Opinion
Request No 2016-SA-0116

The Director General

Maisons-Alfort, 31 July 2018

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

on the proposed TRV by the respiratory route for trichloroethylene (CAS No. 79-01-6)

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 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).

Its opinions are published on its website. This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 31 July 2018 shall prevail.

On 18 March 2016, ANSES received a formal request from the Directorate General for Health (DGS) to conduct the following expert appraisal: selection or development of toxicity reference values (TRVs) for trichloroethylene, perchloroethylene (tetrachloroethylene), ammonia and four chloroanilines.

1. BACKGROUND AND PURPOSE OF THE REQUEST

As part of the risk assessments carried out when examining dossiers concerning classified installations for environmental protection (ICPE) or the management of polluted sites and soils, the Regional Health Agencies (ARSs) or consultancies send questions to the DGS about the choice of TRVs for certain substances. This choice is made in accordance with the procedures defined in information note No. DGS/EA1/DGPR/2014/307 of 31 October 2014 on the methods for selecting chemical substances and choosing TRVs in order to conduct health risk assessments in the framework of impact and management studies for polluted sites and soils. In this note, ANSES is designated as the expert agency for selecting and establishing TRVs.

On 18 March 2016, ANSES received a formal request from the DGS to propose acute, subchronic and chronic TRVs by inhalation (with and/or without a threshold) for trichloroethylene, perchloroethylene, ammonia and four chloroanilines. The purpose of this ANSES opinion and the accompanying collective expert appraisal report is to present the proposed TRVs for trichloroethylene (TCE).

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. Currently, the default assumption is to consider that the relationship between exposure (dose) and effect (response) is monotonic. In the current state of knowledge and by default, it is generally considered that for non-carcinogenic effects, toxicity is only expressed above a threshold dose (ANSES, 2017).

In practice, establishing a TRV involves the following steps:

- identifying and analysing the available toxicity data, based on epidemiological and/or experimental studies;
- identifying the target organ(s) and critical effect;
- identifying the assumption according to which it is established: with or without a threshold dose, depending on the substance's mode of action;
- choosing a good quality scientific study generally enabling establishment of a dose-response relationship;
- defining a critical dose for humans or animals from this study and, if required, in the case of a critical dose obtained in animals, adjusting this dose to humans;
- for a threshold TRV, applying uncertainty factors to this critical dose so as to derive a TRV that is applicable to the entire population in question;
- for a non-threshold TRV, conducting a linear extrapolation to the origin in order to determine an excess risk per unit.

TRVs 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 collective expert appraisal was undertaken by the Expert Committee (CES) on "Characterisation of substance hazards and toxicity reference values" until August 2017 and then by the CES on "Health reference values". The methodological and scientific aspects of the work were presented to the CES. The work was adopted by the CES on "Health reference values" at its meeting on 22 June 2018.

ANSES analyses interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals.
The experts' declarations of interests are made public via the ANSES website (www.anses.fr).

3. ANALYSIS AND CONCLUSIONS OF THE CES

Summary of the toxicological data

- Toxicokinetics

TCE is rapidly absorbed in both animals and humans, regardless of the route of exposure. By inhalation, absorption of TCE through the alveolar epithelium is rapid and can be high (28-80%).
TCE is widely distributed throughout the body by the bloodstream, in humans and animals, regardless of the route of absorption. Due to its high lipid-solubility, it is mainly found in adipose tissue, but also in the liver, kidneys, nervous system and cardiovascular system. It crosses the placental barrier and blood-brain barrier and is found in breast milk.

In humans, 40 to 75% of inhaled TCE is metabolised. This takes place rapidly, by two pathways, mainly in the liver:

- via an <u>oxidative metabolism</u> (involvement of cytochromes P450), leading to the formation of majority metabolites: trichloroethanol (TCOH) (free and conjugated in the form of glucuronide) and trichloroacetic acid (TCA). These metabolites are excreted primarily in the urine. These metabolic pathways may infrequently induce the formation of dichloroacetic acid (DCA), monochloroacetic acid, formic acid, carbon monoxide (CO), oxalic acid and N-(hydroxyacetyl)-aminoethanol.
- to a lesser extent by <u>conjugation with glutathione</u>, leading to the formation of S-1,2-dichlorovinyl cysteine (DCVC), which can then be transformed in different ways either into N-acetyl-dichlorovinyl cysteine (NAcDCVC), thioacyl chloride or chlorothioketene.

