The Director General

Maisons-Alfort, 4 April 2013

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

on "the analysis of the US EPA's toxicity reference values by inhalation for trichloroethylene and perchloroethylene"

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES's public health mission involves ensuring 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).

This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 4 April 2013 shall prevail.

On 31 May 2012, ANSES issued an internal request to conduct an analysis of the toxicity reference values by inhalation developed by the US EPA for trichloroethylene (CAS No. 79-01-6) and perchloroethylene (CAS No. 127-18-4).

1. BACKGROUND AND PURPOSE OF THE REQUEST

A toxicity reference value, or TRV, is a toxicological indicator. When compared with exposure, this indicator can be used for qualifying or quantifying a risk to human health. TRVs are specific to a duration (acute, subchronic or chronic) and route (oral or respiratory) of exposure, and to a type of effect (reprotoxic, carcinogenic, etc.). The way TRVs are established differs depending on the knowledge or assumptions made about the substances' mechanisms of action.

In 2009, within the framework of an internal request on indoor air quality guidelines (IAQGs), the Agency proposed IAQGs for trichloroethylene[1] and perchloroethylene[2] (AFSSET, 2009a and b). The CES on Assessment of the risks related to air environments had nevertheless recommended reconsidering the possibility of setting an acute and long-term IAQG for the non-carcinogenic effects of trichloroethylene and a long-term IAQG for the carcinogenic effects of perchloroethylene, in particular by carrying out a critical analysis of the guideline values and TRVs that would be the subject of new publications.

[1] IAQG for trichloroethylene: 0.8 $mg.m^{-3}$ for subchronic effects with a threshold and 20 $\mu g.m^{-3}$ for carcinogenic effects
[2] IAQG for perchloroethylene: 1380 $\mu g.m^{-3}$ for acute effects, 250 $\mu g.m^{-3}$ for chronic non-carcinogenic effects

In 2010, the WHO proposed IAQGs for nine substances or groups of substances including TCE and PCE. The values proposed by the WHO are similar to those published at that time by the Agency and do not therefore call the Agency's values into question.

In 2011 and 2012, the US EPA proposed new threshold and non-threshold TRVs providing protection from the chronic effects, whether carcinogenic or not, associated with exposure by inhalation to TCE (US EPA, 2011) and PCE (US EPA, 2012).

Following publication of these new values, ANSES commissioned the Expert Committee on Assessment of the risks related to chemical substances (the CES on Chemicals) to analyse the US EPA's TRVs by inhalation of trichloroethylene and perchloroethylene.

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 lies within the sphere of competence of the CES on Chemicals. The CES entrusted the appraisal to the Working Group on Toxicity reference values (2). The methodological and scientific aspects of the work were presented to the CES on 10 January and 21 February 2013. They were adopted by the Expert Committee on Assessment of the risks related to chemical substances at its meeting on 21 February 2013.

3. ANALYSIS AND CONCLUSIONS OF THE CES

■ Trichloroethylene

The TRVs by inhalation for trichloroethylene were analysed both in terms of the establishment method applied by the US EPA and the choices made for establishing these TRVs.

- Analysis of the threshold dose TRV

The chronic threshold TRV by inhalation for trichloroethylene proposed in 2011 by the US EPA (RfC or reference concentration) is described in the table below:

Critical effect and source study	Critical dose	UF	Candidate RfCs	TRV
↘ Thymus weight in female mice exposed for 30 weeks via drinking water Keil *et al*. (2009)	LOAEC = 0.35 $mg.kg^{-1}.d^{-1}$ *Establishment of BMC + transformation into internal dose (PBPK model)* → Internal point of departure 0.139 mg oxidised TCE/$kg^{3/4}$/d *Route-to-route extrapolation + allometric adjustment (PBTK model)* → $LOAEC_{HEC99}$ 0.19 $mg.m^{-3}$	100 UF_L = 10 UF_A = √10 UF_H = √10 UF_S = 1	0.0019 $mg.m^{-3}$	**RfC = 0.002 $mg.m^{-3}$**
↗ Foetal cardiac malformations in rats exposed from gestation day 1 - 22 via drinking water Johnson *et al.* (2003)	*Establishment of BMC + transformation into internal dose (PBPK model)* → BMD_{01} L 0.0142 mg oxidised TCE/$kg^{3/4}$/d *Route-to-route extrapolation + allometric adjustment (PBPK model)* → BMC_{01} L_{HEC99} 0.021 $mg.m^{-3}$	10 UF_L = 1 UF_A = √10 UF_H = √10 UF_S = 1	0.0021 $mg.m^{-3}$	

