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COLLECTIVE EXPERT APPRAISAL:
SUMMARY AND CONCLUSIONS

Related to the establishment of a Toxicity Reference Value (TRV) for inhalation based on the carcinogenic effects of carbon tetrachloride (CAS No. 56-23-5)

Only the French language version of this document shall prevail.

Overview of the question

This work follows an earlier solicited request sent to AFSSET in February 2007 by the Directorate General for Health (DGS) concerning analysis of the method of establishing TRVs for 1,2-dichloroethane (DCE), carbon tetrachloride, chloroform and methylene chloride used by the French National Institute for Industrial Environment and Risks (INERIS) for an application for authorisation from a manufacturing company. In accordance with the initial application, these TRVs concern the carcinogenic effects induced in animals by inhalation.

In response to this request, an analysis of the consistency between the method used by INERIS and that currently advocated by the Working Group (WG) on "Carcinogenic TRVs" was made by the expert *rapporteurs* of the WG. At the end of the initial review, it appeared that, while the overall approach taken by INERIS could be considered satisfactory, the TRVs proposed in this report could not be approved in their present form. In order to proceed with this study, AFSSET proposed to the DGS that these substances be included in the 2008 work programme, to enable validated TRVs to be used. In correspondence dated 25 January 2008, the DGS asked AFSSET to propose TRVs for 1,2-dichloroethane, carbon tetrachloride and chloroform in order to rule on the use of these three values for conducting health risk assessments.

Organisation of the expert appraisal

Since AFSSET conducted its first review of the INERIS "*Rapport d'étude n° 06CR072. Analyse et construction des VTR pour le 1,2-dichloroéthane, le tétrachlorure de carbone, le chloroforme et le chlorure de méthylène*" [Study Report No. 06CR072. Analysis and construction of TRVs for 1,2-dichloroethane, carbon tetrachloride, chloroform and methylene chloride] (September 2007), a new version of this report was published on the INERIS website in September 2007. This version was only slightly modified and only considers comments on the bibliographic process employed. No additional information was provided concerning the toxicity of the substances involved or to help improve the establishment of the TRVs. Consequently, AFSSET had to conduct further expert appraisal work before validating the TRVs for these compounds.

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AFSSET entrusted validation of the TRV for carbon tetrachloride to the Expert Committee (CES) for “Assessment of risks linked to chemical agents”. The CES then mandated three *rapporteur* members of the Working Group on “Carcinogenic TRVs” to conduct the work.

A preliminary meeting was held on 29 February 2008 and the TRV establishment process was submitted to the CES for “Assessment of risks linked to chemical agents” on 20 March 2008. Further to the comments of the CES, a new meeting was held with the expert *rapporteurs* on 15 May 2008.

A report entitled “*Construction d’une VTR fondée sur les effets cancérogènes du tétrachlorure de carbone* [Development of a TRV based on the carcinogenic effects of carbon tetrachloride] (CAS No. 56-23-5)”, prepared by AFSSET and the expert *rapporteurs*, describes the approach, primary data and choices that enabled the TRV for carbon tetrachloride to be established: choice of the critical effect, key study, reference dose and uncertainty factors. This report was submitted to the CES for “Assessment of risks linked to chemical agents” and validated at the meeting on 29 May 2008.

This expert appraisal was therefore done by a group of experts with complementary expertise. It was carried out in accordance with the French Standard NF X 50-110 “Quality in Expertise Activities” to ensure compliance with the following points: competence, independence, and transparency, while at the same time ensuring traceability.

Description of the working method

The establishment of TRVs differs depending on the assumption made or data acquired on the substance’s mechanisms of toxic action. Based on the conclusions reached by INERIS and the supplemental bibliography provided by the *rapporteurs*, the assumption for establishing the carcinogenic TRV for carbon tetrachloride follows a threshold dose relationship. The establishment of a TRV is therefore defined as follows:

$$TRV = \frac{\text{Critical dose}}{UF}$$ where

Critical dose = NOAEL, LOAEL or BMDL

UF = globally applied uncertainty factor

In practice, establishment of the TRV involves the following four steps:

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

This method is detailed in the “*Document de référence pour la construction d’une VTR fondée sur des effets cancérogènes*“ [Reference document for the establishment of a TRV based on carcinogenic effects], which is currently being finalised by the Working Group on “Carcinogenic TRVs” and contains the recommendations of the “*Document de référence pour la construction d’une VTR fondée sur des effets reprotoxiques*” [Reference document for the development of a TRV based on reprotoxic effects] published by AFSSET in July 2007.

Results of the collective expert appraisal

Summary **of toxicity data**

Carbon tetrachloride (CCl_4) is a highly volatile chlorinated hydrocarbon, used as an intermediary in the manufacture of various chemical compounds (refrigerants, solvents). Its uses are currently very limited due to its toxicity and effects on the ozone layer.