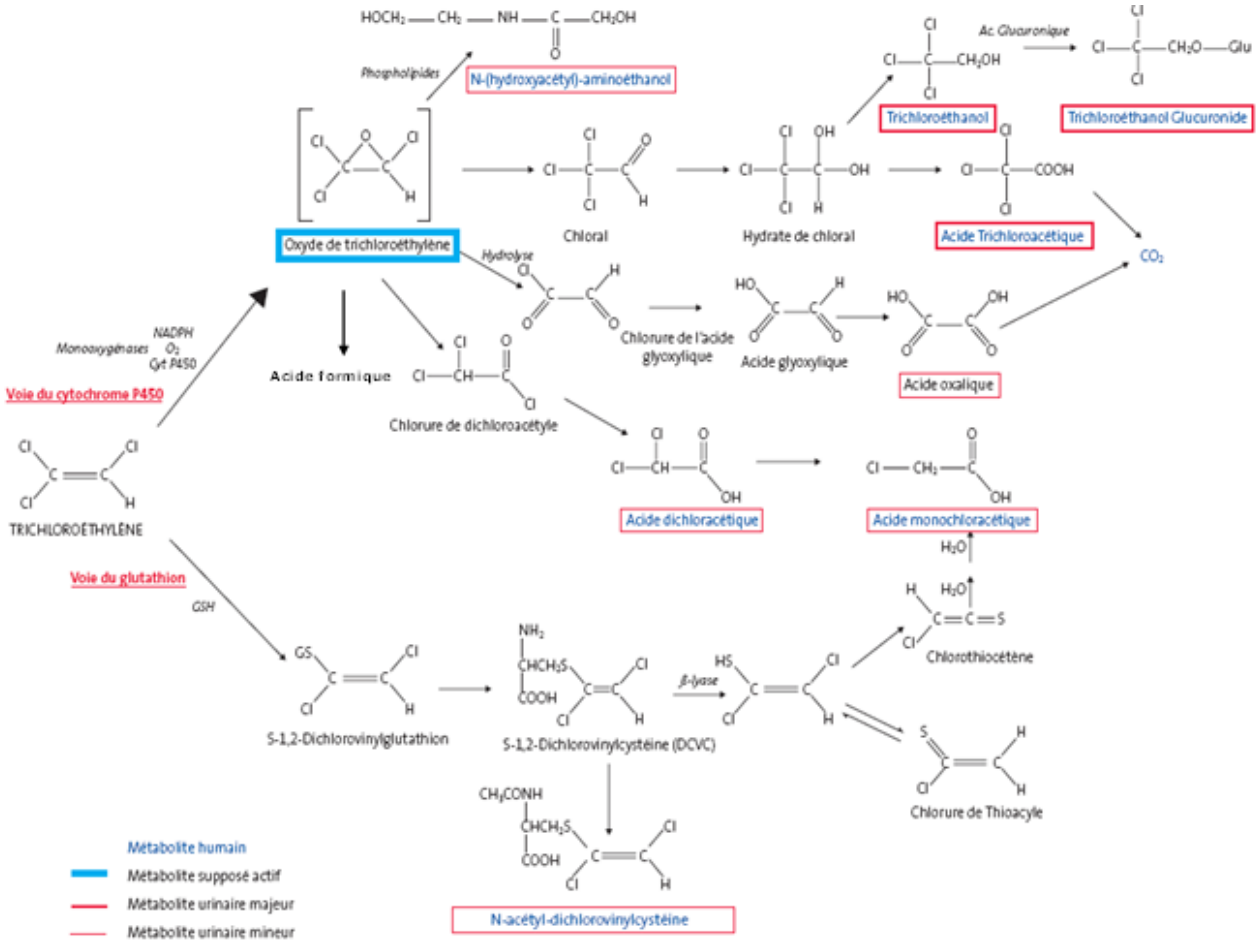

Figure 1: Metabolism of TCE (INRS, 2011)

The elimination pathways of TCE are qualitatively identical in animals and humans, regardless of the route of exposure. Unchanged TCE (10-28% of the dose) and the volatile metabolites (CO_2, CO and TCOH) are eliminated in exhaled air. The main metabolites, TCOH and TCA, are eliminated in the urine (48-85%) and faeces. The minor metabolites (CDA, monochloroacetic acid, N-(hydroxyacetyl)-aminoethanol and NAcDCVC) are eliminated in the urine.

