Committee for Risk Assessment

RAC

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

Adopted

1 December 2022

P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

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1 December 2022

CLH-O-0000007203-83-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: ***n*-hexane**

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

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

The proposal was submitted by **Germany** and received by RAC on **1 December 2021.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **17 January 2022**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **18 March 2022**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Tiina Santonen**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **1 December 2022** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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 Repr. 2 Asp. Tox. 1 STOT SE 3 STOT RE 2 * Skin Irrit. 2 Aquatic Chronic 2	H225 H361f*** H304 H336 H373** H315 H411	GHS02 GHS08 GHS07 GHS09 Dgr	H225 H361f*** H304 H336 H373** H315 H411		STOT RE 2; H373: C ≥ 5%	
Dossier submitters proposal	601-037-00-0	*n*-hexane	203-777-6	110-54-3	**Modify** STOT RE 1	**Modify** H372 (nervous system)		**Modify** H372 (nervous system)		**Delete** STOT RE 2; H373: C ≥ 5%	
RAC opinion	601-037-00-0	*n*-hexane	203-777-6	110-54-3	**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 COM	601-037-00-0	*n*-hexane	203-777-6	110-54-3	Flam. Liq. 2 Repr. 2 Asp. Tox. 1 STOT SE 3 STOT RE 1 Skin Irrit. 2 Aquatic Chronic 2	H225 H361f*** H304 H336 H372 (nervous system) H315 H411	GHS02 GHS08 GHS07 GHS09 Dgr	H225 H361f*** H304 H336 H372 (nervous system) H315 H411			

HUMAN HEALTH HAZARD EVALUATION

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 (**Error! Reference source not found.**) (ATSDR 1999, EPA 2005, WHO/IPCS/EHC 1991). Severity of effects range from reduced motor and sensory nerve conduction velocities to severe quadriparesis[1]. 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 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

[1] Weakness in all four limbs.

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; **Error! Reference source not found.**). 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; **Error! Reference source not found.**) compared to humans (≤ 0.67 mg/L, ≤ 190 ppmV; **Error! Reference source not found.**), 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.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).