free 2,5-hexanedione. No correlation was found between the decreased amplitudes of SAP and exposure to *n*-hexane.	(Neghab et al. 2012)
Surveillanc e (retro-spective)	*n*-Hexane; 500 – 2500 ppmV (1.76-8.8 mg/L) in the patients' work areas; outbreak 1968), <50 ppmV during rescreening	Medical examination on 296 of 1,662 workers from sandal manufacturer by questionnaire and medical/neurological examination, 44 cases were further examined by electromyography, measurement of peripheral nerve conduction velocities, and other tests; no control group. During a rescreening in 1981, again 21 cases with mild *n*-hexane polyneuropathy were clinically and neurophysiologically observed, revealing mostly the same features as in the previous study in 1968.	93 cases of polyneuropathy were found in 1968. Remarkable changes of nerve conduction velocities, especially in the lower rather than the upper extremities. Over a few years since 1968 most of the cases completely recovered, except for a few with mild sensory impairment, after providing for 100 ppm as the maximal allowable concentration of *n*-hexane and well equipped ventilation systems in individual houses. During rescreening mild cases of *n*-hexane polyneuropathy were clinically and neurophysiologically observed in spite of less than	(Iida 1982) (Yamamura 1969, only summar available)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
			50 ppmV *n*-hexane concentration.	
Cohort study (retro-spective)	*n*-Hexane, exposure to n-hexane assessed by walk-through inspections of the factories, interviews with workers and management, and examination of past exposure measurements; levels of *n*-hexane between 0.181 and 2.436 mg/L (51-692 ppmV; median 0.593 mg/L or 168 ppmV)	Relationship between *n*-hexane exposure and neurological symptoms and signs, and sensitivity to peripheral vibration, was investigated in 126 metal can manufacturing workers from two factories, internal control group not exposed to *n*-hexane (63 workers), 63 workers with low or high exposure	Exposed workers were significantly more likely than unexposed workers to report at least one neurological symptom of the limbs. Highly exposed workers were able to detect tuning fork vibration for significantly shorter periods than unexposed workers, independent of the effects of age, height, sex, alcohol, and education; the tuning fork was best able to discriminate between high exposure and unexposed groups, and was free of the influence of confounders.	(Bachmann et al. 1993)
Survaillance (retro-spective)	*n*-Hexane, time weighted average air concentrations: 30 to 110 ppmV (0.11-0.39 mg/L; mean 63 ppmV, 0.22 mg/L) for *n*-hexane; concentrations were higher in the personal air samples from the offset machine workers: 80 to 210 ppmV (0.28-0.74 mg/L; mean	56 workers of an offset printing factory, workers worked 12 hours per day, 6 days per week; mean duration of employment 2-6 years (range 1 month to 12 years); use of cleaning solvents with 14-20% of *n*-hexane; these solvents contained a variable percentage of toluene but no methyl *n*-butyl ketone (MBK) or methyl ethyl ketone (MEK); moistening solutions contained only trace amounts of phosphate at 42 ppm. The printing inks contained 0.6-8.2 pg/g lead, <0.05-0.95 pg/g mercury and no volatile organic compound; time weighted average air concentrations: 30 to 110 ppmV (0.11-0.39 mg/L; mean 63 ppmV, 0.22 mg/L) for *n*-hexane, 57 to 340 ppmV (mean 130 ppmV) for isopropyl alcohol (IPA) and 11 to 46 ppmV (mean 26 ppmV) for toluene; concentrations were higher in the personal air samples from the offset machine workers, 80 to 210 ppmV (0.28-0.74 mg/L; mean 132 ppmV, 0.46	20 workers (36%) developed symptomatic peripheral neuropathy, 26 (46%) developed subclinical neuropathy; reduced amplitude of the sensory action potentials, followed by reduced amplitude of the motor action potentials, reduction in motor conduction velocities and increase in distal latencies; no correlation between the development of neuropathy and the length of employment in printing; nine workers (16%) developed neuropathy just a few months after exposure while five (9%) remained healthy 4 to 8 years after exposure	(Chang et al. 1993)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
	132 ppmV, 0.46 mg/L)	mg/L) for *n*-hexane, 20 to 680 ppmV (mean 235 ppmV) for IPA, and 20 to 84 ppmV (mean 50 ppmV) for toluene; no potentially confounding chemicals detected (e.g., no MBK, no MEK)		
	n-Hexane; air concentrations over eight hours of personal sampling: 109.1 ± 5 ppmV (0.38 mg/L, 2 workers in cement coating), 86.4 ± 3.7 ppmV (0.30 mg/L; 3 workers in nylon winding), 75 ppmV (0.26 mg/L; 1 worker in gas injection)	44 workers from a ball manufacturing factory; classified into three groups according to their degree of solvent exposure (I: 5 workers in cement coating or nylon fibre winding, II: 8 workers in gas injection, III: 31 other jobs; samples of the solvents used contained 14.1% n-hexane, 54% of other saturated hydrocarbons, 3% toluene, 0.8% benzene, 0.1% xylene, no methyl n-butyl ketone, methyl ethyl ketone, carbon disulphide, acrylamide detected; air concentrations over eight hours personal sampling: 109.1 ppmV	5 workers of group I (highest exposure group) developed severe (4) or moderate (1) polyneuropathy (100%); 2 of 8 workers in group II (medium exposure) developed mild polyneuropathy; no cases of polyneuropathy in group III; increasing frequency of polyneuropathy with increasing degree of *n*-hexane exposure; after installation of ventilation systems and enclosed solvent operations, *n*-hexane air concentrations <15 ppmV and no new cases of polyneuropathy in a two year follow up period; before occurrence of polyneuropathy incidences, the factory did not use solvents with *n*-hexane	(Huang et al. 1991)
Case control studies	*n*-Hexane; air concentrations in the working environments between 60 and 810 ppmV (0.21-2.85 mg/L).	27 male patients (15 - 52 years old, mean 22.5 years) with polyneuropathy; worked in leather coat or shoe production for 4 months to 20 years (mean 4.4 years); exposure to glues containing *n*-hexane (46.6-98.9%) was 4 months to 2 years (mean 12.2 months); 24 non-exposed controls without symptoms	Results of needle electromyography and nerve conduction studies were compatible with primarily axonal polyneuropathy with secondary segmental demyelination; motor conduction velocities were the slowest in distal regions of the nerves; in the proximal nerve segments, which were partly tested by magnetic stimulation of the nerve roots, this slowing was much less pronounced; the reduction in mean motor conduction velocities in the forearm segments of ulnar nerves was more than 30% in comparison to the control group means; this reduction was 10% in the neckaxilla segments	(Öge et al. 1994)