- Acute toxicity

In humans exposed by inhalation to high concentrations of TCE, the main target is the central nervous system (CNS). Indeed, during massive exposure (several hundred ppm), states of CNS excitation followed by depression (narcotique syndrome, then coma) have been observed. Neurological damage, particularly to the optic and trigeminal nerves, has also been reported following accidental exposure and may be attributable to dichloroacetylene, a degradation product of TCE in alkaline media.
In several studies conducted under controlled conditions but with small numbers of healthy volunteers, minor neurological effects (dizziness, fatigue, drowsiness) were observed starting at 200 ppm.
Experimental studies confirm that CNS effects are greater following inhalation exposure to TCE.

- Irritation

TCE is a skin and eye irritant and is classified as such by Regulation (EC) No 1272/2008, the "CLP Regulation" (Skin Irrit. 2, H315; Eye Irrit. 2, H319).

- Subchronic and chronic toxicity

In humans, subchronic and chronic inhalation exposure is mainly responsible for the following effects:

- renal effects (proximal tubule impairments);
- neuropsychic disorders (psychosomatic syndrome with anaesthesia, headache, memory disorders, etc., and vegetative syndrome with perspiration, functional disorders, dizziness, etc.);
- liver effects (hepatic necrosis, hepatic steatosis, cirrhosis, hepatitis, Stevens-Johnson syndrome, jaundice extending to severe liver failure, increased cholesterol and bile acid concentrations);
- immunological effects (immunosuppression, autoimmunity).

In animals, TCE mainly induces:

- neurological effects: decreased attention, disrupted sleep cycles, increased spontaneous motor activity, decreased sleep duration, altered neurotransmitters, hearing loss. The results of animal studies corroborate those carried out in humans with regard to neurological effects;
- renal effects: TCE induces toxicity in the renal tubules;
- liver effects (hepatocellular necrosis, hepatomegaly, fatty infiltration, increased liver weight, centrilobular cell hypertrophy, decreased plasma concentrations of cholesterol and albumin).

- Reprotoxicity and effects on development

In men, several occupational studies have observed impaired spermatozoid morphology, hyperzoospermia, a decrease in libido and impaired fertility. A few effects have been observed in women exposed to TCE or solvent mixtures including TCE, such as increased menstrual cycle disruptions or decreased fertility.

In rodents, several recent studies indicate that exposure to TCE:

- disrupts spermatogenesis (impaired sperm count and spermatozoa morphology and motility);
- causes changes in serum testosterone concentrations;
- causes histopathological lesions in the testicles and epididymis;
- decreases the fertilisation capacity of spermatozoa (*in vitro* fertilisation tests with spermatozoa from exposed males);

- decreases the ability of oocytes to be fertilised in females (*in vitro* fertilisation tests with oocytes from exposed females).

Regarding effects on development, some human studies and animal data suggest the possibility of an increased incidence of heart malformations, without finding sufficient evidence of a link between TCE exposure and these malformations.

- Genotoxicity

Based on the results of genotoxicity tests, TCE has low mutagenic potential *in vitro,* while *in vivo* it appears to be weakly genotoxic for somatic cells.

- Carcinogenicity

Numerous epidemiological studies have shown a link between exposure to TCE and the occurrence of various cancers. TCE is mainly associated with the occurrence of **kidney and hepatobiliary cancers, and non-Hodgkin lymphomas (NHL)** (EC, 2004; NRC, 2006; IRSST, 2010; US EPA, 2011; ATSDR, 2014; IARC, 2014a; NTP, 2015). Excess risks of lung, cervical, breast, prostate and oesophageal cancer have also been observed in a few studies. However, according to the IARC, these studies are not sufficient to conduct an assessment of these cancers (IARC, 2014a).