CCl_4 is primarily absorbed in the body by inhalation. It is transformed mainly by cytochrome P450 2E1 to form a trichloromethyl radical. It can also react with oxygen leading to the formation of a highly reactive trichloromethyl peroxy radical which causes a lipid peroxidation phenomenon.

Toxicity data in animals show signs of hepatotoxicity from 10 ppm (increased liver weight, histological alterations, increased hepatic enzymes) leading, at higher doses, to necrosis, fibrosis and cirrhosis of the liver (the target organ). Genotoxicity test results indicate that CCl_4 is genotoxic at doses higher than the cytotoxic doses. Carcinogenicity studies indicate the occurrence of adenomas and hepatocellular carcinomas at 125 ppm in F344 rats and at 25 ppm in BDF1 mice (Nagano *et al.,* 1998). The European Union has therefore classified it as a Category 3 carcinogen (substances which cause concern for humans).

From this information, it is possible to describe the mechanism of carcinogenic action involving hepatic cytotoxicity, genotoxicity observed at the same doses, and necrosis followed by regenerative cell proliferation. These cell divisions bring about increased formation of spontaneous mutations leading to cancer. Liver tumour formation was observed at doses higher than or equal to those inducing toxicity and cell proliferation (dose-effect relationship).

The key events leading to the formation of hepatocellular carcinomas in animals can therefore be identified: hepatotoxicity (histological and biochemical changes), necrosis, regenerative cell proliferation, fibrosis, cirrhosis and hepatocellular carcinomas.

Thus, a threshold TRV based on hepatotoxicity, the critical precursor effect to cancer, may also be proposed to protect against carcinogenic effects.

Analysis and assessment of the choices for establishment of the TRV

Pivotal study

Because of the question posed (carcinogenic effect by inhalation) and the availability of toxicology studies on this route, only inhalation studies were discussed. In 2007, Nagano *et al*. published a 13-week (90-day) toxicity study in F344 rats and BDF_1 mice of both sexes. The animals were exposed for 6 hours a day, 5 days a week, according to five dose groups of 0, 10, 30, 90, 270, 810 ppm. The authors did not detect any local lesions (larynx and nasal cavity, trachea, lungs). Histological results showed large lipid inclusion droplets in liver cells (in rats of both sexes and male mice) and cytoplasmic globules (mice) as well as a release of hepatic cytolytic enzymes from 10 ppm. Fibrosis and cirrhosis phenomena were observed only in rats from 270 ppm. Biochemical monitoring showed signs of nephrotoxicity in rats and haematotoxicity (anaemia) in both animal species tested, from 90 ppm.

This study was carried out with reference to the guideline documents published by the Organisation for Economic Cooperation and Development (OECD) and followed the recommendations for good laboratory practices. The experimental protocol is described in detail and precise information is provided on the purity of the substance administered. The route of exposure is consistent with the methodological choices specified above (inhalation route, a transposition from the oral route being deemed irrelevant). The exposure duration is consistent with the choice of using a critical precursor effect (signs of hepatotoxicity). In addition, this study makes it possible to establish the lowest dose at which the observed effects are significantly higher than in the controls.

For all these reasons, this study is considered acceptable according to its Klimisch rating (valid without restriction) and was chosen as the key study.

Choice of the critical dose
The data support the choice of '**lowest observed adverse effect level**' (**LOAE**L) **at 10 ppm.** At this concentration, several observed effects are correlated, according to the mechanism of action of CCl_4, with precursor events that may be predictive of the occurrence of hepatocellular carcinomas:

- elevated liver enzyme levels (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP])
- cytoplasmic inclusions or globules in hepatocytes (male mice)
- observation of large fatty droplets in hepatocytes (rats, male mice).

These changes are considered as degenerative rather than adaptive morphological alterations, unlike increased liver weight. They may be considered as sensitive signs of the hepatic toxicity of CCl_4 and could be used as the critical precursor effect of the hepatic carcinogenic effect of CCl_4.
The use of a benchmark dose was not feasible for several reasons:

- regarding consideration of several effects (enzymatic and histological changes), the different values associated with these effects do not enable a benchmark dose to be determined;
- regarding precursor effects, it was not considered appropriate to model the entire dose-response relationship from any one of these effects; these effects may be masked at higher concentrations by the appearance of more severe effects. Accordingly, from a certain concentration, hepatotoxicity may be masked by other toxic effects on the cell, causing an artificial decrease in the response associated with this effect.

