Maisons-Alfort, 12 July 2012

The Director General

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

on the development of TRVs by the oral and respiratory routes for the carcinogenic effects of vinyl chloride

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 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 12, 2012 shall prevail.

1. BACKGROUND AND PURPOSE OF THE REQUEST

In 2004, within the framework of the first French National Environment & Health Action Plan (PNSE1) (2004-2008) and Cancer Plan (2003-2007), ANSES began specific work on developing toxicity reference values (TRVs) and enhancing French expertise in this area. Therefore, in accordance with its missions, AFSSET proposed to its scientific partners that a national programme on TRVs be established, investigating reprotoxic chemicals as a first step.

This approach was then extended to the field of chemical carcinogens, which led to the development in 2007 of a method for establishing TRVs based on carcinogenic effects. A pilot phase was conducted to validate the implementation of the proposed method. Benzene, cadmium, ethanol, naphthalene and vinyl chloride were selected as the substances to be studied during this pilot phase. This Opinion concerns the TRVs on vinyl chloride.

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.

"Threshold dose" TRVs are established for substances that cause, above a certain dose, damage whose severity is proportional to the absorbed dose, while "non-threshold dose" TRVs are established for substances for which there is a probability, however small, that even a single molecule entering the body will cause harmful effects for the organism. Threshold TRVs are usually expressed as acceptable or tolerable daily doses or concentrations (Acceptable Daily Intake: ADI, Tolerable Daily Intake: TDI, Tolerable Concentration in Air: TCA, etc.), or reference doses or concentrations (Reference Dose: RfD or Reference Concentration: RfC). Non-threshold TRVs are generally expressed as excess risk per unit (Excess Risk per Unit: ERU, Drinking Water Unit Risk: DWUR, Inhalation Unit Risk: IUR, Reference Concentration: RC, etc.).

In practice, establishing a 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 development of a critical dose from experimental doses and/or epidemiological data;
- application of uncertainty factors to the critical dose to take uncertainties into account, or a linear extrapolation to the origin derived from the critical dose for non-threshold TRVs.

TRVs[1] 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 Agency entrusted examination of this expert appraisal to the Expert Committee (CES) on Assessment of the risks related to chemical substances. The CES mandated the Working Group on Toxicity reference values to conduct this expert appraisal. Their work was submitted at regular intervals to the CES. The report produced by the Working Group takes account of the observations and additional information provided by the CES members. The report entitled "Vinyl chloride: Development of TRVs by the oral and respiratory routes based on its carcinogenic effects" was validated by the CES on 8 December 2011.

3. ANALYSIS AND CONCLUSIONS OF THE CES

The principal source of exposure to vinyl chloride in the environment is the production of PVC (Polyvinyl Chloride). Another important source of exposure is the microbiological degradation through anaerobic dehalogenation in soil and underground water systems of perchloroethylene (PCE), trichlorethylene (TCE) and cis- and trans-1,2-dichloroethylene (DCE) (used as solvents, in degreasing operations, as surface treatments and in dry cleaning). The microbiological degradation of these compounds produces vinyl chloride in

[1] Method of establishing toxicity reference values for carcinogenic chemicals, ANSES Scientific Edition, March 2010

the subsoil, which is then released into the ambient air and water resources in the form of emanations *via* soil gases.

Vinyl chloride is absorbed rapidly and at a high level by ingestion (demonstrated in animals) and by inhalation (demonstrated in humans and animals). The studies carried out in animals (principally rats) show that vinyl chloride is rapidly metabolised by the action of the cytochromes P450, with the isoenzyme CYP2E1 being responsible for most of the metabolisation. The most reactive metabolite generated is chloroethylene epoxide (CEO), which rearranges spontaneously into chloroacetaldehyde (CAA). Saturation of the metabolism of vinyl chloride by CYP2E1 was observed in rats, monkeys and humans at exposure levels equivalent to those leading to liver tumours in rats (ATSDR, 2006)[2].

The International Agency for Research on Cancer (IARC) states that there is sufficient proof linking exposure to vinyl chloride with angiosarcomas of the liver and hepatocellular carcinomas, as well as with an increase in the risk of lung cancer and malignant neoplasms of the conjunctive and soft tissues.