Case control study	*n*-Hexane (eight-hour time-	2 age-matched groups compared consisting of 14 control and 14 exposed workers employed in a factory	Prevalence of headaches, dysesthesia of limbs, muscle weakness and complaints	(Sanagi et al. 1980)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
	weighted average exposure: 58±41 ppmV (0.204 mg/L), co-exposure to acetone (39±30 ppmV)	producing tungsten carbide alloys; exposure measured with 22 personal samples taken from the breathing zone over a period of two years; exposure duration from 1 to 12 years with an average of 6.2 years; questionnaires and clinical neurological examinations	about hearing deficits was higher in the exposed group compared to the control; no significant differences in the nerve conduction velocities of the right median, ulnar, and posterior tibial nerves but a statistically significant decrease was detected in the posterior tibial nerve, co-exposure to acetone as potential confounding factor	
Case study	*n*-Hexane; two one hour air samples: *n*-hexane 55 ppmV, benzene 9.65 ppmV; toluene, carbon disulfide, acrylamide, methyl n-butyl ketone (MBK) and triorthocresyl phosphate (TOCP) were not detected; analysis of the colouring agents showed absence of arsenic and lead	Study on 5 workers from press-proofing factory with chronic *n*-hexane exposure, 12 h shifts per day; evoked potentials (EPs) studied, supposed to be useful in detecting the CNS dysfunctions; main ingredients of 3 types of organic solvents used were 65% of *n*-hexane and 100% of benzene and C15-Cl9 hydrocarbons.	Reduction in the nerve conduction velocity was in general correlated with the severity of clinical involvement; data indicate that the spinal cord and the brainstem might be affected in chronic *n*-hexane intoxication	(Huang and Chu 1989)
Case study	*n*-Hexane;	26 exposed workers from a shoe factory; only 10 workers exposed to solvents, 11 to 12 months exposure, up to 11 h daily. Organic vapour gas chromatography analysis, at the place of work, showed 40% of n-hexane, 55% of hexane isomers, 4% of heptane, 1% of cyclohexane, and traces of methylethylketone and toluene. 10 workers clinically diagnosed as suffering a polyneuropathy were biological studies of the urine and a neurophysiological evaluation (NCS and EMG)	10 diagnosed with polyneuropathy were the only ones directly exposed to manually used adhesives; clinical neuropathy was diagnosed when at least two of the following conditions were observed: (1) diminution of tendon reflex in the limbs or absence of achilles reflex; (2) decreased tactile sensibility in the lower extremities; (3) reduced vibratory sensibility below the knee; and (4) alteration of walking capacity	(Pastore et al. 2002)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
Case study	*n*-Hexane	20 healthy workers who were exposed for prolonged periods to solvents containing *n*-hexane; in all, 20 urinary 2,5-HD concentrations were above the 5 mg/L biological exposure index (BEI) recommended by the American Conference of Governmental Industrial Hygienists	Neurographic studies revealed significant differences in the amplitude of sensory nerve action potentials (SNAP) recorded from the sural (mean 14.0 pV), median (mean 17.3 pV), and ulnar (mean 7.9 pV) nerves when compared with normal values from healthy adults of the same age range, examined under identical conditions; the amplitude of the SNAP in sural and median nerves correlated significantly with the number of years worked	(Pastore et al. 1994)
Case study	*n*-Hexane	3 women exposed to an adhesive agent containing 80.4% of *n*-hexane; job: to stick together shaped pieces of raincoats, after spreading glue on them	3 women developed a predominantly motor polyneuropathy following exposure to an adhesive agent containing 80.4% of n-hexane; irregular and swollen myelin sheaths and segmental swelling of axons with dissolution of neurotubules and evident increase of neurofilaments were frequently observed; muscles showed denervation atrophy and focal degenerative myopathic changes.	(Scelsi et al. 1980)
Case study		Aim of this study was to investigate the effects of n-hexane on visual function and to determine the duration of any symptoms related to workplace exposure to *n*-hexane; 26 workers from leather industry who were diagnosed as having polyneuropathy following *n*-hexane exposure; FM-100 Hue test was performed with the 26 workers and compared with a control group of 50 people who had not been exposed to *n*-hexane	All 26 cases reported upper and lower limb weakness, leg pain, asthenia, paraesthesiae in the hands and arms and difficulty with walking; mean total error score for colour discrimination in the exposed group was 168.3 (SD=70.5) for the right eye and 181.5 (SD=103.0) for the left eye and for the control group 36.0 (SD=19.8) and 35.6 (SD=18.2) for the right and left eye respectively; differences between total and partial error scores for exposed and control group were statistically significant ($p<0.001$; increased total error scores in the exposed group); possible relationship between *n*-hexane exposure	(Issever et al. 2002)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
			and defects in colour vision	
Case report	*n*-Hexane	3 female workers wiping glue off furniture with rags soaked in a solvent which contained *n*-hexane; air concentration of *n*-hexane 650 ppmV (2.29 mg/L, average), peaks of up to 1,300 ppmV (4.58 mg/L)	Motor and sensory impairment were noted in all 3 women with an onset 2-4 months after beginning employment; burning sensation in the face, numbness of the distal extremities, and an insidious, progressive distal symmetrical weakness in all extremities; nerve conduction velocities were 26 - 45 m/s in the right and left ulnar nerve (normal range in the general population, 49-75), 23 m/s in the left peroneal nerve (normal range, 40-60)	(Herskowitz et al. 1971; only summary available)

