afsset
french agency for environmental and occupational health safety

COLLECTIVE EXPERT APPRAISAL: SUMMARY AND CONCLUSIONS

Related to the establishment of a Toxicity Reference Value (TRV) for inhalation based on the carcinogenic effects of 1,2-dichloroethane (CAS No. 107-06-2)

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 followed 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 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. Indeed, in the case of 1,2-dichloroethane, the TRV failed to take into account data produced after 2005 that challenged the choice of critical effect (study by Nagano *et al.* 2006). 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 version of this report was published on the INERIS website in 2006. 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.

afsset
253 Avenue du Général Leclerc – 94701 Maisons-Alfort Cedex – SIREN No. 180092348
☎ +33 (0)1 56 29 19 30 – 🖷 +33 (0)1 43 96 37 67 – Website: http://www.afsset.fr – E-mail: afsset@afsset.fr

AFSSET entrusted validation of the TRV for 1,2-dichloroethane 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.
The process for establishing the TRV was submitted to the CES for “Assessment of risks linked to chemical agents” on 23 October and 27 November 2008. Further to the comments of the CES, new meetings were held with the *rapporteurs*.

A report entitled “*Elaboration d’une VTR fondée sur les effets cancérogènes du 1,2-dichloroéthane*” [Development of a TRV based on the carcinogenic effects of 1,2-dichloroethane], prepared by AFSSET and the expert *rapporteurs*, describes the approach, primary data and choices that enabled the TRV for 1,2-dichloroethane (DCE) to be established: choice of the critical effect, key study and critical dose considered as the point of departure for the extrapolation to low doses. This report was submitted to the CES for “Assessment of risks linked to chemical agents” and validated at the meeting on 26 February 2009.
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 DCE follows a no-threshold dose relationship. The effect appears irrespective of the dose received, and the probability of occurrence increases with the dose. The TRV is then expressed in the form of an ERU (excess risk per unit), and is defined as the additional probability, compared to an unexposed subject, that an individual will develop a disease (in this instance, cancer), if he is exposed over his entire lifetime to a unit dose of the substance.

In practice, establishment of the 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 (or dose-effect) relationship;
- choice or establishment of a critical dose from experimental doses and/or epidemiological data;
- extrapolation to the origin (low doses).

This method is detailed in the “*Document de référence pour la construction d’une VTR fondée sur des effets cancérogènes*” [Reference document for the development of a TRV based on carcinogenic effects].

Results of the collective expert appraisal

Summary of toxicity data

DCE is a chemical compound consisting of two atoms of chlorine. It is in the form of a highly volatile colourless liquid, used as an industrial and laboratory solvent.
In 1993 the European Union classified this compound as a Category 2 carcinogen (Substances which should be regarded as if they are carcinogenic to man

DCE is rapidly absorbed by inhalation and ingestion and distributed throughout all compartments of the body, mainly in fatty tissue. In rats, DCE is metabolised by means of two mechanisms. The first is the major metabolic pathway involving saturable microsomal oxidation mediated by cytochromes P450 2E1 and 2B1, which lead to the formation of chloroacetaldehyde and 2-chloroethanol; these reactions are followed by conjugation with glutathione.
The second mechanism is triggered when the first metabolic pathway is saturated. It consists of direct conjugation with glutathione to form *S*-2-chloroethyl-glutathione, which can be converted by a non-enzymatic route into a glutathione episulfonium ion capable of forming adducts with proteins and DNA (US Agency for Toxic Substances and Disease Registry [ATSDR], 2001).
Non-metabolised DCE is eliminated by exhalation. Metabolites are excreted through the urine (primarily thiodiacetic acid and thiodiacetic acid sulfoxide (RIVM, 2001; IARC 1987).

The mechanism of carcinogenic action of DCE may involve the formation of genotoxic metabolites. However, the saturation threshold of the first metabolic pathway is unknown in humans. In the absence of this information, and following the precautionary principle, the assumption used for establishing the carcinogenic TRV for DCE will be the no-threshold dose.

Genotoxicity tests with and/or without metabolic activation show that DCE causes the formation of gene mutations *in vitro* and DNA damage *in vivo* (IARC, 1987). In rats and mice exposed *in vivo*, the formation of adducts to DNA has been reported in the lungs, liver, kidneys and stomach (ATSDR, 2001).

In humans, no conclusions can be drawn about the risk of cancer related to DCE by oral and respiratory routes because of co-exposures to other chemical compounds.

In animals, four studies on the inhalation route have yielded conflicting results.

