The Director General

Maisons-Alfort, July 4, 2014

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

regarding the establishment of TRVs by respiratory route for n-Hexane (CAS No.110-54-3)

ANSES undertakes independent and pluralistic scientific expertise.
ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are made public. This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated July 4, 2014 shall prevail.

On 24 April 2013, ANSES issued an internal request to establish a toxicity reference value (TRV) by inhalation for n-Hexane (CAS No. 71-43-2).

1. BACKGROUND AND PURPOSE OF THE REQUEST

Since 2004, ANSES has been working to establish toxicological reference values (TRVs) and develop related methodologies.

In the context of the request received from the Directorate General for Health (DGS, 2009) on "endocrine disruptors and category 3 reprotoxic substances", a health risk assessment (HRA) was published in April 2014 for five chemical substances including n-Hexane (ANSES, 2014). In its recommendations, ANSES recommended establishing a TRV by respiratory route for n-Hexane further to the aforementioned expert appraisal.
According to the methodology used in this expert appraisal, an overall approach assessing dose-response relationships was adopted by relating the critical dose (NOAEL or LOAEL[1]) to a margin of safety taking into account inter-individual variability and, if applicable, inter-species variability and the use of a LOAEL. In the case of n-Hexane, the expert appraisal undertaken based on all of the available data led to the following three effects being selected:

- effects on fertility (reduced testis weight, atrophy of seminiferous tubules)
- effects on development (increase in the number of early and late foetal resorptions)
- neurotoxic effects (changes in peripheral nervous system conduction); effects on the peripheral nervous system were considered the most sensitive effects.

[1] LOAEL = Lowest Observed Adverse Effect Level; NOAEL = No Observed Adverse Effect Level

A TRV is a generic term encompassing all of the types of toxicological indicators that are used to establish a relationship between a dose and an effect (toxic with a threshold effect) or between a dose and a likelihood of effect (toxic without a threshold effect). TRVs are specific to an exposure time (acute, subchronic or chronic), an exposure route (oral or respiratory) and a type of effect (reprotoxic, carcinogenic, etc.). The establishment of TRVs differs based on knowledge or assumptions of substances' mechanisms of action.

“Threshold” TRVs are used for substances that, above a certain dose, cause damage whose severity is proportional to the absorbed dose, while “non-threshold” TRVs are used for substances for which there is likelihood, even very small, that a single molecule penetrating the body will cause harmful effects to that body.

In practice, the establishment of a TRV includes the following stages:

- analysis of the available data,
- choice of the critical effect,
- identification of the establishment assumption, with or without a dose threshold, based on the substance's mode of action,
- choice of a study of good scientific quality enabling the establishment of a dose-response relationship,
- choice or establishment of a critical dose based on experimental doses and/or epidemiological data; for any critical dose obtained in animals, adjustment of this dose to humans,
- application of uncertainty factors to the critical dose to take into account uncertainties for threshold TRVs or linear extrapolation from the origin using the critical dose for non-threshold TRVs.

The establishment of TRVs adheres to a highly structured and stringent approach that involves collective expert appraisals relying on expert judgement (AFSSET, 2010).

2. ORGANISATION OF THE EXPERT APPRAISAL

This expert appraisal was carried out in accordance with the French standard NF X 50-110 “Quality in Expertise – General Requirements of Competence for Expert Appraisals (May 2003)”.

It falls within the sphere of competence of the Expert Committee (CES) on “Assessment of the risks related to chemical substances”. ANSES entrusted the expert appraisal to the working group on “Toxicological Reference Values II” (TRV WG). The methodological and scientific aspects of the work were presented to the CES on 19 December 2013. They were adopted by the CES on “Assessment of the risks related to chemical substances” in its meeting of 19 December 2013.

ANSES analyses interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals.
The experts’ declarations of interests are made public on ANSES's website (www.anses.fr).

3. ANALYSIS AND CONCLUSIONS OF THE CES

Both animal and human studies were taken into account to characterise the toxicological profile of n-Hexane.

n-Hexane is known for its neurotoxic effects in humans. Acute respiratory exposure to high concentrations of n-Hexane (generally greater than 1,000 ppm) in humans causes damage to the central nervous system (CNS) by first inducing a euphoric state with sensations of inebriation followed by drowsiness with headaches, dizziness and nausea. Irritation of the eyes and respiratory mucosa has also been observed.

Many subchronic and chronic respiratory toxicity studies report primarily neurological effects in professional sectors where n-Hexane exposure levels are very high. Neurological impairment manifested as polyneuritis, which starts with sensory (paraesthesia) and then motor (leg and arm weakness) difficulties. At a more advanced stage, impairment of motor skills primarily affecting the lower limbs has been observed with flaccid paralysis and varying degrees of muscular atrophy. In the most severe cases, impairment of the central nervous system expressed as dysarthria, unsteady gait and vision problems (macular oedema) has been observed. This damage results from degeneration of the peripheral and central systems mainly affecting the distal portion of longer, large-diameter axons with decreased nerve conduction velocity. Similar effects have also been observed in animals (US EPA, 2005; INRS, 2008; Environment Canada, 2009).