HEC: Human equivalent concentration, LOAEC: Lowest Observed Adverse Effect Concentration, BMC: Benchmark concentration, BMCL: Lower limit of the confidence interval of the BMC at 95%, PBPK: Physiologically-based pharmacokinetic, UF_A: Inter-species uncertainty factor, UF_H: Inter-individual uncertainty factor, UF_S: Uncertainty factor related to sub-chronic to chronic transposition, UF_L: Uncertainty factor related to the use of a LOAEC

Several limitations relating to the establishment of the US EPA's RfC were identified:

- The establishment method used by the US EPA to derive its RfC is unusual. It involves calculating candidate RfCs *a priori* and then seeking the study supporting the lowest RfC. The approach used does not follow the US EPA's methodological guides to establishing TRVs and is not in agreement with the approach proposed by ANSES for establishing TRVs (AFSSET, 2010).

- Due to this unconventional establishment method, the choice of key studies is not primarily based on the quality of the studies or the relevance of the effects, but on the lowest RfC value obtained. Thus, the study by Johnson *et al.* has significant limitations that were described by the US EPA. With regard to the study by Keil *et al.*, this also has limitations: study not following the guidelines even though it followed good laboratory practices, lack of control of water consumption, effects on the thymus not directly sought. This last point raises the question of the feasibility of transposing this immunological effect to humans. Indeed, the variation in thymus weight in mice is not a specific marker of immunotoxicity. The National Toxicology Program (NTP) therefore advises against using this effect to derive the RfC, especially since the study by Keil *et al.* shows other auto-immune effects at the

same dose. According to the NTP, these effects could have been selected as the critical effect because they have a functional significance and a stronger link between the auto-immune effect observed in animals and the auto-immune effects induced by TCE in humans.

- The BMC established on the basis of the study by Johnson *et al.* has certain limitations (the highest dose was excluded, no effect was observed at the first dose tested).

- The US EPA established its RfC by taking the average of two candidate RfCs established from different studies, of limited quality and demonstrating different effects. The WG on TRVs believes that this approach does not ultimately guarantee that a high quality TRV will be obtained.

In view of the limitations identified, the WG on TRVs recommends not adopting the US EPA's RfC.

- Analysis of the TRV without a threshold dose

The no-threshold TRV by inhalation for trichloroethylene proposed by the US EPA in 2011 is described in the table below:

Critical effect and source study	Establishment method	Value of the TRV
Renal carcinoma, non-Hodgkin's lymphoma and liver tumours Charbotel *et al.*, 2006	Linear extrapolation to the origin Adjustment of the excess risk calculated for kidney cancer for the potential risk of tumours at multiple sites (liver, non Hodgkin's lymphoma) (factor 4: calculations based on US EPA, 2011; Raaschou-Nielsen *et al*., 2003)	**$4.1.10^{-6}$ $(\mu g.m^{-3})^{-1}$** Concentrations associated with different levels of risk: **10^{-4}: 20 $\mu g.m^{-3}$** **10^{-5}: 2 $\mu g.m^{-3}$** **10^{-6}: 0.2 $\mu g.m^{-3}$**

Several limitations related to the establishment of the US EPA's ERU were identified:

- As with the establishment of the threshold TRV, the US EPA used a method of establishment that did not follow the US EPA's methodological guides to establishing TRVs by establishing ERUs from all the studies demonstrating a dose-response relationship.