In 2012, the **IARC** classified TCE in **Group 1** (carcinogenic to humans) based on epidemiological evidence supported by mechanistic data (IARC, 2014a). Case-control studies have provided conclusive evidence of an association between exposure to TCE and the risk of renal adenocarcinoma (Charbotel *et al.,* 2006; Moore *et al.,* 2010). Cohort studies and a meta-analysis have reported a modest increase in the relative risk of kidney cancer (Boice *et al.,* 2006; Zhao *et al.*, 2005; Raaschou-Nielsen *et al.*, 2001; Scott and Jinot, 2011). In humans, the level of evidence is limited for the association between non-Hodgkin lymphoma, liver cancer and exposure to TCE. TCE is a multi-site carcinogen in rats and mice exposed by the oral and respiratory routes: an increase in hepatic, renal, pulmonary, testicular and haematopoietic tumours was observed in several studies.
In 2001, the **European Commission** classified TCE in **Group 2, which became 1B under Regulation No 1272/2008 (the CLP Regulation)**, for kidney cancers and non-Hodgkin lymphomas, based on studies showing kidney cancers in rats, also observed in epidemiological studies showing an association between exposure to TCE and kidney cancer (Henschler *et al.*, 1995; Vamvakas *et al.*, 1998; Blair *et al.*, 1998) and non-Hodgkin lymphoma (Axelson *et al.*, 1994; Anttila *et al.*, 1995; Blair *et al.*, 1998; Boice *et al.*, 1999).

In animals, only the studies by Henschler *et al.* (1980), Fukuda *et al.* (1983) and Maltoni *et al.* (1986,1988), available in the literature, studied carcinogenicity for the respiratory route. They show lung, liver and lymphoma tumours in mice and renal and Leydig cell tumours in Sprague-Dawley rats.

Acute TRV

- Choice of the critical effect

In humans, short-term inhalation exposure to TCE causes effects on the **central nervous system (CNS)**. Indeed, during high exposure (several hundred ppm), a state of CNS excitation followed by depression (narcotic syndrome, then coma) has been observed. Several studies conducted in volunteers under controlled conditions observed minor neurological effects (dizziness, fatigue, drowsiness) from 200 ppm (Stewart *et al.,* 1970 and 1974; Vernon and Ferguson, 1969 and 1970). No significant signs of CNS depression were observed at this concentration or at lower concentrations, except in the study by Salvini *et al.* (1971), which observed impaired performance

at 110 ppm. The effects observed at the lowest concentrations, starting at 27 ppm, were subjective effects (drowsiness) reported by volunteers, without any significant change in neurobehavioural tests (Nomiyama and Nomiyama, 1977).

Numerous studies in animals have also noted the **neurotoxicity** of TCE. These effects appear particularly early, with NOAECs[1] ranging from 120 to 2400 ppm. It is often difficult to conclude as to their significance or harmfulness, as in many cases the studies are old and were carried out with different protocols from those that would be used today.

A few reports have observed hepatic and renal effects, but these were more sporadic, and at high concentrations (2800 ppm) (EC, 2004; US EPA, 2008 and 2011; ATSDR, 2014).

In conclusion, the critical effect retained was the effect on the central nervous system (CNS).

- Analysis of the existing TRVs

As no acute TRV by inhalation is available, the CES opted to establish one.

- Establishment

The CNS effects were observed in both animal and human studies.
In animals, the available studies cannot be used to establish a TRV (inadequate range of doses tested, difficulty interpreting results, poor description of experimental protocols, etc.).
The human studies showing neurotoxic effects following short-term exposure are accident reports and controlled studies. The studies tend to indicate an absence of acute effects in humans at concentrations below 100 ppm and for exposure of 1 to 6 hours. It is not possible to use accident reports – which often involve a single individual exposed to very high levels – to establish a TRV. There are a few controlled studies in humans that mainly explored effects on the central nervous system. The study by Winneke *et al.* (1982) is one of the only ones to show objective effects (change in auditory evoked potentials) at a relatively low concentration (50 ppm). It is supported by the study by Salvini *et al.* (1971), in which a decrease in performance in all neurobehavioural tests was observed in students and workers at 110 ppm. Nevertheless, these studies are old and suffer from methodological limitations such as a small number of subjects, lack of a control group, a small number of doses tested, etc., making it difficult to exploit them for the establishment of an acute TRV.

The CES was unable to identify quantitative data in the literature on short-term inhalation exposure of sufficient quality to establish an acute TRV, despite the existence of neurological effects. The CES is therefore not proposing an acute TRV.

