The Director General

Maisons-Alfort, France, 15 June 2009

OPINION

of the French Agency for Environmental and Occupational Health Safety

Relating to establishing carcinogenic HTVs by inhalation for carbon tetrachloride, chloroform and 1,2-dichloroethane

The mission of Afsset (French Agency for Environmental and Occupational Health Safety), is to help ensure environmental and occupational health safety and assess potential health risks in these areas. It provides the competent authorities with all information on these risks as well as the expertise and technical support required to draft legislative and statutory provisions and implement risk management strategies.

In the scope of the French National Health & Environment Plan 2004-2008 (NEHAP), in 2003, Afsset was requested on its own initiative to propose a method of establishing human toxicity values (HTVs) based on reprotoxic effects. In the framework of the Cancer Plan in 2004, these studies were widened to calculate HTVs based on carcinogenic effects. On 25 July 2007, Afsset was given the task of establishing HTVs by its funding ministries.

Presentation of the question

The expert appraisals were made following an initial solicited request to Afsset in February 2007 by the Directorate General for Health (DGS) asking for the analysis of the method used by the French National Research and Safety Institute (INRS) for establishing HTVs. Indeed, in the framework of a licence request for industrial use, INRS established HTVs for 1,2-dichloroethane, carbon tetrachloride, chloroform and dichloromethane. In accordance with the initial request from the manufacturer concerned, these HTVs were concerned with carcinogenic effects by inhalation.

In response to this solicited request, the consistency between the method followed by INRS and that recommended at the time by the "carcinogenic HTVs" working group had been analysed by the expert rapporteurs from the working group. From this initial analysis, it seemed that while, on the whole, the approach followed by INRS could be judged satisfactory, the HTVs proposed in the report could not be approved as they stood. With a view to pursuing this expert appraisal, Afsset suggested to DGS to add these substances to its work programme 2008, so that the HTVs could be validated by the CES (Committee of Specialised Experts) "Assessment of risks linked to chemical substances". By post on 25 January 2008, the DGS asked Afsset to propose HTVs for 1,2-dichloroethane, carbon tetrachloride and chloroform with a view to deciding on the use of these three values in the practice of the assessment of health risks.

French Agency for Environmental and Occupational Health Safety
253 av. du Général Leclerc 94701 Maisons-Alfort Cedex - France
Tel. +33 (0)1.56.29.19.30 Fax +33 (0)1.43.96.37.67 E-mail afsset@afsset.fr
www.afsset.fr

Context

The HTVs are indicators allowing a qualitative or even quantitative relationship to be established between exposure to a chemical substance and an effect on human health.

They are specific to a substance, a duration and a route of exposure. They are established in different ways according to the hypothesis formulated or data acquired on the toxic action mechanisms of the substance: HTVs come under two categories. First, a "threshold dose" HTV, used for substances for which the severity of the damage caused, above a certain dose, is proportional to the dose absorbed, and, second, a "non-threshold dose" HTV, used for substances for which there exists the probability, however slight, that a single molecule entering the body will cause harmful effects to the body.
The "threshold dose" HTVs are expressed as daily admissible intakes. These values correspond to an estimate of the amount of substance to which an individual can theoretically be exposed without noticing any harmful effects on health.
"Non-threshold" HTVs are expressed in the form of a potency slope factor. These values correspond to the additional probability, in comparison to a non-exposed subject, that an individual develops a pathology (in this case, cancer) if exposed to a single dose of the substance during their entire lifetime.
HTVs are used in the framework of assessing health risks. It is most often about preventing the occurrence of an effect in an exposed population or of estimating a risk according to the exposure levels of the studied population.

Organisation of the appraisal

These appraisal activities are a result of work by a group of experts with complementary competences. They have been carried out in compliance with the French Standard NF X 50-110 "Quality in Appraisal Activities" with a view to cover the following points: competence, independence, transparency and traceability.
Afsset reported the validation of the HTVs for chloroform, carbon tetrachloride and 1,2-dichloroethane to the CES "Assessment of risks linked to chemical substances". For this work, three rapporteur members of the "Carcinogenic HTVs" working group were nominated.