The unit ppmV is converted to mg/L according to the Guidance on the Application of the CLP Criteria: mg/l = ppmV x MW (86.18 $g \cdot mol^{-1}$) × 1/24,450 (conversion factor: 0.00352).

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

The evidence of target organ toxicity through repeated exposure to *n*-hexane was obtained from animal testing and epidemiological data. None of the animal tests on repeated dose toxicity was carried out in full compliance with EU Regulation (EC) No 440/2008 or current OECD guidelines for the testing of chemicals (Table 7: Summary table of animal studies on STOT RE.). One study was similar to OECD TG 413 (Dunnick 1991). The information provided is sufficient to conclude that *n*-hexane produces significant toxicity (i.e., polyneuropathy) in animals following repeated exposure through inhalation and oral ingestion. Significant neurotoxic effects observed in 90-day (or longer) repeated-dose inhalation studies conducted in experimental animals were seen at concentrations ≥ 1.76 mg/L (500 ppmV; lesions to the olfactory epithelium (Dunnick 1991); decreased nervous system specific proteins in rat tail nerves (Huang et al. 1989); axonal degeneration and abnormal gait (Ulrich 1983a, b)) with clear neurological deficits (e.g., decreased locomotor activity) at 3.52 mg/L (1000 ppmV) (Dunnick 1991) or 4.22 mg/L (1200 ppmV) (Huang et al. 1989). Valid inhalation tests according to current guidelines with concentrations below 500 ppmV (including the dose range below guidance values for classification) are not available. Also in an oral exposure study, neurotoxic effects (hind limb paralysis, multifocal axonal swellings, adaxonal myelin infolding, and paranodal myelin retraction) were observed at the highest dose (3973 mg/kg bw/d) during 120 days daily exposure (Krasavage et al. 1980).

Multiple studies reporting human data suggest that the incidence of peripheral neuropathy can be attributed to prolonged occupational exposure to *n*-hexane (Table 8: Summary table of human data on STOT RE.) (ATSDR 1999, EPA 2005, WHO/IPCS/EHC 1991). Severity of effects range from reduced motor and sensory nerve conduction velocities to severe quadriparesis. Kutlu et al. (2009) demonstrated that 83.3% of patients with *n*-hexane induced neuropathy recovered completely within 12 months after cessation of exposure. Drawback of epidemiological studies is that the exposure groups may be exposed to mixtures of solvents and hence confounding factors cannot be excluded in all cases. In particular co-exposure to ketones (e.g., methyl *n*-butyl ketone) may aggravate the effect of *n*-hexane (Ladefoged et al. 1994, Noraberg and Arlien-Soborg 2000). Methyl *n*-butyl ketone (CAS# 591-78-6; classified as STOT RE 1, H372) is metabolized to the same neurotoxic metabolite as *n*-hexane (2,5-hexanedione) and causes the same

neurotoxic effects (Governa et al. 1987, Granvil et al. 1994, LoPachin and Gavin 2015). Thus, 2,5-hexanedione is most likely responsible for the neurotoxic effects of *n*-hexane (LoPachin and Gavin 2015). Co-exposure to potentially confounding factors has been documented in the following studies from the summary in Table 8: Summary table of human data on STOT RE.: Sanagi et al. 1980, Mutti et al. 1982, Neghab et al. 2012. Co-exposure to potentially confounding factors is unlikely in the following studies (e.g., no detection or only detection of trace amounts of acetone or methyl n-butyl ketone): Wang et al. 1986, Huang and Chu 1989, Huang et al. 1991, Chang et al 1993, Pastore et al. 2002.

The following observations from workers at 16 press proofing factories, where cleaning solvents containing 10–65% of *n*-hexane were used, indicate that *n*-hexane is driving the observed neurological effects (Wang et al. 1986):

i) increased frequency of polyneuropathy as well as abnormal motor nerve conduction velocity (MCV) with higher *n*-hexane concentration in the cleaning solvents;
ii) association between the frequency of polyneuropathy and *n*-hexane air concentrations > 100 ppmV (> 0.352 mg/L);
iii) consistent improvement of neurological symptoms of workers who went back to work and no new cases of polyneuropathy after *n*-hexane was removed and ventilation improved;
iv) other organic chemicals known to cause polyneuropathy (Juntunen and Haltia 1982) were not present in significant amounts.

In Huang et al. (1991), higher exposure levels of *n*-hexane coincided with higher incidences of polyneuropathy in workers and no polyneuropathy cases occurred before the factory started using *n*-hexane and after a new ventilation system was installed which reduced the *n*-hexane air concentrations below 15 ppmV (0.05 mg/L). Governa et al. (1987) found a significant correlation between the urinary level of the *n*-hexane metabolite 2,5-hexanedione and electroneuromyographic changes as marker for polyneuropathy of forty shoe factory workers. However, urinary 2,5-hexanedione levels alone are not sufficient to prove sole exposure to *n*-hexane because it is also a metabolite of the solvent methyl *n*-butyl ketone (Granvil et al. 1994). The latter solvent has not been used in the press proofing workshops studied in Wang et al. (1986). Such potential confounding factors were also excluded in the study by Chang et al. (1993). In this study, from 56 workers of an offset printing factory, 20 workers (36%) developed symptomatic peripheral neuropathy and 26 (46%) developed subclinical neuropathy (total: 82%) at *n*-hexane concentrations in the personal air samples from the offset machine workers from 80 to 210 ppmV (0.28-0.74 mg/L; mean 132 ppmV, 0.46 mg/L) and 20 to 680 ppmV (mean 235 ppmV) for isopropyl alcohol, and 20 to 84 ppmV (mean 50 ppmV) for toluene; no potentially confounding chemicals detected (no methyl *n*-butyl ketone or methyl ethyl ketone; Table 8: Summary table of human data on STOT RE.). Also more recent case studies support the polyneuropathy-inducing effects of the *n*-hexane metabolite 2,5-hexanedione (Baslo et al., 2021; Sun et al., 2020).

More detailed information and numerical data demonstrating the correlation between the incidences and magnitude of adverse neurological effects and *n*-hexane exposure as well as details on co-exposure to other toxic substances are presented in the confidential Annex I.

All available animal studies (Dunnick 1991, Huang et al. 1989, Krasavage et al. 1980, Takeuchi et al. 1980, Ulrich 1983a, b) accurately reproduce the clinical and pathological findings in humans (e.g., reduced MCV, motor dysfunction, impaired peripheral nerve function, limb paralysis). Effects in animals occur after exposure to higher concentrations of *n*-hexane (≥ 1.76 mg/L, ≥ 500 ppmV; Table 7: Summary table of animal studies on STOT RE.) compared to humans (≤ 0.67 mg/L, ≤ 190 ppmV; Table 8: Summary table of human data on STOT RE.), however no lower concentrations were tested in animals. In the study by Wang et al. (1986) the most sensitive effect was a significant reduction in the MCV among workers exposed to air concentrations ≤ 0.08 mg/L (≤ 23 ppmV). Effect concentrations for adverse neurological effects in animal studies are about 10-times higher than the guidance value for Category 1 classification (C ≤ 0.2 mg/L/6h/d). However, *n*-hexane exerts neurotoxic effects via its metabolite 2,5-hexanedione and 2,5-hexanedione is considered as an important *n*-hexane metabolite in humans (Governa et al. 1987, Perbellini et al. 1981) present in human urine in concentrations 20–30-times higher than 2-hexanol, another *n*-hexane metabolite (MAK 1982). In contrast, animal studies identified 2-hexanol as the main metabolite (Perbellini et al. 1981, MAK 1982) with urine concentrations 3-times higher than concetrations of 2,5-hexanedione (MAK 1982). Additionally, some authors claim that humans (with longer nerves) are more susceptible than rodents (with

