Opinion
Request No 2016-SA-0162

The Director General

Maisons-Alfort, 22 December 2017

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

on the development of a chronic TRV by the respiratory route for carbon tetrachloride (CAS No. 56-23-5)

ANSES undertakes independent and pluralistic scientific expert assessments.
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 the necessary information concerning these risks as well as the requisite expertise 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 published on its website. This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 22 December 2017 shall prevail.

On 22 July 2016, ANSES received a formal request from the Directorate General of Health to determine health values for carbon tetrachloride in water intended for human consumption and in indoor air (Bas-Rhin *département*).

1. BACKGROUND AND PURPOSE OF THE REQUEST

The Regional Health Agency (ARS) for Alsace-Champagne-Ardennes-Lorraine was consulted regarding the presence of carbon tetrachloride (CCl_4) in water intended for human consumption (WIHC) and in the indoor air of homes in the *département* of Bas-Rhin, following a spill from a tanker truck in the municipality of Benfeld in 1970.
Regarding the population's exposure to CCl_4 by inhalation, air samples were taken in 2005 from inside homes located in the municipality of Benfeld in the vicinity of the spill area. The maximum concentrations measured at the time were of the order of 1.6 µg/m^3. The local authority is considering having new measurements taken in indoor air.
In this context, due to the existence of several toxicity reference values (TRVs) for inhalation in the scientific literature, and in order to be able to propose appropriate management measures if necessary, ANSES was asked to determine a chronic TRV for inhalation.

A toxicity reference value, or TRV, is a toxicological indicator for qualifying or quantifying a risk to human health. It establishes the link between exposure to a toxic substance and occurrence of an

adverse health effect. TRVs are specific to a duration (acute, subchronic or chronic) and route (oral or respiratory) of exposure. The way TRVs are established differs depending on the knowledge or assumptions made about the substances' mechanisms of action. Currently, the default assumption is to consider that the relationship between exposure (dose) and effect (response) is monotonic. In the current state of knowledge and by default, it is generally considered that for non-carcinogenic effects, toxicity is only expressed above a threshold dose (ANSES, 2015a).

In practice, establishing a threshold TRV involves the following four steps:

- choice of the critical effect;
- choice of a good quality scientific study generally enabling establishment of a dose-response relationship;
- choice or establishment of a critical dose from experimental doses and/or epidemiological data;
- application of uncertainty factors to the critical dose to account for uncertainties.

TRVs are established according to a highly structured and rigorous approach involving collective assessments by groups of specialists.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)".

The expert appraisal falls within the sphere of competence of the Expert Committee (CES) on "Characterisation of substance hazards and toxicity reference values" (hereinafter referred to as the CES "Substances"). The methodological and scientific aspects of the work were presented to the CES between December 2016 and June 2017. It was adopted by the CES "Substances" at its meeting on 22 June 2017.

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 *via* the ANSES website (https://dpi.sante.gouv.fr).

3. ANALYSIS AND CONCLUSIONS OF THE CES

3.1. Summary of the health effects

3.1.1. Acute effects

In humans, the main effects observed are depression of the central nervous system, hepatic disorders progressing to liver failure, and renal lesions that may progress to reversible tubulopathy. These effects are observed independently of the route of exposure. However, inhalation was the main route of exposure implicated in poisonings or accidents reported in the literature. In cases of accidental or deliberate oral poisoning, gastric irritation has also been reported. By the dermal route, CCl_4 causes the formation of transient erythema.
In animals, the effects observed following inhalation mainly concern the liver (increased enzymatic activity, steatosis).

3.1.1. Chronic non-carcinogenic effects

In humans, studies conducted in the workplace have reported hepatic events (increased transaminases) and neurological disorders (headache, dizziness, nausea). Some studies report liver and kidney toxicity potentiated by alcohol consumption (INERIS, 2005).

In animals (rats, mice and dogs), liver damage was observed with the oral and inhalation routes: increased liver weight, increased liver biochemical parameters, cirrhosis, fibrosis and necrosis. Centrolobular vacuolation was also noted, consistent with the localisation of cytochromes P (CYP) 2E1, the main enzymes of CCl_4 metabolism. Hepatotoxicity occurs in rats and mice at concentrations of 10 ppm (64 $mg \cdot m^{-3}$) and the severity of the effects increases with the dose.

In 2007, Nagano *et al.* (2007b) published a 13-week (90-day) toxicity study in both sexes of F344 rats and BDF1 mice. Animals were exposed for 6 hours/day, 5 days/week in five dose groups: 0, 10, 30, 90, 270, 810 ppm. The authors investigated liver and kidney lesions, and changes in haematological parameters. Organ weight (absolute and relative) was also monitored.