Histopathological examination of nerves in animals suggests that the substance acts by a sequence of events involving axonal inflammation leading to alteration of the neurofilaments followed by secondary myelin retraction (ATSDR, 1999). 2,5-hexanedione is believed to be responsible for neurotoxicity by binding to amino groups of lysine in proteins (INRS, 2008).

Regarding the effects of n-Hexane on reproductive function, no studies in humans on the toxicity of n-Hexane to reproduction and fertility have been referenced in the literature. n-Hexane was classified by the European Commission as toxic to reproduction category 3 (current category 2 according to the CLP[2] Regulation) due to a potential risk of impaired fertility. The decision relied on the observation of effects in male rats after exposure by inhalation or ingestion to n-Hexane or its metabolite, 2,5-hexanedione, in reprotoxicity studies. The effects were expressed as histological changes in the testes and epididymis and as changes in sperm characteristics (Environment Canada, 2009). Neurotoxic effects due to exposure to n-Hexane have been observed at lower concentrations than those inducing impaired fertility.

n-Hexane is not classified as a potential endocrine disruptor, according to the European data of BKH and DHI (DHI, 2007; BKH, 2002). No information on the mechanism of action involved in reproductive effects was found in the literature. No specific studies have been undertaken to assess the hormonal properties of n-Hexane. Therefore, no causal relationship between observed effects on fertility and development and endocrine disruption can be established in the current state of knowledge.

Regarding the genotoxicity and carcinogenicity of n-Hexane, several short-term genotoxicity tests have been undertaken *in cellulo* and most of them have been negative (US EPA, 2005). In light of the results of several *in vivo* genotoxicity tests, n-Hexane is not considered genotoxic *in vivo*. In the current state of knowledge, it is not possible to assess the carcinogenic nature of n-Hexane. No studies in favour of carcinogenicity related to the use of n-Hexane alone have been identified (US EPA, 2005).

The available data do not describe any susceptible populations for reproductive effects. The US EPA (2005) suggests that juvenile rats may be less susceptible than adults to neurological

[2] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures.

effects (related to the decreased size of axons, greater growth and repair of peripheral nerves and a lower level of CYP 2E1, suggesting decreased production of 2,5-hexanedione).

Establishment of a chronic TRV by inhalation

Analysis of current TRVs

Three chronic TRVs by inhalation are available (ATSDR, 1999; OEHHA, 2000; US EPA, 2005). However, the experts considered that these TRVs had a number of limitations and therefore decided to establish a new TRV.

Choice of the critical effect

The experts chose the effects on the peripheral nervous system shown in both epidemiological and experimental studies as the critical effect. Indeed, peripheral neurotoxicity is recognised as being the most sensitive effect associated with exposure by inhalation to n-Hexane in humans and animals. The lowest LOAEC related to exposure by inhalation is 700 $mg.m^{-3}$ (200 ppm), based on changes in peripheral nerve conduction in male rats, in the context of a 24-week study published by Ono *et al.* (Ono *et al*., 1982).

Choice of the key study

The experts chose the study by Huang *et al.* (1989) as the key study, supported by the experimental study by Ono *et al*. (1982) and the epidemiological studies by Sanagi *et al*. (1980) and Chang *et al.* (1993).

Choice of the critical dose

The US EPA (2005) derived a BMC and BMCL[3] from the study by Huang *et al.* (1989). The US EPA experts did not have access to the study's individual data. They estimated the values (means and standard deviations for reduced motor nerve conduction velocity) graphically, based on Figure 2 in the article by Huang *et al.* (1989). Since the experts did not have the individual results from this study, they chose to use the BMCL of the US EPA of 122 ppm (430 $mg.m^3$) to derive the TRV.

Dosimetric adjustment

A human equivalent concentration (HEC) was calculated based on the recommendations of the US EPA (1994) which developed various dosimetric adjustments for the respiratory route based on the physicochemical properties of the inhaled substance (particle or gas, highly soluble or poorly soluble in water) and the site where critical effects are observed (respiratory or extra-respiratory).

n-Hexane is considered a category 3 gas (US EPA, 1994) because:
- it primarily has extra-respiratory effects (systemic toxicity)
- it has little action on the respiratory tract
- it is rapidly transferred from the lungs to blood circulation.