[1] No observed adverse effect level

- **Subchronic and chronic TRVs**
  - Choice of the critical effect

TCE can exert effects on the **immune system**, both in humans and animals, which will affect not only immunity cells/tissues but also other tissues/organs, thus causing systemic effects.
The immunosuppressive effects of TCE, observed in humans and animals, have been demonstrated by the many different impacts of TCE on the immune system. However, the consequences on susceptibility to viral and bacterial infections or on the occurrence of tumours of viral origin are unclear. These effects are therefore not relevant for consideration as critical effects.
Autoimmune effects are also observed in humans and animals. An association between exposure to TCE and occurrence of scleroderma has been found in individuals with production of antinuclear autoantibodies. These individuals had severe and widespread skin damage accompanied by systemic problems. The effects observed here are indeed adverse effects. However, studies in humans are often carried out in the context of multiple exposures of occupational origin, which complicates any interpretation as to the direct role of TCE. The animal studies were conducted on rodent models liable to develop autoimmune diseases and the biological effects measured in these studies often remain sporadic, meaning that these studies cannot be used as key studies. Therefore, these effects cannot be selected as the critical effects.

The **neurological effects** observed were a nerve conduction disorder on the trigeminal nerve and impaired vestibular function. Impaired vestibular function cannot be retained, despite the quality of the studies describing it, because these were mainly acute toxicity studies with high doses (several thousand ppm).
Nerve conduction disorders have been observed in both humans and animals. The studies in humans are also of good quality, but the confounding factors (exposure to other solvents), or lack of exposure data are limiting factors. One good-quality animal study (Arito *et al.*, 1994) does not show a dose-response relationship for an exposure duration of 6 weeks and cannot be used to establish a chronic TRV.

The **renal effects**, mainly at the tubular level, also occur at low doses. In humans, studies show changes in urinary markers, particularly tubular impairment. Animal inhalation studies show effects such as increased kidney weights in rats and mice from 75 ppm (30-day study, Kjellstrand *et al.*, 1983b) and renal tubular cell karyocytomegaly from 300 ppm in male rats exposed for 104 weeks (Maltoni *et al.*, 1988). The same renal tubular lesions as described by Maltoni *et al.* (1988) in male rats (cytomegaly and karyomegaly) were also observed, but at higher doses, in female rats and in mice of both sexes following oral exposure (NCI, 1976; NTP, 1988 and 1990).
The main mechanism of TCE nephrotoxicity is related to metabolites derived from the glutathione conjugation pathway, such as DCVC and its metabolites. Based on predictions from PBPK models in mice, rats and humans, it seems that TCE conjugation by glutathione is greater in humans than in animals, suggesting higher nephrotoxicity in humans compared to rodents (US EPA, 2011; ATSDR, 2014).

The CES selected the renal effect as the critical effect for establishing chronic and subchronic TRVs because of a well-identified nephrotoxicity mechanism, the appearance of this effect at low doses in animals, the plausibility of its transposition to humans and the existence of a good-quality study (Maltoni *et al.*) supported by several other studies in two animal species (rat and mouse).

  - Analysis of the existing chronic TRVs

Four organisations have proposed chronic threshold TRVs for exposure by inhalation: RIVM (2001), OEHHA (2003), US EPA (2011) and ATSDR (2014). The TRVs from the RIVM and OEHHA were not selected because:

- they are not based on renal effects;
- they are based on effects that according to the RIVM may indicate adaptation to TCE exposure and not an adverse effect (change in the ratio between liver weight and body weight), and on subjective effects that may correspond either to subacute effects or to chronic effects for the OEHHA (non-specific and non-objective mild neurological symptoms).

In 2011, the US EPA proposed a "reference concentration" (RfC). This TRV was adopted by the ATSDR in its working document (ATSDR, 2014). The establishment method used by the US EPA to derive its RfC consisted in calculating candidate RfCs a priori and then searching for the study corresponding to the lowest RfC. The approach adopted does not follow the US EPA's methodological guidelines for establishing TRVs and is not in line with the approach proposed by ANSES for developing TRVs (ANSES, 2017). Because of this atypical establishment method, the key studies were chosen not for their quality or the relevance of the critical effects, but based on the lowest RfC value obtained. The US EPA ultimately established its RfC by averaging two candidate RfCs established from two different studies, of limited quality and showing different effects. The CES on "Health Reference Values" considers that averaging studies of limited quality does not help obtain a better quality final value.
Given that the US EPA's establishment method was not validated by the CES on "Health Reference Values" and that the quality of the selected studies was limited, the US EPA's RfC was not selected, and **the CES therefore opted to establish a chronic TRV by inhalation**.