The authors did not detect any lesions of the upper airways (nasal cavity, larynx, trachea, lungs). Histological results showed the inclusion of large fatty droplets in liver cells (male and female rats and male mice) and cytoplasmic globules (mice), as well as the release of liver cytolysis enzymes from 10 ppm. Fibrosis and cirrhosis were observed only in rats at 270 ppm. A phenomenon of hepatic collapse with necrosis was observed in mice at 30 ppm and above. Biochemical monitoring showed signs of nephrotoxicity in rats and haematotoxicity (anaemia) in both animal species tested, starting at 90 ppm.

3.1.1. Carcinogenic effects

In humans, no studies by the oral route are available. Many inhalation studies have been conducted in the workplace to assess a possible relationship between an increased incidence of cancer or mortality and CCl_4 exposure.

The International Agency for Research on Cancer (IARC) has classified CCl_4 in Group 2B (possibly carcinogenic to humans) (IARC, 1999) based on insufficient evidence in humans and sufficient evidence in animals.

In animals, via both the oral and inhalation routes, experimental studies (rats, hamsters and mice) have provided convincing evidence of the relationship between oral exposure to CCl_4 and the risk of liver cancer (INERIS, 2007).
Oral gavage carcinogenicity studies by the NTP[1] show:

- in mice (B6C3F1, male and female), hepatocarcinomas and pheochromocytomas,
- in rats (Osborne Mendel, male and female), no cancer was observed at the tested doses but there were signs of liver toxicity: hyperplasia, cirrhosis, fat inclusions and regenerative nodules.

Nagano *et al.* (2007a) conducted an inhalation carcinogenesis study in 50 female and 50 male F344 rats and 50 female and 50 male BDF1 mice exposed for 6 hours/day, 5 days/week for 104 weeks. In mice, a significant decrease in survival was noted at doses of 25 and 125 ppm. Changes in some blood haematological and biochemical parameters, including liver enzymes, and in urine analysis were observed at the two highest doses. In male mice, liver changes, cysts and liver degeneration were noted at 25 and 125 ppm. In the spleen, an increase in haemosiderin deposition at 25 ppm and extramedullary haematopoiesis at 125 ppm was observed. In female mice, liver changes included ceroid deposition, thrombus, necrosis, degeneration and cysts at doses of 25 and 125 ppm.

The incidence of hepatocellular adenomas was significantly increased at doses of 25 and 125 ppm in male mice, and at 5 and 25 ppm in female mice. The incidence of hepatocellular carcinomas was significantly increased at doses of 25 and 125 ppm in male and female mice. The incidence of

[1] National Toxicology Program

adrenal pheochromocytomas was higher in male mice at both 25 and 125 ppm and in female mice at 125 ppm.

3.1.2.Genotoxicity

More than 80 studies on CCl_4 genotoxicity have been published. All the results tables compiled by the US EPA (2010) were used to carry out a global study to weight the studies according to the relevance of the results, using the framework proposed by ECHA (2015) (assessment of the weight of evidence).
A critical review of all the data led to the conclusion that CCl_4 probably does not act according to a direct genotoxic mode of action and that oxidative DNA damage is more indirect/secondary to lipoperoxidation phenomena.

The mode of action of hepatic carcinogenesis has not yet been well established. The hypothesis of CCl_4 metabolism by CYP 2E1 to a trichloromethyl radical and other reactive oxygen species (ROS) whose products of lipid peroxidation lead to sustained cytotoxicity and proliferation by cell regeneration is proposed as a mode of action.

The CES "Substances" considers CCl_4 to be a threshold carcinogen (indirect genotoxicity).

3.2. Development of a chronic TRV by inhalation

3.2.1. Choice of the critical effect

Among the effects of CCl_4, the CES considers that the hepatic carcinogenic effect is the most sensitive health effect. Among the chronic and carcinogenic effects reported**,** increased liver adenomas and carcinomas in rats and mice are described as being the most sensitive effects, occurring at the lowest levels of exposure. These were therefore selected as the critical effect.

3.2.2. Analysis of the existing TRVs

Among the existing chronic TRVs (tables below), the CES "Substances" considered that none of these values could be selected because of the choice of critical effect (considered irrelevant, non-threshold effects) and/or the choice of a key study deemed to be of insufficient quality. The CES experts therefore proposed establishing a chronic TRV by inhalation.