- The study by Maltoni *et al.* (1980) showed, in female rats exposed to 0, 5, 10, 50 and 250 parts per million (ppm) for 7 hours per day, 5 days per week, for 78 weeks and then observed for several additional weeks, a significant increase in the cumulative incidence of benign and malignant breast tumours ($p<0.05$, Fisher test) (39/90, 65/90, 43/90, 58/90 and 52/90).
- The study by Cheever *et al.* (1990), in which male and female rats were exposed to 50 ppm with and without disulfiram (a CYP450 inhibitor, including CYP2E1), for 7 hours per day, 5 days per week for two years, showed a significant increase in the combined incidence of benign and malignant mammary gland tumours only in the group exposed in the presence of disulfiram ($p<0.05$, Fisher test) (21/50 *versus* 34/48)[1].
- In 1998 Nagano *et al.* first published a report based on a study of the toxicity of six halogenated compounds, and then in 2006 an article in a peer-reviewed journal (Journal of Occupational Health) covering DCE alone. In the 1998 study, the animals were exposed for 6 hours per day, 5 days per week for 104 weeks to 0, 10, 40 and 160 ppm for F344 rats and to 0, 10, 30 and 90 ppm for BDF_1 mice. They showed haemangiosarcomas of the liver in male mice (0/50, 4/49, 6/50 and 5/50). Fibroadenomas of the mammary gland were observed in male (0/50, 0/50, 1/50 and

[1] This study suggests that the *in vivo* carcinogenicity of DCE may be linked to a larger quantity of episulfonium ions generated due to the inhibition of the primary metabolic pathway (i.e. P450-dependent metabolism).

5/50) and female (4/50, 1/50, 6/50 and 13/50) rats. Fibromas of the subcutaneous tissue were also reported in male rats (6/50, 9/50, 12/50 and 15/50).

- The 2006[2] study by Nagano *et al.* clarified the results from 1998. According to the authors, the increased incidence of haemangiocarcinomas of the liver in male mice was unlikely to be correlated to an exposure to DCE, given that no significant dose-response relationship was identified in this study (Peto test, $p > 0.05$). In female rats, the combined incidence of adenomas (3/50, 5/50, 5/50 and 11/50), fibroadenomas (4/50, 1/50, 6/50 and 13/50) and adenocarcinomas (1/50, 2/50, 0/50 and 5/50) of the mammary gland showed a significant dose-response relationship (Peto test, $p< 0.01$). The combined incidence increased significantly at the dose of 160 ppm not only compared to the unexposed group ($p < 0.01$, Fisher test) but also compared to historical controls (at 40 and 160 ppm).

Analysis and assessment of the choices for establishment of the TRV

Pivotal study

No epidemiological study can be used for establishing a TRV for DCE (due to characterisation bias in the exposures).

As indicated in the INERIS report, at low doses, the major metabolic pathway became saturated more rapidly after oral exposure than after inhalation exposure, which leads to the assumption of equivalence of the routes being ruled out, and consequently to rejecting studies where exposure was not via the respiratory route.

The study by Cheever *et al* (1990) was not chosen because only a single dose was tested (in the presence or absence of an inhibitor).

The study by Maltoni *et al.* (1980) showed no significant increase in the incidence of tumours either in the rats or the mice (except when they were combined). Moreover, in this study, the animals were exposed for a period of 78 weeks and then observed for several additional weeks, which does not correspond to the protocol recommended by the Organisation for Economic Cooperation and Development (OECD) guidance documents (animals must be exposed for 104 weeks).

Among the studies of carcinogenicity by inhalation, only the study by Nagano *et al.* (2006) showed a significant increase ($p < 0.01$, Fisher test) in the incidence of tumours at different sites (notably, the liver, peritoneum, lungs and mammary glands). In this study, F344 rats and BDF_1 mice (50 animals per batch and sex) were exposed for 6 hours per day, 5 days per week for 104 weeks at 0, 10, 40 and 160 ppm for the rats and at 0, 10, 30 and 90 ppm for the mice.

This study was carried out with reference to the guidance documents published by the 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).

[2] This study had not been published at the time INERIS completed its study report.

Choice of the critical effect
Statistical analyses of the study by Nagano *et al.* (2006) show a significant increase in the incidence of breast tumours (benign and/or malignant) both in male (fibroadenomas) and female (adenomas, fibroadenomas and adenocarcinomas) rats.
In addition, this study shows a significant dose-response relationship between the combined increased incidence of benign and malignant breast tumours and the concentration of DCE in the air, which is not the case for haemangiosarcomas of the liver (the critical effect chosen by INERIS).
Furthermore, since the fact that some benign tumours evolve into malignant tumours cannot be ruled out, it was decided to use the combination of benign and malignant breast tumours for establishing the no-threshold dose TRV for DCE.

Choice of the critical dose and extrapolation to lower doses
In the case of establishing a no-threshold dose TRV, the choice of a 'Point of departure' (POD) is determined by modelling the experimental data corresponding to a benchmark dose. Thus, the data were adjusted according to the models developed by the US EPA (http://www.epa.gov/ncea/bmds/), software version 1.4.1c for dichotomous data (gamma, logistic, multi-stage, probit, and Weibull models).
The model chosen was the one best suited to the data by the method of maximum likelihood ($p > 0.1$). Ultimately, the probit model was chosen for estimating the lower limit of the confidence interval at 95% of a dose corresponding to a 10% increase in response compared to the unexposed group ($BMD_{10}L_{95}$) at 40 ppm or 164.4 $mg.m^{-3}$.
The linear extrapolation performed subsequently consisted of a straight line plotted from the POD to the origin representing the risk of developing cancer according to the dose.