- The key study (Charbotel *et al*., 2006) seems to have been conducted well with a good retrospective assessment of exposure, in particular production of a cumulative index that takes dermal and inhalation exposure into account. The results show an association between high cumulative exposure during a period of employment and the risk of kidney cancer, which remains significant after adjustment for smoking and the body mass index. In contrast, the significance disappears when the model takes into account exposure to cutting oils and petroleum. However, the odds ratio remains high. It cannot be excluded that a more robust study could have led to a statistically significant result. Due to the concomitant exposure to TCE and cutting oils and petroleum, the authors acknowledge that the possible role of these confounding factors cannot be excluded.

- The reconstruction of exposure is very well documented, but nevertheless remains a difficult exercise, complicated here by the fact that the exposure of certain cases or controls may be very old, dating from periods during which the quality of the TCE used may have varied. The use of combined exposure (inhalation and dermal) makes it difficult to use these figures to establish a TRV by inhalation.

- Regarding the establishment method, the application of American mortality tables associated with French incidence data is questionable.

- Finally, adjusting the excess risk calculated for kidney cancer for the potential risk of tumours at multiple sites by applying a multiplying factor is debatable, since this is not common practice when establishing TRVs. Cumulating the tumours in various organs is unusual and not recommended by the experts of the CES on Chemicals and the WG on TRVs 2.

Accordingly, the **group of experts recommends not adopting the ERU proposed by the US EPA in 2011**.

- **Perchloroethylene**

  - Analysis of the threshold dose TRV

The chronic threshold TRV by inhalation proposed by the US EPA in 2012 is described in the table below:

Critical effect and source studies	Critical dose	UF	Value of the TRV
Neurotoxicity (reaction time, cognitive effects) in 65 workers employed in a dry-cleaners Echeverria *et al*., 1995	LOAEC 56 $mg.m^{-3}$	1000 UF_H 10 UF_L 10 UF_D 10	**RfC 0.04 $mg.m^{-3}$** (average interval between 0.056 and 0.015 $mg.m^{3}$)
Neurotoxicity (colour vision) in 35 workers employed in a dry-cleaners Cavalleri *et al.,* 1994	LOAEC 15 $mg.m^{-3}$	1000 UF_H 10 UF_L 10 UF_D 10	

LOAEC: Lowest Observed Adverse Effect Concentration, UF_H: Inter-individual uncertainty factor, UF_L: Uncertainty factor related to the use of a LOAEC, UF_L: Uncertainty factor related to the lack of data

Several limitations relating to the establishment of the US EPA's RfC were identified:

- The choice of key studies. These have already been the subject of a critical analysis in the collective expert appraisal report "Assessment of health effects and methods for measuring levels of workplace exposure for perchloroethylene" (ANSES, 2010).

- The establishment approach, which is not in line with the method of establishing TRVs used by the WG on TRVs. Firstly, the method does not provide for the averaging of several TRVs. Secondly, given the data from the different studies, the dose of 15 $mg.m^{-3}$ from the study by Cavalleri *et al*. cannot be regarded as a LOAEC. This value is regarded as a NOAEC in other studies based on a sufficient number of subjects. The observed effect is in fact very sensitive, as is the test used. Moreover, the absence of any cumulative aspect (lack of correlation with the duration of exposure) suggests an acute effect and it seems difficult to establish a chronic TRV based on this type of effect.

- The uncertainty factors such as those applied by the US EPA for PCE: a UF_L of 3 would have been sufficient to go from the LOAEC to the NOAEC, a factor of 5 for the inter-individual difference would also have been necessary, from the very fact of the sensitivity of the anomaly observed, and finally, the value of 10 for the UF_D seems excessive given the number of studies in humans and animals available for this substance.

In conclusion, the ANSES experts recommend not adopting the RfC proposed by the US EPA as the chronic TRV for PCE.