shorter nerves) to *n*-hexane induced neurofilament-filled axonal swellings after exposure because there is supposed to be a greater probability for cross-linking in the longer axons of humans in comparison to the shorter nerves in rodents (Dunnick 1991, Friede et al. 1984, Graham and Gottfried 1984). The higher susceptibility of longer axons is also supported by a recent review, stating that initial changes occur in the largest and longest axons in peripheral nerves and the spinal cord, with similar changes in shorter nerve fibres at a later stage (Spencer and Chen, 2021). Thus, it seems plausible that humans are more susceptible than rodents to neurotoxic effects of *n*-hexane.

The neurotoxic effect of 2,5-hexanedione is documented in numerous *in vivo* and *in vitro* studies as discussed in the mini review by LoPachin and Gavin (2015) and adduct formation with specific lysine residues on neurofilament subunits and other proteins is identified as likely mechanism of toxic action leading to neuropathies. This strengthens the case that long-term inhalation exposure to *n*-hexane is causing the observed epidemiological evidence of neurotoxic effects in humans.

10.12.2 Comparison with the CLP criteria

According to Annex I of regulation (EC) No 1272/2008 (section 3.9.2.), substances that have produced significant specific target organ toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant specific target organ toxicity in humans following repeated exposure, shall be classified in STOT RE Category 1. Substances are classified in Category 1 for target organ toxicity (repeated exposure) on the basis of reliable and good quality evidence from human cases or epidemiological studies or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health (i.e., no indication that mechanisms of action are not relevant for humans), were produced at generally low exposure concentrations. Only in exceptional cases human evidence can also be used to place a substance in Category 2: (a) when the weight of human evidence is not sufficiently convincing to warrant Category 1 classification; and/or (b) based on the nature and severity of effects. When well-substantiated human data are available showing a specific target organ toxic effect that can reliably be attributed to repeated or prolonged exposure to a substance, the substance shall normally be classified. Positive human data, regardless of probable dose, predominates over animal data (Annex I of regulation (EC) No 1272/2008, section 3.9.2.10.2.).

As outlined in section 10.12.1, human evidence from several epidemiological studies (Table 8: Summary table of human data on STOT RE.) as well as experimental data in animals (Table 7: Summary table of animal studies on STOT RE.) demonstrate that long-term inhalation exposure to *n*-hexane can reliably be attributed to significant adverse neurological effects. The possible occupational co-exposure to other agents causing neurotoxic effects decreases the power of the epidemiological studies. However, all epidemiological studies indicated effects on the peripheral nervous system (i.e., polyneuropathy) at *n*-hexane concentrations occurring in these occupational settings, i.e., ≤ 0.67 mg/L (190 ppmV). In one study, a significant reduction in the motor nerve conduction velocity among workers exposed to air concentrations ≤ 0.08 mg/L (≤ 23 ppmV) was observed. Significant neurotoxic effects observed in 90-day (or longer) repeated-dose animal studies occur at concentrations ≥ 1.76 mg/L (500 ppmV) (i.e., above the guidance value for Category 1 classification for vapour inhalation exposure: C ≤ 0.2 mg/L/6h/d), but positive human data, regardless of probable dose, overrule animal data and no lower dose than 1.76 mg/L was tested in the animal studies. In addition, there is some evidence to suggest that humans are more sensitive to the neurotoxicity of *n*-hexane than rats. Additionally, neurological effects in humans (e.g., quadriparesis due to polyneuropathy) are reversible only after a prolonged time after cessation of exposure (83% of patients recover within 12 months) substantiating the high severity of effect. Evidence for severe neurotoxicity of *n*-hexane in humans, as detailed in section 10.12.1, requires STOT RE Category 1 classification because an exemption for Category 1 classification due to insufficiently convincing human data and/or low severity of effects is not justified.

10.12.3 Conclusion on classification and labelling for STOT RE

Conclusively, neurotoxicity of *n*-hexane is unequivocally documented in rats, and, based on human data suggesting an even higher sensitivity than rats, of high relevance for human health. Thus, *n*-hexane complies with the CLP criteria for STOT RE Category 1 due to sufficient evidence of significant toxicity in humans as outlined in the section above.

For the current harmonised classification (STOT RE 2) a specific concentration limit of C ≥ 5 % is applied. For STOT-RE, a specific concentration limit (SCL) may only be set for substances inducing target organ toxicity at a dose level or concentration clearly (more than one order of magnitude) below the guidance value according to CLP Annex I, Table 3.9.2 and shall be calculated according to the following equation: SCLCat.1 = (effective dose/guidance value) × 100%.

The guidance value for the proposed STOT RE 1 classification is C ≤ 0.2 mg/L/6h/day (vapour inhalation exposure). The lowest effective dose in animal studies (1.76 mg/L) is about one magnitude higher than the guidance value (Table 7: Summary table of animal studies on STOT RE.). The lowest effective dose observed in human studies was 0.08 mg/L (Table 8: Summary table of human data on STOT RE.) and hence still less than one order of magnitude below the guidance value. Thus, establishment of a specific concentration limit is not indicated for *n*-hexane and the generic concentration limits should be applied.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The current CLP Annex VI STOT entry for n-hexane is STOT RE 2*; H373. The DS proposed to modify this to STOT RE 1; H372 (nervous system). This was based on human evidence. The CLH report contained also *in vivo* animal data for n-hexane showing neurotoxicity, but these effects occurred at doses above the guidance value range for classification.

The human data includes epidemiological cohort and case-control studies, as well as medical surveillance or case studies. They show consistently adverse neurological effects, such as polyneuropathy, decreased motor nerve conduction velocities, increased distal latencies, limb weakness, dysesthesia, paraesthesia, hypoesthesia, sleepiness and dizziness.