The reports "Establishing a HTV based on carcinogenic effects of chloroform" and "Establishing a HTV based on the carcinogenic effects of carbon tetrachloride" were submitted to the CES "Assessment of health risks linked to chemical substances" on 20 March 2008 and validated on 29 May 2008.

The report "Calculation of a HTV based on the carcinogenic effects of 1,2-dichloroethane" was submitted to the CES on 23 October and 27 November 2008 and validated on 26 February 2009.

Carcinogenic HTV of chloroform (CASRN 67-66-3)

Chloroform is a very volatile chlorinated hydrocarbon used in the bleaching of paper pulp or in chemical synthesis. It is also emitted from reactions with nitrogenous organic matter during the chlorination of water.
The available genotoxicity data show that neither chloroform nor its metabolites appear in such a way as to interact directly with DNA. In humans, hepatotoxic effects have been detected in workers exposed to numerous products including chloroform. In rodents, the effects of chloroform by ingestion and by inhalation have shown that the target organs are the liver, the kidneys and the nasal epithelium.

Chloroform is classified as a category 3 carcinogen by the European Union according to Directive 67/548/EC[1]. The International Agency for Research on Cancer (IARC) classified it as group 2B ("The chemical is a possible human carcinogen").

In accordance with the conclusions of the collective expert appraisal, Afsset proposes, a threshold dose HTV based on the critical precursory effects of cancer, in order to protect from the carcinogenic effects of chloroform by inhalation.

Critical effect	Critical dose*	UF*	HTV
Cellular proliferation in the proximal renal tubules in the BDF1 male mouse 13-week subchronic toxicity study in the BDF1 mouse Templin *et al*. 1998[2]	NOAEL = 5 ppm or 25 mg.m $^{-3}$ or after partial time adjustment (6h/24h) $NOAEL_{AJ}$** = 6.3 mg.m $^{-3}$ No determination of BMD***	**100** UF_A 10 UF_H 10 UFs 1	**HTV = 63 µg.m^{-3}**

* UF: Overall uncertainty factor (applied), UF_A: inter-species variability, UF_H: individual variability, UF_S: use of a subchronic study
** $NOAEL_{AJ}$ = adjusted NOAEL to the time of exposure
*** BMD: benchmark dose

Carcinogenic HTV of carbon tetrachloride (CASRN 56-23-5)

Carbon tetrachloride (CCl_4) is a very volatile chlorinated hydrocarbon used as an intermediate in the manufacturing of a range of chemical compounds (refrigerants, solvents). Its uses are limited today due to its toxicity and its effects on the ozone layer.
The toxicity data in animals show signs of hepatotoxicity (target organ) leading to, at high doses, necrosis, fibrosis then cirrhosis of the liver. The results of genotoxicity tests show that CCl_4 is genotoxic at doses higher than cytotoxic doses.
Carbon tetrachloride is classified as a category 3 carcinogen by the European Union according to Directive 67/548/EC[1]. The International Agency for Research on Cancer (IARC) classified it as group 2B ("The chemical is a possible human carcinogen").

In accordance with the conclusions of the collective expert appraisal report, Afsset proposes, a threshold HTV based on the precursory critical effects, in order to protect from the carcinogenic effects of CCl_4 by inhalation.

[1] As such, and in accordance with Article R. 4411-6 of the "Code du travail" (French Labour Code), the substance is considered a hazardous chemical. According to Articles R. 4412-15 and R. 4412-16, labour regulations encourage the removal of chemical risks or, if this is not possible, their substitution. If all else fails, the risk must be reduced to a minimum by the use of appropriate protection measures.
[2] Templin MV, Constan AA, Wolf DC, Wong BA and Butterworth BE. Patterns of chloroform-induced regenerative cell proliferation in BDF1 mice correlate with organ specificity and dose-response of tumor formation. *Carcinogenesis* 1998; 19(1), 187-193.