According to the recommendations of the US EPA (1994), the dosimetric adjustment applied by default for a category 3 gas is as follows:

$$BMCL_{HEC} = BMCL \times (Hb/g)_{rats} / (Hb/g)_{humans}$$

[3] BMC: Benchmark concentration, BMCL: 95% lower bound on the BMC

where (Hb/g) is the blood/air partition coefficient for n-Hexane

Since the $(Hb/g)_{rats}$ / $(Hb/g)_{humans}$ ratio is greater than 1 (2.86), the default value of 1 which is more conservative has been applied.

$$BMCL_{HEC} = BMCL \times 1 = 430\ mg.m^{-3}$$

Time adjustment

The animals were exposed for 12 hours per day for 24 weeks. To take into account the discontinuity in exposure, time adjustment was applied:

$$BMCL_{HEC\ ADJ} = 430 \times 12/24 = 215\ mg.m^{-3}$$

Choice of uncertainty factors

- Inter-species variability (UF_A)

Dosimetric adjustment was applied, making it possible to calculate a human equivalent concentration using the equation given above. To take into account toxicodynamic variability and residual uncertainties, an additional uncertainty factor of 2.5 was established.

$UF_A = 2.5$

- Intra-species variability (UF_H)

UF_H is applied to take into account intra-species variability.
One study suggests that weaned rats may be less susceptible to the neurotoxic effects of n-Hexane than adult rats (US EPA, 2005). Moreover, CYP 2E1 is responsible for metabolism of various substances including n-Hexane and acetone. Polymorphism of CYP 2E1 could lead to inter-individual differences in toxicity. The neurotoxic effects of n-Hexane are believed to be the result of its metabolism to the toxic metabolite 2,5-hexanedione by CYP 2E1. In addition, differences in the development and maturity of phase I and phase II enzymes (specifically CYP 2E1) between adults and children have been shown in several studies. These data suggest that there may be differences in metabolism of n-Hexane within the human population and between adults and children.
Therefore, an uncertainty factor of 10 was applied.

$UF_H = 10$

- Exposure time in the source study (UF_S)

In the study by Huang *et al.* (1989), the animals were exposed for 16 weeks, which corresponds to subchronic exposure. n-Hexane is not considered a cumulative toxin, but it nonetheless cannot be ruled out that chronic exposure may cause effects at lower concentrations than with subchronic exposure. Therefore, a UF_S of 3 was applied to take into account this uncertainty.

$UF_s = 3$

Thus, an **overall uncertainty factor of 75** was applied to derive the TRV.

TRV calculation

$TRV = 215 / 75 = 3\ mg.m^{-3}$

Critical effect and source study	Establishment method	UF	TRV
Neurotoxicity (decreased nerve conduction velocity in motor nerves) Huang *et al*., 1989: study in rats	BMCL = 122 ppm Time adjustment $BMCL_{ADJ}$ = 61.4 ppm (215 $mg.m^{-3}$) Allometric adjustment BMC_{HEC} = 215 $mg.m^{-3}$	75 UF_A = 2.5 UF_H = 10 UF_S = 3	**TRV = 3 $mg.m^{-3}$** **Confidence level medium/high**

Confidence level:

A **medium/high** confidence level was assigned to this TRV:

- **Critical effect: medium**

  The toxicity of n-Hexane to the peripheral nervous system is well established. However, it cannot be ruled out that reprotoxic effects related to *in utero* exposure or effects on the central nervous system (CNS) may occur at lower concentrations than those causing effects on the peripheral nervous system. Such effects have not been found to date but no studies have properly assessed the effects of n-Hexane on the CNS or on neurodevelopment.

- **Key study: high**

  The experimental study by Huang *et al.* (1989) is supported by the study by Ono *et al*. (1982) and by the epidemiological studies by Sanagi *et al*. (1980) and Chang *et al.* (1993).

- **Critical dose: high**

  A BMCL was derived from the study by graphic estimation. Allometric adjustment made it possible to calculate a human equivalent dose and an overall residual uncertainty factor of 75 was applied to this value (UF_A = 2.5; UF_H= 10; UF_S = 3).

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) endorses the conclusions and recommendations of the Expert Committee (CES) on "Assessment of the risks related to chemical substances" which cover the establishment of toxicological reference values by inhalation for n-Hexane.

Critical effect and source study	Establishment method	UF	TRV
Neurotoxicity (decreased nerve conduction velocity in motor nerves) Huang *et al*., 1989: study in rats	BMCL = 122 ppm Time adjustment $BMCL_{ADJ}$ = 61.4 ppm (215 $mg.m^{-3}$) Allometric adjustment BMC_{HEC} = 215 $mg.m^{-3}$	75 UF_A = 2.5 UF_H = 10 UF_S = 3	**TRV = 3 $mg.m^{-3}$** **Confidence level medium/high**

Furthermore, the Agency points out that no studies published to date have adequately assessed the effects of n-Hexane on the central nervous system or on neurodevelopment. These could occur at lower concentrations than those having effects on the peripheral nervous system.

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KEYWORDS

n-Hexane, toxicity reference value, inhalation

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