Many genotoxicity studies have been carried out in humans including research into chromosomal aberrations on lymphocyte cultures of exposed workers. The results of these studies reveal numerous chromosomal aberrations and micronuclei, as well as an increase in sister chromatid exchanges observed in exposed workers as compared with non-exposed controls. *In vitro* studies show that vinyl chloride can have mutagenic effects, especially after metabolic activation.

The ingestion and inhalation of vinyl chloride lead to the development of cancers in laboratory animals. Vinyl chloride is mutagenic, it forms DNA adducts, and there is no threshold dose to the associated dose-effect relationship.

1. *Carcinogenic TRV for the oral route*

- *Choice of the critical effect*

In animals, studies of chronic toxicity by ingestion in rodents show an increase in the incidence of liver angiosarcomas, neoplastic liver nodules and hepatocellular carcinomas.
In view of all these elements taken from experimental data, the experts chose the increase in the incidence of liver tumours as the critical effect on which to base an oral TRV.

- *Choice of the study*

Although there is sufficient proof of a link between exposure to vinyl chloride and liver angiosarcomas and hepatocellular carcinomas in humans, these studies do not give grounds for establishing a dose-response relationship, because of the poor characterisation of the exposure. The experts therefore preferred to use animal studies as more suitable for establishing TRVs.

The chosen approach was therefore to determine a Point of Departure (POD) for different endpoints based on the results of studies deemed to be the most relevant and capable of demonstrating a dose-response relationship for at least one type of tumour: liver, lung and/or mammary gland tumours. Several studies were thus examined.

[2] ATSDR: Toxicological profile for Vinyl Chloride. 2006:274 p.

The study by Feron *et al.*[3] chosen as the key study by the experts and carried out on Wistar rats consisted of the oral administration of vinyl chloride (in the form of PVC powder) by incorporating it in their feed for 135 weeks in the male rats and 144 weeks for the female rats. At exposure doses of 1.7, 5.0 and 14.1 mg/kg/day, dose-response relationships were observed for liver tumours: angiosarcomas, hepatocellular carcinomas and neoplastic nodules, and pulmonary angiosarcomas (see Table 1). Pre-neoplastic lesions were observed at the lowest doses with a higher incidence: neoplastic nodules and altered hepatocellular foci, explaining the formation of hepatocellular tumours, and angiosarcomas derived from sinusoidal cells.

Table 1: Dose-response relationship, study by Feron *et al.* (1981)

Study by Feron *et al.* (1981), exposure to vinyl chloride in female Wistar rats				
Liver tumours	Doses of daily exposure ($mg.kg^{-1}.j^{-1}$)*			
	0	1.7	5	14.1
Angiosarcomas	0/57	0/58	2/59	9/57
Hepatocellular carcinomas	0/57	4/58	19/59	29/57
Neoplastic nodules	2/57	26/58	39/59	44/57
Combined angiosarcomas, hepatocellular carcinomas and neoplastic nodules	2/57	28/58	49/59	56/57

This study, given a Klimisch score of 2 (reliable with restriction) in the OECD document (SIDS, 2001), was also chosen as the critical study by the US EPA, the Dutch National Institute for Public Health and the Environment (RIVM) and the WHO to establish the TRV by ingestion for carcinogenic effects.

- *Choice of the critical dose*

The key study states that there is a significant dose-response relationship between an increase in angiosarcomas, hepatocellular carcinomas and neoplastic nodules and the daily dose of exposure to vinyl chloride (see Table 1). The available data were modelled using the US EPA's Benchmark Dose software, BMDS 2.1.1.

The aim of the approach is to estimate the dose that corresponds to a defined level of response or a defined percentage of additional response compared to a control. This level or percentage is called the Benchmark Response (BMR). This is predominantly the BMDL, i.e. the benchmark dose lower confidence limit, which is considered to be a reference dose. The experimental data were fitted by the models developed by the US EPA for dichotomous data (gamma, logistic, multistage, probit, Weibull models, etc.).