Also in sub-chronic mouse and rat studies, similar adverse neurological effects have been observed. No guideline studies were available, but the dataset includes one study similar to OECD TG 413. In addition, several non-guideline studies are included. The observed effects include hindlimb paralysis, foot drop, unsteady or otherwise abnormal gait, decreased locomotor activity, muscular atrophy, reduced motor nerve conduction velocity (MCV) and prolonged distal latencies. Histopathological changes in the nervous system have also been observed.

For n-hexane's current harmonised classification (STOT RE 2), a specific concentration limit of C ≥ 5 % is applied. Now, the DS proposes that for mixtures containing n-hexane, establishment of a specific concentration limit is not indicated, and the generic concentration limits should be applied (i.e. STOT RE 1 for mixtures containing n-hexane at concentrations ≥ 10%, and STOT RE 2 for mixtures containing n-hexane at concentrations ≥ 1% and <10%).

Comments received during consultation

The two commenting MSCAs and one Company-Downstream user agreed with the proposal. Two Industry or trade associations agreed with the proposal otherwise, but

suggested alternative concentration limits for the classification, as described in some more detail below.

The two Industry or trade associations mentioned above commented that n-hexane may be present as a constituent in light hydrocarbon solvents (e.g. technical hexane) at varying concentrations depending on the boiling range of the solvent, but also in solvent-based formulations. They pointed out that in the past decades, the industry has built a legacy of solvents, formulations and industrial processes on the basis of the current 5% specific concentration limit. They stated that a change in the concentration limit would cause a lot of additional work and disruption for the industry, without increasing safety. In the detailed comment, they argue that the available human studies (key study Wang *et al.*, 1986 in line with the others) show no neurotoxicity at n-hexane concentrations <10%. In addition, they argued that a review of the human data indicates that the no effect level for neurological effects, including reductions in MCV, is in the range of 140-300 mg/m^3. In summary, they expressed that the consequences of changing the current concentration limit of 5% to the proposed GCL of 1 % would be disproportionate to any evidence of health benefits. As a pragmatic approach to support managing this change in classification, they proposed alternative concentration limits for n-hexane, resulting in the following scheme:

> 10% n-hexane – STOT RE 1 (H372)

< 10% but > 5% - n-hexane – STOT RE 2 (H373)

< 5% n-hexane – no classification.

One Company-Downstream user gave a general comment that the proposed new classification of n-hexane should also be applied to technical hexane, registered under REACH as "Hydrocarbons, C6, n-alkanes, iso-alkanes, cyclics, n-hexane rich" (EC 925-292-5), as consumers can mostly be exposed to this technical hexane instead of pure n-hexane (CAS 110-54-3; EC 203-777-6).

Two Company-Downstream users sent a bibliographic report on the reprotoxic and endocrine disruptive effects of n-hexane and its toxic metabolite 2,5-hexanedione. One of them also commented that n-hexane should be classified as Repr. 1A, H360 (May damage fertility on human) and EDC Human - suspected (Endocrine Disruptor compound for human).

One individual provided a scientific paper (Cheng *et al.*, 2012: Exposure to 2,5-hexanedione can induce neural malformations in chick embryos) and commented that based on it, n-hexane should be classified for Repr. 1B, H360 with a warning related to teratogenic effect.

Please see the RCOM for the RAC replies to these comments.

Assessment and comparison with the classification criteria

Animal data

As mentioned by the DS, none of the available animal tests on repeated dose toxicity were carried out in full compliance with current OECD guidelines for the testing of chemicals.

One study was similar to OECD TG 413, but it was not performed in compliance with GLP

(Dunnick, 1991). This was a sub-chronic (13-week) inhalation toxicity study in B6C3F1 mice (18 M + 18 F, 10 animals for core studies and 8 animals for neurobehavioral studies). Whole body inhalation exposure to 99% n-hexane (vapour) was carried out daily for 5 d/week, 6 h/day at the concentration levels of 0, 1.76, 3.52, 14.08 and 35.2 mg/L. In addition, an additional 3.52 mg/L group was exposed for 22 h/d, 5 d/week. The only observed clinical sign was decreased locomotor activity in the female mice in this 3.52 mg/L/22 h/d exposure group and the 35.2 mg/L/6h/d group. In the histopathological examination, paranodal swellings in the tibial nerve were observed in the 35.2 mg/L/6 h/d exposed males and females, and the 3.52 mg/L/22 h/d exposed females. Inflammation and regeneration of the respiratory epithelium and olfactory epithelium, and metaplasia was characterized by replacement of olfactory cells with a ciliated respiratory epithelium in mice exposed to 35.2 mg/L/6h/d. Olfactory lesions were generally limited to the olfactory epithelium in the dorsal meatus (anterior olfactory region); lesions of the olfactory turbinates in the posterior portion of the nasal cavity were less numerous and less severe. Similar lesions, but of less severity, were also seen in females in the 14.08 mg/L/6h/d group and 3.52 mg/L, 22 hr, exposure group females. Females in the 3.52 and 1.76 mg/L/6h/d groups showed minimal olfactory epithelium changes. At lower exposure concentrations, the nasal lesions were almost always limited to the olfactory epithelium and rarely involved the respiratory epithelium. Males in the 3.52 mg/L, 22 hr exposure group, and 3.52 mg/L/6h/d group, had minimal lesions. Males in the 14.08 mg/L/6h/d group and 1.76 mg/L/6h/d group did not show nasal lesions.

Also five non-guideline studies are available:

- *Krasavage et al., 1980*: non-GLP in CD(SD)BR rats, 5 M/group. Dosing: 99% n-hexane, oral gavage daily (5 days/week) at 0, 568, 1135 or 3973 mg/kg bw/d for 90 or 120 days (highest dose group).
- *Takeuchi et al., 1980:* non-GLP in Wistar rats, 7 M/group. Dosing: 99% n-hexane, whole body inhalation (vapour) 12 h/day (7 days/week) at 0 or 10.56 mg/L for 16 weeks. Motor nerve conduction velocity (MCV) and the distal latency were measured before the beginning of the exposure and after exposure for 4, 8, 12, and 16 weeks.
- *Ulrich, 1983a* (study not available to the DS): GLP in Sprague-Dawley rats, 19 M/group. Dosing: pure n-hexane or mixed hexanes, dynamic whole body inhalation (vapour) 22 h/d (7 days/week) at 0, 0.44, 0.88, 1.76, 5.28 mg/L for 6 months.
- *Ulrich, 1983b* (study not available to the DS): GLP in Sprague-Dawley rats, 20/group (sex not specified). Dosing: Pure *n*-hexane or mixed hexanes, dynamic whole-body inhalation (vapour) 22 h/d (7 d/week) at 1.76 or 3.52 mg/L for 24 weeks.
- *Huang et al., 1989*: non-GLP in Wistar rats, 8 M/group. Dosing: >99% n-hexane, inhalation (vapour) 12 h/day (daily, no further information) at 1.76, 4.22 or 10.56 mg/L for 16 weeks.