According to the experimental protocol used in the study by Nagano *et al.* (2007), the dose ranges tested do not enable a 'no observed adverse effect level' (NOAEL) to be defined. Indeed, the lowest concentrations tested correspond to the LOAEL described above. Nevertheless, some organisations (the Dutch National Institute for Public Health and the Environment [RIVM], the US Agency for Toxic Substances and Disease Registry [ATSDR], and the California Office of Environmental Health Hazard Assessment [OEHHA]) which have established TRVs for CCl_4 have chosen studies that enable NOAELs of 5 ppm to be determined on the basis of biological and histochemical changes observed in rats. However, these studies were not chosen for inclusion in this assessment because their quality was considered to be inadequate for establishing a TRV.
The critical dose is therefore a LOAEL of 10 ppm.

Choice of the uncertainty factors (UF)

- UF_A (inter-species variability): the factor chosen is the maximum factor of 10 because the critical dose comes from an animal study and there is insufficient evidence in humans to clarify this variability.
- UF_H (individual variability): the factor 10 is chosen by default when using studies conducted in animals and when there is a lack of data to clarify the variability of the human species.
- UF_L (use of a LOAEL): using a factor of 3 was proposed because the LOAEL determined corresponds to the appearance of low-severity precursor effects. Furthermore, NOAELs of 5 ppm have been proposed from studies conducted in rats based on signs of hepatotoxicity.

- UF_S (use of a sub-chronic study): the recommendations shown in the reference document for the establishment of a TRV based on carcinogenic effects that is being finalised are factors of 1, 3 or 10 depending on the case. In the key study selected (study by Nagano *et al.* 2007), the animals were exposed for 13 weeks. However, an inhalation study of carcinogenesis by Nagano *et al.* (1998) was carried out with the same animal strains and under the same experimental conditions, in which significant increases in the incidence of liver tumours were observed from 125 ppm in rats and 25 ppm in mice when exposed for 6 hours a day, 5 days a week, for two years at doses of 5, 25 and 125 ppm. Thus, the factor is 1.
- Time adjustment: in a study conducted in 1981 by David *et al.*, the authors concluded that the hepatotoxicity of CCl_4 seems more dependent on the concentration than the duration of exposure. Thus, short-term/high concentration exposure may induce more effects than long-term/low concentration exposure. However, dose fractionation appears to reduce toxicity. Therefore, the available data for this compound are contradictory. The United States Environmental Protection Agency (EPA), OEHHA and ATSDR apply a linear adjustment of the number of hours and days of administration by default, even for precursor effects. This implementation strategy is based on the choice of adopting the TRV establishment method that offers most protection for human health (following the precautionary principle). Thus, the proposed LOAEL will be corrected by an adjustment of 5d/7d and 6h/24h.

The Expert Committee (CES) for "Assessment of risks linked to chemical agents" accepted the report of the collective expert appraisal at its meeting on 10 July 2008 and informed the Directorate General of AFSSET.

The CES emphasised the fact that this TRV was established from an animal study, and on the assumption that there is a similar mode of action in humans and animals (F344 rats and BDF_1 mice).

Conclusions of the collective expert appraisal

- Carbon tetrachloride has been the subject of numerous studies in animals but its carcinogenic effects on the liver have not been demonstrated in humans to date.
- The effects observed in animals (rats and mice) are relevant for humans and signs of hepatic cytotoxicity have been chosen as the critical precursor effect of carcinogenic effects.

A TRV threshold can thus be proposed for the carcinogenic effects of carbon tetrachloride.

Critical effect	Critical dose*	UF*	TRV
Hepatoxocity (histological and enzymatic changes) Thirteen-week sub-chronic toxicity study in rats and mice Nagano *et al.* 2007	LOAEL = 10 ppm = 63.9 $mg.m^{-3}$ No NOAEL No BMD established Time adjustment: **$LOAEL_{ADJ}$** = 11.4 $mg.m^{-3}$**	**300** UF_A 10 UF_H 10 UF_L 3 UF_S 1	**TRV = 38 $\mu g.m^{-3}$** Confidence level Data collection: medium (insufficient in humans) Study: high TRV: medium (establishment of a LOAEL)

*UF_A: inter-species variability; UF_H: individual variability; UF_L: uncertainty about the LOAEL; UF_S: use of a sub-chronic study

**$LOAEL_{ADJ}$ = LOAEL adjusted to exposure time

Recommendations of the CES

Due to the similarity of the mechanism of action (metabolism, target organs, etc.) with other compounds likely to be encountered with carbon tetrachloride, particularly chloroform, the CES advises against the use of TRVs in isolation when managing risk.

Concerning the time adjustment, as a precaution and by default (pending the outcome of work being done on time adjustment by the Working Group on “TRVs”) the CES recommends applying this adjustment for the development of this TRV.

Maisons-Alfort, 10 July 2008

On behalf of the Expert Committee (CES) for
“Assessment of risks linked to chemical agents”,

Chairman of the CES

M. Michel Guerbet