- Analysis of the TRV without a threshold dose

The inhalation cancer slope factor for perchloroethylene proposed by the US EPA in 2012 is described in the table below:

Critical effect and source study	Establishment method	Value of the TRV
Hepatocellular adenomas and carcinomas in male Crj:BDF1 mice JISA, 1993	Calculation of a $BMC_{10}L_{95}$ PBTK model (Chiu and Ginsberg, 2011)	**$2.6.10^{-7}$ ($\mu g.m^{-3}$)$^{-1}$** Concentrations associated with different levels of risk: **10^{-4}: 400 $\mu g.m^{-3}$** **10^{-5}: 40 $\mu g.m^{-3}$** **10^{-6}: 4 $\mu g.m^{-3}$**

The experts of the WG on TRVs recommend adopting the no-threshold TRV proposed by the US EPA for carcinogenic effects by inhalation on the basis of the criteria explaining the approach and the choice of the critical effect, the key study, the critical dose and the calculation of the excess risk per unit.

- The US EPA selected hepatocellular adenomas and carcinomas observed in male mice as the critical effects in order to derive their TRV, unlike the preliminary report of 2008 which based the RfC on mononuclear cell leukaemia.

- In the current state of knowledge, it is not possible to precisely identify the mechanism of action of PCE in the onset of liver tumours. Therefore, taking account of the method of establishing TRVs for carcinogenic effects, the experts of the WG on TRVs by default believe that the mechanism of carcinogenic action of PCE is without a threshold, in agreement with the choice of the US EPA. It should be emphasised that this approach helps ensure the highest level of protection.

- The experts of the WG on TRVs do not reject the JISA study (1993) as the key study. However, this study is unpublished and not all its data are available.

- The ANSES experts consider that, strictly from a calculation point of view, the BMCL provided by the US EPA is of good quality, and meets the conditions of application of this theory.

- The experts of the WG on TRVs consider that the PBTK model of Chiu and Ginsberg (2011) can be used for route-to-route and animal-to-human extrapolations.

The experts note however that uncertainties remain, especially concerning the mode of carcinogenic action of PCE in the liver, the extrapolation to humans of the liver tumours observed in mice, the share in humans of the metabolic pathway of GSH conjugation.

The experts also stress that not all the data from the key study are available. Therefore, due to these uncertainties, the WG on TRVs has assigned an average/low overall confidence level to the no-threshold TRV proposed by the US EPA and recommends reviewing these conclusions depending on the results of the assessments under way.

4. 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 analysis of toxicity reference values by inhalation developed by the US EPA for trichloroethylene and perchloroethylene and adopts the no-threshold TRV for perchloroethylene.

In view of the uncertainties identified, the French Agency for Food, Environmental and Occupational Health & Safety recommends reviewing the no-threshold TRV by inhalation for perchloroethylene depending on the results of the assessments under way.

In the current state, the reference values recommended by ANSES for these substances are as follows:

Substance	**IAQGs** (reference)	**OELs** (reference)	**TRV by inhalation**
Trichloroethylene	Short term IAQG: / Intermediate IAQG: 800 µg.m^{-3} Long-term IAQG - non-carcinogenic effects: / Long-term IAQG - carcinogenic effects: 2 µg.m^{-3} for a risk level of 10^{-6} (AFSSET, 2009)	8h OELV: 38.3 mg.m^{-3} STEL: 191.4 mg.m^{-3} (ANSES, 2011 draft)	/
Perchloroethylene	Short term IAQG: 1380 µg.m^{-3} Intermediate IAQG: / Long-term IAQG - non-carcinogenic effects: 250 µg.m^{-3} Long-term IAQG - carcinogenic effects: / (AFSSET, 2010)	8h OELV: 138 mg.m^{-3} STEL: 275 mg.m^{-3} (ANSES, 2010)	ERU by inhalation: $2.6.10^{-7}$ (µg/m^{3})$^{-1}$ (400 µg.m^{-3} for a risk of 10^{-4}, 40 µg.m^{-3} for a risk of 10^{-5}, 4 µg.m^{-3} for a risk of 10^{-6})

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KEYWORDS

Trichloroethylene, perchloroethylene, tetrachloroethylene, toxicity reference value, US EPA, inhalation

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