The model that offered the best fit with the experimental data was selected, using the AIC measure (the Akaike Information Criterion is a measure for selecting the most suitable model for determination of the BMD or BMC, the model with the lowest AIC being used). The

[3] Feron VJ, Hendriksen CF, Speek AJ, Til HP, Spit BJ: **Lifespan oral toxicity study of vinyl chloride in rats**. *Food Cosmet Toxicol* 1981, **19**:317-333.

gamma model was selected to estimate the lower limit of the 95% confidence interval of a dose corresponding to a 10% increase in response (increased incidence of angiosarcomas, hepatocellular carcinomas and neoplastic nodules) compared to the non-exposed group. The $BMD_{10\%}$ and $BMD_{10\%}L_{95\%}$ were calculated because the 10% threshold is generally used in studies of carcinogenicity.

The **$BMD_{10\%}$** was equal to **0.31** $mg.kg^{-1}.j^{-1}$**,** and the **$BMD_{10\%}L_{95\%}$** to **0.26** $mg.kg^{-1}.j^{-1}$**.**

- *Time and dose adjustments*

The US EPA has developed physiologically-based pharmacokinetic (PBPK) models[4] for different exposure routes (oral and respiratory), for modelling the fate of vinyl chloride in the bodies of different species (rats, mice and humans). The US EPA uses a model to determine equivalent doses or concentrations in humans. This involves converting the external exposure dose in an animal to an internal dose using a PBPK model. The conversion factor to be applied is then determined considering the lifetime external exposure dose in humans that would be required to produce an equivalent quantity of metabolites in the liver.

The external exposure dose in the animal is thus converted into an internal dose using a PBPK model developed by the US EPA. The external exposure dose required to produce an equivalent quantity of metabolites in the liver is then calculated for humans. For a calculated critical dose ($BMD_{10\%}L_{95\%}$ = 0.26 $mg.kg^{-1}.d^{-1}$), the corresponding internal dose would be 5.9 mg/L.
In humans, daily exposure to a concentration of vinyl chloride of 0.16 mg/kg would lead to a quantity of metabolites formed in the liver of approximately 5.9 mg/L.

- *Calculation of the TRV*

The slope or excess risk per unit is calculated using the following formula:

$slope = BMR/BMD_{10\%}L_{95\%}$

As the BMR is 10%, this means an excess risk per unit of 0.1/0.16, or **$0.625\ (mg/kg/d)^{-1}$.**

2. *<u>Carcinogenic TRV for the respiratory route</u>*

- *Choice of the critical effect*

Studies of chronic toxicity by inhalation show a dose-response relationship between exposure to vinyl chloride and the formation of tumours in the liver, lungs or mammary glands. In view of all these elements in animals, the experts chose the increase in the incidence of liver tumours as the critical effect on which to base a respiratory TRV.

- *Choice of the study*

Although there is sufficient proof of a link between exposure to vinyl chloride and liver angiosarcomas and hepatocellular carcinomas in humans, these studies do not give grounds

[4] US EPA (IRIS): **Toxicological review of Vinyl Chloride**. 2000:74 p

for establishing a dose-response relationship, because of the poor characterisation of the exposure. The experts therefore preferred to use animal studies as more suitable for establishing TRVs.

The chosen approach was therefore to determine a Point of Departure (POD) for different endpoints based on the results of studies deemed to be the most relevant and capable of demonstrating a dose-response relationship for at least one type of tumour: liver, lung and/or mammary gland tumours. Several studies were thus examined.

The study by Hong *et al.*[5], chosen by the experts as the key study, was carried out on rats and mice exposed to vinyl chloride by inhalation over different durations (a maximum of 45 weeks for the rats and a maximum of 28 weeks for the mice), followed by a 52-week monitoring period post-exposure. In the rats, this study shows a dose-related increase in the incidence of certain tumours (liver angiosarcomas, hepatocellular carcinomas and neoplastic nodules). In the mice, this study shows a dose-related increase in the incidence of liver angiosarcomas, hepatocellular tumours and bronchoalveolar tumours (see Table 2).