In Krasavage *et al.* (1980), three animals died immediately after the oral gavage. In Takeuchi *et al.* (1980), two animals died during the study (close to the end of the

exposure period).

In these five studies, the following neurotoxic (clinical) signs were observed: hindlimb paralysis, unsteady or otherwise abnormal gait, foot drop, muscular atrophy, reduced motor nerve conduction velocity, significantly prolonged distal latencies. In addition, changes in nervous system-specific proteins in tail nerve tissues were observed, specifically a significant decrease in beta-S-100 protein, which is a marker for neurological damages, diseases and neurotoxicity.

In addition, the following neurohistopathological changes were observed in these five studies: multifocal axonal swellings; adaxonal (meaning innermost layer of the myelin sheath) myelin infolding; paranodal myelin retraction; paranodal swellings in the myelinated fibers of the tibial nerve and dorsal trunk of the tail nerve; excessive number of neurofilaments, vesicles, multivesicular bodies, mitochondria, myelin figures, and dense bodies in the paranodal axoplasm; no neurotubules; denervated neuromuscular junctions in the muscles; muscle fibers of irregular shape and size that seemed to have an increased number of nuclei (probably indicating regenerative proliferation), and had disordered myofilaments, zig-zagging of the z-band, and invaginations of the plasma membrane; axonal degeneration; myelin vacuolation, muscle atrophy; severe impairment of peripheral nerves, including remarkable paranodal swellings and demyelination as well as remyelination in the myelinated nerve fibers.

As summarised by the DS, the information provided is sufficient to conclude that *n*-hexane produces significant toxicity (i.e., polyneuropathy) in animals following repeated exposure through inhalation and oral ingestion. Significant neurotoxic effects observed in 90-day (or longer) repeated-dose inhalation studies conducted in experimental animals were seen at concentrations ≥ 1.76 mg/L (500 ppmV; lesions to the olfactory epithelium (Dunnick, 1991); decreased nervous system specific proteins in rat tail nerves (Huang *et al.*, 1989); axonal degeneration and abnormal gait (Ulrich, 1983a, b) with clear neurological deficits (e.g., decreased locomotor activity) at 3.52 mg/L (1000 ppmV) (Dunnick, 1991) or 4.22 mg/L (1200 ppmV) (Huang *et al.*, 1989). Valid inhalation tests according to current guidelines with concentrations below 500 ppmV (including the dose range below guidance values for classification) are not available. Also in an oral exposure study, neurotoxic effects (hind limb paralysis, multifocal axonal swellings, adaxonal myelin infolding, and paranodal myelin retraction) were observed at the highest dose (3973 mg/kg bw/d) during 120 days daily exposure (Krasavage *et al.*, 1980).

Human data

The human data includes epidemiological studies (four retrospective occupational cohort studies and three retro- or prospective case-control studies) and nine medical surveillance or case studies.

Cohort studies (retrospective)

- *Wang et al., 1986:* reported an occupational exposure study in 16 factories (period not given), covering 59 press proofing workers employed for at least 2 months (57 M / 2 F, mean age 25.8 years with a standard deviation of 10.2 years). Exposures were to cleaning solvents containing n-hexane at concentrations ranging form 10–65%; no other organic chemicals known to cause polyneuropathy were present in significant amounts. Two air measurements were made on two different workers in 14/16 factories, using personal air samplers. N-

hexane air concentrations of up to 0.67 mg/L were measured. Prolonged exposures were due to overtime work. Referent neurological data were from 150 healthy individuals (50 from three age groups: 10–35, 36–50, and 51–80 years, sex not stated).

- *Mutti et al., 1982:* considered occupational exposure in a shoe factory. A total of 95 shoe factory workers (24 M, 71 F) aged 16–58 years (mean 29.6) were involved and were xposed to hydrocarbon mixtures containing n-hexane, cyclohexane, methyl ethyl ketone, and ethyl acetate. The N-hexane concentration was quantified in 108 breathing zone samples: 0.24 mg/L (69 ppmV) in the mildly exposed group and 0.47 mg/L (134 ppmV) in the highly exposed group. The exposure time varied from 1-25 years (mean 9.1. Co-exposure to cyclohexane and methyl ethyl ketone may have enhanced the neurotoxicity. Comparison was made to 52 unexposed workers from the same factory and gender, age, and employment time were similar in the exposed and referent groups.

- *Neghab et al., 2012*: Reported on occupational exposure in 6 shoe making workshops, and followed long term inhalation exposure In 27 asymptomatic male workers. 84 air samples were collected from the breathing zone of 12 selected workers (7 samples per worker). Based on air monitoring, exposures to cyclohexane, pentane, toluene and ethyl acetate. Time-weighted average (TWA) exposure of workers to n-hexanewas 0.08 mg/L (24 ppmV). In addition, urinary concentrations of free 2,5-hexanedione were determined. Co-exposure to cyclohexane may have enhanced the neurotoxic effects. Comparison was made to a group of 20 age- and sex-matched normal controls with no history of exposure to any neurotoxic agent. Physical examinations were carried out as well as conventional needle electromyographic examinations and sensory and motor nerve conduction studies of upper and lower extremities.

- *Bachmann et al., 1993*: reported on 63 workers in two metal can manufacturing factories. Low or high exposure to n-hexane was assessed by walk-through inspections of the factories, interviews with workers and management, and past exposure measurements with levels of n-hexane between 0.181 and 2.436 mg/L (51-692 ppmV; median 0.593 mg/L or 168 ppmV). An internal control group not exposed to n-hexane (63 workers) was included and the relationship between n-hexane exposure and neurological symptoms and signs, and sensitivity to peripheral vibration was investigated.

Case-control studies

- *Governa et al., 1987*: Prospective occupational exposure in 4 small shoe factories. 40 workers chosen randomly, aged 16–58 years (mean 31.3). Exposure was to glue or solvent that contained over 50% n-hexane for about 7 h/d, with no protective equipment. Exposure time was 1 to 28 years (mean: 12.4). Air concentrations were not measured but 1 urine sample per study subject was taken at the end of the weekly shift. Reference values were obtained from 41 unexposed individuals; the threshold value of 7.5 mg/L of the n-hexane metabolite 2,5 -hexanedione was derived from the observation that the majority of electroneuromyography (ENM) effects was seen above this value.