Table 1: Threshold TRVs available for chronic inhalation exposure

	Chronic threshold TRV			
Organisation	**RIVM**	**ATSDR**	**OEHHA**	**US EPA**
Year	2001	2005	2000	2010
TRV	TRV	MRL*	REL*	RfC*
Value of the TRV	60 µg·m^{-3}	180 µg·m^{-3}	40 µg·m^{-3}	100 µg·m^{-3}
Critical effect	Hepatic effects	Increased liver weight, histological and biochemical changes	Increased liver weight, histological changes	Fatty changes in the liver
Species	Rat	Rat	Guinea pig	Rat
Route of exposure	Inhalation	Inhalation	Inhalation	Inhalation
Critical dose	NOAEC = 5 ppm (30 mg·m^{-3})	NOAEC = 5 ppm (30 mg·m^{-3})	NOAEC = 5 ppm (30 mg·m^{-3})	$BMC_{10\%}L_{95\%\ HEC}$ = 14.3 mg·m^{-3} based on the use of a PBPK model
Adjustments	$NOAEL_{ADJ}$ = 6.4 mg·m^{-3}	$NOAEL_{ADJ}$ = 5.4 mg·m^{-3}	$NOAEC_{ADJ}$ = 5.4 mg·m^{-3} $NOAEC_{HEC}$ = 10.2 mg·m^{-3}	
UF	100 UF_A = 10 UF_H = 10	30 UF_A = 3 UF_H = 10	300 UF_A = 10 UF_H = 10 UF_L = 3 UF_S = 3	100 $UF_{A\text{-}TD}$ = 3 UF_H = 10 UF_D = 3
Reference	Undisclosed source, Adams *et al.,* 1952	Adams *et al*., 1952	Adams *et al*., 1952	Nagano *et al.,* 2007a

* MRL: Minimum Risk Level; REL: Reference Exposure Level; RfC: Reference Concentration

Table 2: Non-threshold TRVs available for chronic inhalation exposure

Organisation	**OEHHA**	**US EPA**
Year	2000	2010
TRV	ERUi*	ERUi
Value of the TRV	$4.2 \cdot 10^{-5}$ (µg·m^{-3})$^{-1}$	$6 \cdot 10^{-6}$ (µg·m^{-3})$^{-1}$
Critical effect	Increased incidence of liver tumours (hepatocellular carcinomas and hepatomas)	Increased incidence of liver tumours (hepatocellular carcinomas and hepatomas)
Species	Hamster, mouse	Mouse
Route of exposure	Inhalation	Inhalation
Establishment	Route-to-route extrapolation	Linear extrapolation to the origin
Reference	Della Porta *et al*., 1961; Edwards *et al.*, 1942	Nagano *et al.,* 2007a

* ERUi: Excess Risk per Unit by Inhalation

3.2.3. Choice of the key study

The CES experts selected the study by Nagano *et al.* (2007a) as the key study. Nagano *et al.* (2007a) conducted an inhalation carcinogenesis study in 50 female and 50 male F344 rats and BDF1 mice, exposed for 6 hours/day, 5 days/week for 104 weeks. The authors showed:

- in mice (BDF1, male and female), liver adenomas and carcinomas and pheochromocytomas, from 5 ppm
- in rats (F344, male and female), liver adenomas and carcinomas, from 125 ppm.

3.2.4. Choice of the critical concentration

The relationship between the increase in liver adenomas and carcinomas in mice and the daily exposure concentration to CCL_4 was modelled using the US EPA's BMDS 2.1.1.1 software: development of a Benchmark Dose (BMD). The calculated critical concentrations were:

$BMD_{10\%}$ = 4.2 ppm and $BMD_{10\%}L_{95\%}$ = 2.6 ppm

3.2.5. Temporal and allometric adjustments

The animals were exposed for 6 hours per day, 5 days per week. To take account of the discontinuity of the exposure, a time adjustment was made:

$$BMDL_{ADJ} = 2.6 \text{ ppm} \times 6/24 \times 5/7 = 0.46 \text{ ppm} = 2.91 \text{ mg}\cdot\text{m}^{-3}$$

According to the recommendations of the US EPA (1994), CCl_4 is considered to be a gas with systemic action. The dose adjustment applied by default was as follows:

$$\textbf{critical concentration}_{Human} = \textbf{critical concentration}_{Animal} \times (Hb/g)_{Animal}/(Hb/g)_{Human}$$

Where (Hb/g): blood/air partition coefficient
As the CCl_4 blood/air partition coefficients for humans and rats are not known, the US EPA proposes using the default value of 1 (US EPA, 1994).