Critical effect	Critical dose*	UF*	HTV
Hepatotoxicity (histological and enzymatic changes) 13-week subchronic toxicity study in the F344 rat and the BDF1 mouse Nagano *et al.* 2007[3]	LOAEL = 10 ppm = 63.9 mg.m $^{-3}$ Absence of NOAEL No determination of BMD** Time adjustment: $LOAEL_{aj}$*** = 11.4 mg.m $^{-3}$	300 UF_A 10 UF_H 10 UF_L 3 UF_S 1	**HTV = 38 µg.m^{-3}**

* UF: Overall uncertainty factor (applied), UF_A: inter-species variability, UF_H: individual variability, UF_L: uncertainty in the LOAEL, UF_S: use of a subchronic study
** BMD: benchmark dose
*** $LOAEL_{AJ}$ = adjusted LOAEL to the time of exposure

Carcinogenic HTV of 1,2-dichloroethane (CASRN 107-06-2)

1,2-dichloroethane (DCE) is a very volatile colourless liquid used as a solvent in the chemical and pharmaceutical industry and as an intermediate in the synthesis of vinyl chloride.
In rats, DCE is metabolised following two routes: a main route CYP P450 2E1 and 2B1-dependent, followed by a second route of glutathione conjugation, if the main route is saturated. This second route leads to the formation of glutathione episulfonium ions capable of forming adducts with proteins and DNA. Genotoxicity tests show that DCE leads to the formation of genetic mutations *in vitro* and DNA alterations *in vivo*. In animals, DCE leads to the formation of tumours in multiple organs (lungs, subcutaneous tissue, mammary glands).
DCE is classified as a class 2 carcinogen by the European Union by Directive 67/548/EC[1]. The International Agency for Research on Cancer (IARC) classified it as group 2B ("The chemical is a possible human carcinogen").

In accordance with the conclusions of the collective expert appraisal report, Afsset proposes a non-threshold VTR based on the carcinogenic effects of DCE by inhalation.

Critical effect	Critical dose	HTV
Increase in the incidence of tumours in mammary glands Carcinogenesis study (104 weeks) in the F344 rat and the BDF1 mouse Nagano *et al.* 2006[4]	$BMD_{10}L_{95}$* = 40 ppm = 164.4 mg.m $^{-3}$ Time adjustment: $BMD_{10}L_{95aj}$** = 40 x 6/24 x 5/7 = 7.14 ppm = 29.3 mg.m $^{-3}$ Allometric adjustment: x 1	After linear extrapolation of the original: **HTV = 0.014 (ppm)$^{-1}$ = 3.4.10^{-3} (mg.m^{-3})$^{-1}$** 0.3 µg.m^{-3} for a risk of 10^{-6} 3 µg.m^{-3} for a risk of 10^{-5} 30 µg.m^{-3} for a risk of 10^{-4}

* BMD_{10} L_{95}: lower limit of the 95% confidence interval of the benchmark dose corresponding to an increase in the response in comparison with the non-exposed group of 10%
** $BMD_{10}L_{95aj}$: BMD_{10} L_{95} adjusted to the time of exposure

[3] Nagano K, Umeda Y, Saito M, Nishizawa T, Ikawa N, Arito H, Yamamoto S and Fukushima S. Thirteen-week inhalation toxicity of carbon tetrachloride in rats and mice. *J Occup Health* 2007; 49:249-259
[4] Nagano K, Umeda Y, Senoh H *et al*. Carcinogenicity and chronic toxicity in rats and mice exposed by inhalation to 1,2-dichloroethane for two years. J *Occup Health*. 2006 Nov; 48(6):424-36.

Recommendations

With regards to 1,2-dichloroethane, the Agency recommends initiating research on the determination of the saturation threshold of the first metabolic route in humans, which leads to the activation of the second route forming genotoxic metabolites.

It would also be advisable to widen the knowledge on the mechanism of carcinogenic action of DCE, especially on the nature of the formed adducts. This would allow susceptible groups of the population to be identified (window of vulnerability, enzymatic polymorphism, and so on).

Generally, during an overall risk assessment in a multi-exposure context, it would be advisable to take into account the sum of the risks of the compounds as they have the same target organs and the same mechanisms of toxic action (for instance, carbon tetrachloride and chloroform).

Finally, it is often assumed that at the time of establishing a HTV, a time adjustment is applied as a precaution, taking into account the absence of scientific data. The future work of the "HTV" working group guided by Afsset should allow factors on the application conditions of such an adjustment during the establishing of HTVs to be introduced.

The Director General

Martin GUESPEREAU