- *Öge et al., 1994:* reported on 27 male patients of age 15–52 (mean 22.5 years)

with polyneuropathy that had worked in leather coat or shoe production for 4 months to 20 years (mean 4.4 years). Exposure to glues containing n-hexane (46.6-98.9%) had been 4 months to 2 years (mean 12.2 months). n-Hexane; air concentrations reported in the working environments were between 60 and 810 ppmV (0.21-2.85 mg/L). 24 non-exposed controls without symptoms.

- *Sanagi et al., 1980*: reported on 14 workers employed in a factory producing tungsten carbide alloys. N-hexane exposure (8 h TWA) of 58±41 ppmV (0.204 mg/L), was measured with 22 personal samples taken from the breathing zone over a period of two years. Exposure duration was from 1 to 12 years with an average of 6.2 years and there was co-exposure to acetone (39±30 ppmV). An age-matched control group of 14 non-exposed workers employed in the factory was included in the study which usedquestionnaires and clinical neurological examinations.

Medical surveillance and case studies

- *Yamamura, 1969 and Iida, 1982* (only the summary was available to the DS): A total of 296/1,662 workers from a sandal manufacturer were assessed by questionnaire and medical/neurological examination. Some 44 cases were further examined by electromyography, measurement of peripheral nerve conduction velocities, and other tests. N-Hexane air levels: 500 – 2500 ppmV (1.76-8.8 mg/L) in the patients' work areas in an outbreak in 1968). <50 ppmV during rescreening in 1981, when 21 cases were studied. There was no control group.

- *Chang et al., 1993:* reported a surveillance study of 56 workers of an offset printing factory who worked 12 h/day, 6 days/week. The mean employment duration was 2-6 years (range 1 month to 12 years). Exposure was to cleaning solvents with 14-20% of n-hexane. In addition, the solvents contained a variable percentage of toluene, but no potentially confounding chemicals were detected (e.g., no methyl n-butyl ketone (MBK) or methyl ethyl ketone (MEK)). Moistening solutions contained only trace amounts of phosphate at 42 ppm. The printing inks contained 0.6-8.2 pg/g lead, <0.05-0.95 pg/g mercury and no volatile organic compound. For n-hexane, TWA air concentrations were 30 to 110 ppmV (0.11-0.39 mg/L; mean 63 ppmV, 0.22 mg/L). Concentrations were higher in the personal air samples from the offset machine workers, for n-hexane 80 to 210 ppmV (0.28-0.74 mg/L; mean 132 ppmV, 0.46 mg/L).

- *Huang et al., 1991:* reported that 44 workers from a ball manufacturing factory were classified into three groups according to their degree of solvent exposure;

  I. 5 workers in cement coating or nylon fibre winding,

  II. 8 workers in gas injection,

  III. 31 workers carrying out other jobs.

  Exposure was to solvents and samples from those used contained 14.1% n-hexane, 54% of other saturated hydrocarbons, 3% toluene, 0.8% benzene, 0.1% xylene. No methyl n-butyl ketone, methyl ethyl ketone, carbon disulphide oracrylamide were detected. N-Hexane air concentrations over eight hours were assessed by personal sampling:

I. 2 workers in cement coating 109.1 ± 5 ppmV (0.38 mg/L), and 3 workers in nylon winding 86.4 ± 3.7 ppmV (0.30 mg/L,

II. 1 worker in gas injection 75 ppmV (0.26 mg/L.

- *Huang and Chu, 1989*: reported 5 workers from a press-proofing factory with chronic n-hexane exposure, working 12 h shifts per day. The main ingredients of 3 types of organic solvents used were 65% of n-hexane and 100% of benzene and C15-Cl9 hydrocarbons. Concentrations in air were measured in two one hour long samplings yielding n-hexane at 55 ppmV and benzene at 9.65 ppmV. Toluene, carbon disulfide, acrylamide, methyl n-butyl ketone (MBK) and triorthocresyl phosphate (TOCP) were not detected. Analysis of the colouring agents showed an absence of arsenic and lead. Evoked potentials (EPs) were studied to detect CNS dysfunctions.

- *Pastore et al., 2002*: reported on 26 exposed workers from a shoe factory, of which 10 were exposed to solvents for 11–12 months exposure, up to 11 h daily. Organic vapour gas chromatography analysis at the factory showed 40% of n-hexane, 55% of hexane isomers, 4% of heptane, 1% of cyclohexane, and traces of methylethylketone and toluene. Biological studies of the urine and a neurophysiological evaluation were carried out (NCS and EMG).

Additionally, the DS reviewed Pastore *et al.*, 1994, Scelsi *et al.*, 1980, Issever *et al.*, 2002, and Herskowitz *et al.*, 1971.

As summarised by the DS, multiple studies reporting human data suggest that the incidence of peripheral neuropathy can be attributed to prolonged occupational exposure to *n*-hexane (Table 8: Summary table of human data on STOT RE.) (ATSDR 1999, EPA 2005, WHO/IPCS/EHC 1991). Severity of effects range from reduced motor and sensory nerve conduction velocities to severe quadriparesis[2]. Kutlu *et al.*, (2009) demonstrated that 83.3% of patients with *n*-hexane induced neuropathy recovered completely within 12 months after cessation of exposure.

RAC agrees with the DS that the following observations from Wang *et al.*, (1986) indicate that *n*-hexane is driving the observed neurological effects:

i. increased frequency of polyneuropathy as well as abnormal motor nerve conduction velocity (MCV) with higher *n*-hexane concentration in the cleaning solvents;
ii. association between the frequency of polyneuropathy and *n*-hexane air concentrations > 100 ppmV (> 0.352 mg/L);
iii. consistent improvement of neurological symptoms of workers who went back to work and no new cases of polyneuropathy after *n*-hexane was removed and ventilation improved;
iv. other organic chemicals known to cause polyneuropathy (Juntunen and Haltia, 1982) were not present in significant amounts.