3.2.6. Choice of uncertainty factors

The TRV was calculated from CCl_4 using the following uncertainty factors (ANSES, 2015):

- Inter-species variability (UF_A): 2.5

The dose adjustment performed enabled a human equivalent concentration to be calculated, using the previous equation. To account for toxicodynamic variability and residual uncertainties, an additional uncertainty factor was set at 2.5 according to WHO/IPCS recommendations (WHO/IPCS, 2005) and based on ANSES practices.

- Subchronic to chronic transposition (UF_S): 1, because a 104-week study was used
- Inter-individual variability (UF_H): 10
- Use of a BMDL, LOAEL/C or NOAEL/C ($UF_{B/L}$): 1, because the dose-response relationship made it possible to determine a benchmark dose.
- Inadequacy of the data (UF_D): 1, because there were many studies on CCl_4.

An overall uncertainty factor of 25 was thus used for establishing the TRV.

3.2.7. Confidence level

The overall confidence level was assigned to this TRV based on the following criteria:

- Level of confidence in the type and quality of the data: **High.**

The literature review revealed that there were many studies on CCl_4.

- Level of confidence in the choice of the critical effect and the mode of action: **Moderate**

The effect is well described by the authors, and found in other publications. However, this effect has not been confirmed in humans.

- Level of confidence in the choice of the key study: **High**.

This is a well detailed study that follows the guidelines.

- Level of confidence in the choice of the critical dose: **High**.

It was possible to establish a BMD.

Thus, the overall level of confidence for this TRV is moderate-high.

3.2.8. Proposed chronic TRV by inhalation

A chronic TRV by inhalation was proposed for carbon tetrachloride based on hepatocellular adenomas and carcinomas in mice. A moderate-high confidence level was assigned to this TRV.

Critical effect (key study)	Critical concentration	UF	Chronic TRV by inhalation
Hepatocellular adenomas and carcinomas in mice Nagano *et al.* (2007a)	$BMD_{10\%}L_{95\%}$ = 2.6 ppm Temporal adjustment $BMDL_{ADJ}$ = 2.6 ppm x 6/24 x 5/7 = 0.46 ppm = 2.91 $mg \cdot m^{-3}$	25 UF_A = 2.5 UF_H = 10	**TRV = 0.11 $mg \cdot m^{-3}$** (or 0.0184 ppm) **Confidence level Moderate-high**

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions and recommendations of the CES "Substances" on the formulation of a chronic TRV by inhalation for carbon tetrachloride.

In 2009, ANSES (AFSSET, 2009) had developed a chronic threshold TRV for the respiratory route for the carcinogenic effects of carbon tetrachloride. Signs of hepatic cytotoxicity were identified as the critical precursor effect of the carcinogenic effects. Based on the 13-week subchronic toxicity study in rats and mice (Nagano et *al.,* 2007b), the experts had selected an associated critical concentration of 10 ppm (LOAEC). This TRV is no longer consistent with the TRV establishment methodology (ANSES, 2017), and has therefore been revised to reflect:

- application of the **UF_A** factor (inter-species variability). A factor of 10 had been used in 2009. According to the TRV establishment methodology, a dosimetric adjustment is recommended, with a default value of 1 ($UF_{A\text{-}TK}$). To account for residual uncertainties regarding toxicodynamics, an uncertainty factor was set at 2.5 ($UF_{A\text{-}TD}$) according to WHO/IPCS recommendations (WHO/IPCS, 2005) and based on ANSES practices;
- changes in recent years regarding the choices of critical dose/concentration that can be used as a point of departure (POD). A benchmark dose was calculated ($BMD_{10\%}L_{95\%}$ = 2.6 ppm) based on the carcinogenic effect. It is lower than the one that had been determined for hepatic cytotoxicity (LOAEC = 10 ppm).

The chronic TRV by inhalation for CCl_4 is described in the table below.

Critical effect (key study)	Critical concentration	UF	Chronic TRV by inhalation
Hepatocellular adenomas and carcinomas in mice Nagano *et al.* (2007a)	$BMD_{10\%}L_{95\%}$ = 2.6 ppm Temporal adjustment $BMDL_{ADJ}$ = 2.6 ppm x 6/24 x 5/7 = 0.46 ppm = 2.91 mg·m^{-3}	25 UF_A = 2.5 UF_H = 10	**TRV = 0.11 mg·m^{-3}** (or 0.0184 ppm)
			Confidence level Moderate-high

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KEYWORDS

Toxicity reference value, TRV, carbon tetrachloride, chronic, inhalation

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