Table 2: Dose-response relationship, study by Hong *et al.* (1981)

Study by Hong *et al.* (1981), exposure to vinyl chloride in male CD-1 mice				
Liver tumours	Concentration of exposure (ppm)*			
	0	50	250	1000
Angiosarcomas	0/28	0/8	7/12	5/12
Hepatocellular carcinomas	4/28	1/8	0/12	1/12
Combined angiosarcomas and hepatocellular carcinomas	4/28	1/8	7/12	6/12

- *Choice of the critical dose*

The key study reveals a significant dose-response relationship between the increase in angiosarcomas and hepatocellular carcinomas and the concentration of daily exposure to vinyl chloride (see Table 2). The available data were modelled using the US EPA's Benchmark Dose application, BMDS 2.1.1.

The model that offered the best fit with the experimental data was selected, using the AIC measure. The logistic model was selected to estimate the lower limit of the 95% confidence interval of a dose corresponding to a 10% increase in response (increased incidence of angiosarcomas and hepatocellular carcinomas) compared to the non-exposed group. The $BMD_{10\%}$ and $BMD_{10\%}L_{95\%}$ were calculated because the 10% threshold is generally used in studies of carcinogenicity.

[5] Hong CB, Winston JM, Thornburg LP, Lee CC, Woods JS: Follow-up study on the carcinogenicity of vinyl chloride and vinylidene chloride in rats and mice: tumor incidence and mortality subsequent to exposure. *J Toxicol Environ Health* 1981, 7:909-924.

The **$BMD_{10\%}$** was equal to **78.8 ppm,** and the **$BMD_{10\%}L_{95\%}$** to **33 ppm.**

- *Time and dose adjustments*

A factor of 2.6 was used to convert the value from a concentration in ppmV to a concentration in mg/m^3. A time adjustment was performed that consisted in determining a continual exposure to vinyl chloride for the animals based on the study carried out 6 hours per day, 5 days per week, and 6 months out of 24, which corresponds to applying a weighting value of (6/24)*(5/7)*(6/24).

As described above, the external exposure dose in the animal is converted into an internal dose using a PBPK model developed by the US EPA[6]. This can then be used to calculate the external exposure dose in humans required to produce an equivalent quantity of metabolites in the liver.

Thus, for a calculated critical dose ($BMD_{10\%}L_{95\%}$ = 33 ppm), the corresponding internal dose would be 28 mg/L.
In humans, daily exposure to a concentration of vinyl chloride of 10 ppm, or 26 mg/m^3, would lead to the formation of approximately 28 mg/L metabolites in the liver.

- *Calculation of the TRV*

The slope or excess risk per unit is calculated using the following formula:

$$\textbf{Slope} = \textbf{BMR}/\textbf{BMD}_{10\%}\textbf{L}_{95\%\ \textbf{HED}}$$

For a BMR of 10%, this would give an excess risk per unit of 0.1/26, or **$0.0038\ (mg/m^3)^{-1}$.**

[6] US EPA (IRIS): **Toxicological review of Vinyl Chloride**. 2000:74 p

4. CONCLUSIONS AND RECOMMENDATIONS OF THE COLLECTIVE EXPERT ASSESSMENT ON TRVS FOR VINYL CHLORIDE

The Working Group established two carcinogenic TRV's, the first for the oral route, the second for the respiratory route.

TRV for the oral route

Critical dose	TRV by the oral route	Effects taken into account	Species	Critical study
$BMD_{10\%}L_{95\%}$	**0.625 (mg/kg/d)$^{-1}$**	Liver tumours: angiosarcomas, hepatocellular carcinomas and neoplastic nodules	Rats	Feron *et al.*, 1981

TRV for the respiratory route

Critical dose	TRV by the oral route	Effects taken into account	Species	Critical study
$BMD_{10\%}L_{95\%}$	**0.0038 (mg/m^3)$^{-1}$**	Liver angiosarcomas and hepatocellular tumours	Mice	Hong *et al.*, 1981

5. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions and recommendations of the CES on Assessment of the risks related to chemical substances on the development of toxicity reference values for vinyl chloride, and adopts these TRVs.

The Director General

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KEY WORDS

Vinyl chloride, toxicity reference values, critical dose, uncertainty factors, general population.