As described by the DS, in Huang *et al.* (1991), higher exposure levels of *n*-hexane coincided with higher incidences of polyneuropathy in workers and no polyneuropathy

[2] Weakness in all four limbs.

cases occurred before the factory started using *n*-hexane and after a new ventilation system was installed, which reduced the *n*-hexane air concentrations below 15 ppmV (0.05 mg/L). Governa *et al.* (1987) found a significant correlation between the urinary level of the *n*-hexane metabolite 2,5-hexanedione and electro-neuromyographic changes as marker for polyneuropathy of forty shoe factory workers. However, urinary 2,5-hexanedione levels alone are not sufficient to prove sole exposure to *n*-hexane, because it is also a metabolite of the solvent methyl *n*-butyl ketone (Granvil *et al.*, 1994). The latter solvent has not been used in the press proofing workshops studied in Wang *et al.* (1986). Such potential confounding factors were also excluded in the study by Chang *et al.* (1993). In this study, from 56 workers of an offset printing factory, 20 workers (36%) developed symptomatic peripheral neuropathy and 26 (46%) developed subclinical neuropathy (total: 82%) at *n*-hexane concentrations in the personal air samples from the offset machine workers from 80 to 210 ppmV (0.28-0.74 mg/L; mean 132 ppmV, 0.46 mg/L) and 20 to 680 ppmV (mean 235 ppmV) for isopropyl alcohol, and 20 to 84 ppmV (mean 50 ppmV) for toluene; no potentially confounding chemicals detected (no methyl *n*-butyl ketone or methyl ethyl ketone; Table 8: Summary table of human data on STOT RE.). Also more recent case studies support the polyneuropathy-inducing effects of the *n*-hexane metabolite 2,5-hexanedione (Baslo *et al.*, 2021; Sun *et al.*, 2020).

In epidemiological studies, the exposure groups may be exposed to mixtures of solvents and hence confounding factors cannot be excluded in all cases. Regarding n-hexane, as described by the DS, in particular co-exposure to ketones (e.g., methyl *n*-butyl ketone) may aggravate the neurotoxic effect (Ladefoged *et al.*, 1994; Noraberg and Arlien-Soborg, 2000). Methyl *n*-butyl ketone (CAS# 591-78-6; classified as STOT RE 1, H372) is metabolized to the same neurotoxic metabolite as *n*-hexane (2,5-hexanedione) and causes the same neurotoxic effects (Governa *et al.*, 1987; Granvil *et al.*, 1994; LoPachin and Gavin, 2015). Thus, 2,5-hexanedione is most likely responsible for the neurotoxic effects of *n*-hexane (LoPachin and Gavin, 2015). Co-exposure to potentially confounding factors has been documented in the following studies: Sanagi *et al.* (1980), Mutti *et al.* (1982), Neghab *et al.* (2012). Co-exposure to potentially confounding factors is unlikely in the following studies (e.g., no detection or only detection of trace amounts of acetone or methyl n-butyl ketone): Wang *et al.* (1986), Huang and Chu (1989), Huang *et al.* (1991), Chang *et al.* (1993), Pastore *et al.* (2002).

RAC agrees with the DS that it is possible that humans are more susceptible to n-hexane neurotoxicity than rodents. As described by the DS, n-hexane exerts its neurotoxic effects via the metabolite 2,5-hexanedione, which is considered an important *n*-hexane metabolite in humans (Governa *et al.*, 1987; Perbellini *et al.*, 1981). This metabolite is present in human urine in concentrations 20–30-times higher than 2-hexanol, another *n*-hexane metabolite (MAK 1982). In contrast, animal studies identified 2-hexanol as the main metabolite (Perbellini *et al.* 1981, MAK 1982) with urine concentrations 3-times higher than concetrations of 2,5-hexanedione (MAK 1982). Additionally, some authors claim that humans (with longer nerves) are more susceptible than rodents (with shorter nerves) to *n*-hexane induced neurofilament-filled axonal swellings after exposure because there may be a greater probability for cross-linking in the longer axons of humans in comparison to the shorter nerves in rodents (Dunnick, 1991; Friede *et al.*, 1984; Graham and Gottfried, 1984). The higher susceptibility of longer axons is also supported by a recent review, stating that initial changes occur in the largest and longest axons in peripheral nerves and the spinal cord, with similar changes in shorter nerve fibres at a

later stage (Spencer and Chen, 2021).

Comparison with the classification criteria

Similar neurotoxic effects have been observed in both animals and humans after prolonged exposure to n-hexane. Histopathological observations also support the neurotoxicity of n-hexane. As described by the DS, effects in animals occur after exposure to higher concentrations of *n*-hexane (≥ 1.76 mg/L, ≥ 500 ppmV; Table 7: Summary table of animal studies on STOT RE.) compared to humans (≤ 0.67 mg/L, ≤ 190 ppmV; Table 8: Summary table of human data on STOT RE.), although lower concentrations were not tested in animals.

According to Annex 1, section 3.9.2.1., the classification criteria for Category 1 are: "*Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations.*" In addition, according to Annex I, section 3.9.2.10.2: "*When well-substantiated human data are available showing a specific target organ toxic effect that can be reliably attributed to repeated or prolonged exposure to a substance, the substance shall normally be classified. Positive human data, regardless of probable dose, predominates over animal data.*"

The lowest LOAEL observed in the animal studies, of 1.76 mg/L/6h/day after 90-day (or longer) inhalation exposure, is almost one order of magnitude higher than the guidance value for classification in Cat. 1, C ≤ 0.2 mg/L/6h/day for a 90-day study. Therefore, the animal data do not fulfil the classification criteria. However, the available human data is considered sufficiently convincing for classification of n-hexane in STOT RE 1: reliable human data of good quality are available, and they demonstrate significant and severe toxic effects in humans.

A specific concentration limit (SCL) may only be set for substances inducing specific target organ toxicity in animal studies at a dose level or concentration clearly (more than one order of magnitude) below the guidance value for cat 1 classification (CLP Annex I, Table 3.9.2). The human data does not provide such reliable exposure information that could be used to support a lower SCL for n-hexane. Thus, establishment of a specific concentration limit is not indicated for *n*-hexane and the generic concentration limits should be applied.

In conclusion, RAC agrees with the DS that for **specific organ toxicity, repeated exposure,** classification as **STOT RE 1, H372 (nervous system)** is warranted for n-hexane.

10.13 Aspiration hazard

The classification for respiratory sensitisation was not part of the assessment.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

The classification for environmental hazards was not part of the assessment.

12 EVALUATION OF ADDITIONAL HAZARDS

The classification for additional hazards was not part of the assessment.

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14 ANNEXES

A confidential Annex I is available.