Thus, for a $BMR_{10\%}$ (benchmark response), the slope is determined by the $BMR_{10\%}/BMD_{10}L_{95}$ ratio, i.e. 10%/40, which is a slope of 0.0025.

Calculation of the TRV
Choice of the critical dose: $BMD_{10}L_{95}$ = 40 ppm = 164.4 $mg.m^{-3}$
Time adjustment: $BMD_{10}L_{95ADJ}$ = 40 x 6/24 x 5/7 = 7.14 ppm = 29.3 $mg.m^{-3}$
Allometric adjustment: x 1
According to US EPA criteria, DCE is a Category 3 gas, and the formula for determining a human equivalent concentration (HEC) for a gas with low water solubility and low reactivity in contact with lung tissue causing an extrarespiratory effect, is the following:

$$Dose_{HEC} = Dose \times \lambda_A/\lambda_H$$

where λ_A represents the coefficient of gas transfer between air and blood in animals and λ_H, the coefficient of gas transfer between air and blood in humans.
In the absence of data on these factors, the US EPA recommends considering the λ_A/λ_H ratio as equal to 1.
Linear extrapolation to low doses: for a $BMR_{10\%}$, the slope is determined by the $BMR_{10\%}/BMD_{10}L_{95}$ ratio, i.e., 0.1/7.14 ppm = 0.1/29.3 $mg.m^{-3}$

→ **TRV = 0.014 $(ppm)^{-1}$ = 3.4.10^{-3} $(mg.m^{-3})^{-1}$**

therefore, for a risk of 10^{-6}: it would be necessary to be exposed to a dose of 7.10^{-5} ppm or 0.3 $\mu g/m^3$

Conclusions and recommendations of the collective expert appraisal

A chronic no-threshold TRV for inhalation can be proposed for the carcinogenic effects of 1,2 DCE.

Critical effect	Critical dose	TRV
Increased incidences of mammary gland tumours Carcinogenicity study (104 weeks) in F344 rats and BDF1 mice Nagano *et al.* 2006	$BMD_{10}L_{95}$ = 40 ppm = 164.4 mg.m^{-3} Time adjustment: $BMD_{10}L_{95ADJ}$ = 40 x 6/24 x 5/7 = 7.14 ppm = 29.3 mg.m^{-3} Allometric adjustment: x 1	After linear extrapolation to the origin: **TRV = 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}

A 'medium' overall confidence level can be attributed to this TRV for the following reasons:

- the assumption of the threshold dose mechanism of action (saturable metabolic pathway and formation of genotoxic metabolites) was ultimately not chosen given a lack of information on the saturation threshold level in humans. As a precaution, the no-threshold development hypothesis was ultimately chosen;
- The key study chosen is of good quality and enables a dose-response relationship to be described;
- the chosen critical effect combines benign and malignant breast tumours in animals, which is not currently recommended by the scientific community. Nevertheless the combined incidence of tumours corresponds well to an increasing dose-response relationship;
- modelling data makes it possible to determine a critical dose of good quality serving as the point of departure for extrapolation to the area of low doses;
- the protective criterion for human health of the TRV thus established is increased, due to the development assumptions chosen and the combination of tumour types.

The Expert Committee (CES) for "Assessment of risks linked to chemical agents" accepted the report of the collective expert appraisal at its meeting on 26 February 2009 and informed the Directorate General of AFSSET.

The CES recommends initiating research to determine the threshold for saturation of the primary metabolic pathway of DCE in humans, which causes the activation of a secondary pathway forming genotoxic metabolites.
It will also be necessary to improve knowledge of the mechanism of carcinogenic action of DCE and particularly on the nature of the adducts formed. This would help identify sensitive population groups (window of vulnerability, enzyme polymorphism, etc.).

Maisons-Alfort, 26 February 2009

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

Chairman of the CES

M. Michel Guerbet

Bibliography

ATSDR. Toxicological profile for 1,2 Dichloroethane. 2001

International Agency for Research on Cancer (IARC). IARC Monographs volume 71. 1,2-dichloroethane. 1987.

Cheever K.L., Cholakis J.M., el Hawari A.M., *et al.* Ethylene dichloride: the influence of disulfiram or ethanol on oncogenicity, metabolism, and DNA covalent binding in rats. *Fundam Appl Toxicol*, 1990; 14: 243-61.

French National Institute for Industrial Environment and Risks (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.

French National Institute for Industrial Environment and Risks (INERIS). - *Fiche de données toxicologiques et environnementales des substances chimiques. Le 1,2-dichloroéthane* [Toxicological and environmental data sheet for chemical substances. 1,2-dichloroethane], 2006.

Maltoni C., Valgimigli L. and Scarnato C. (1980) - Long-term carcinogenic bioassays on ethylene dichloride administered by inhalation to rats and mice. Ethylene dichloride: A potential health risk? Banbury Report No. 5. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, pp. 3-33.

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.

National Toxicology Program (NTP). Bioassay of 1,2 dichloroethane for possible carcinogenicity. 1978.

Rijksinstituut voor Volksgezondheid en milieu (RIVM, the Dutch National Institute for Public Health and the Environment). Re-evaluation of human-toxicological maximum permissible risk levels. RIVM Report 711701 025. Mars 2001.