- Establishment of a chronic TRV
  - Choice of the key study

The human studies on renal toxicity suffer from many limitations, including poor exposure assessment (few measurements taken and protocols lacking detail, co-exposure factors unknown), a very wide exposure interval, a small number of subjects, a possible absence of control groups, difficulties interpreting the results, etc. The CES therefore deemed that they were of insufficient quality to derive a TRV.

Only two studies in animals (rats and mice) exposed by the respiratory route showed renal effects following subchronic or chronic exposure by inhalation: Kjellstrand *et al.* (1983b) and Maltoni *et al.* (1988). After analysing the animal studies, the CES selected the study by Maltoni *et al.* (1988). The authors observed renal tubular cell karyocytomegaly at 300 and 600 ppm in male rats (significant results for both doses, $p < 0.01$). This effect was not observed in historical controls or in rats exposed for 8 weeks. The authors indicate that this renal impairment may be considered a precursor effect of kidney cancer, and has been observed in rats with renal adenocarcinoma.

Although observed only in male rats, the CES therefore retained the renal tubular cell karyocytomegaly found in the study by Maltoni *et al.* (1988) at 300 ppm.

  - Choice of the critical dose

The data from the Maltoni *et al.* study demonstrate a dose-response relationship between karyocytomegaly and exposure to TCE. This was modelled with the software proposed by EFSA (EFSA, 2017), which uses the PROAST software (PROAST version 65.7) developed by the RIVM to establish a "Benchmark Concentration" (BMC).
The aim of this approach is to estimate the concentration 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) and corresponds to an excess risk of 10% (BMR recommended by ANSES and EFSA for quantal data). The confidence level associated with the BMCL is 90%.

EFSA proposes using the "model averaging" approach (based on the publication by Wheeler and Bailer, 2008), which takes into consideration all the models describing the experimental data and weights them according to their Akaike information criterion (AIK). Models that best describe the data have a higher weighting: the lower the AIC the better the fit is considered to be.

The calculated critical concentration is **$BMC_{10\%}L_{90\%}$ = 238 ppm**

- Temporal adjustment

Rats were exposed for 5 days per week and 7 hours per day for 104 days (i.e. a lifetime for rodents). To take the discontinuity of the exposure into account, a time adjustment was made: **$BMC_{10\%}L_{90\%\ ADJ}$** = 238 x 5/7 days x 7/24 hours = **49.6 ppm**

- Dose adjustment

Several PBPK models have been developed to model the fate of TCE in the body in rats and humans for different routes of exposure. The PBPK model of Covington *et al*. (2006) was used.
Using the PBPK model enabled the external exposure dose in animals to be converted to an internal dose in animals. In this case, it is the blood concentration of S-1,2-dichlorovinyl cysteine (DCVC) expressed in mg/L, as DCVC is probably the metabolite responsible for the nephrotoxic effects. It is assumed that the internal concentration in rats is equivalent to the internal concentration in humans. Conversely, the human PBPK model will convert the internal exposure dose into an external dose in humans that will constitute the human equivalent $BMC_{10\%}L_{90\%\ ADJ}$ ($BMC_{10\%}L_{90\%\ HEC\ ADJ}$).
Thus, for a calculated critical concentration ($BMC_{10\%}L_{90\%\ ADJ}$ = 49.6 ppm) in rats, the associated blood concentration of DCVC is $9.4 \cdot 10^{-4}$ mg/L in rats. This internal dose corresponds to an external TCE exposure concentration of 43.7 ppm in humans (human equivalent concentration = HEC). Thus, daily exposure of rats to a TCE concentration of 49.6 ppm would correspond to an exposure concentration in humans of 43.7 ppm.
The CES therefore selected the $BMC_{10\%}L_{90\%\ HEC\ ADJ}$ of 43.7 ppm based on DCVC.

- Choice of uncertainty factors

The TRV was calculated from the $BMC_{10\%}L_{90\%\ HEC\ ADJ}$ using the following uncertainty factors, leading to an **overall uncertainty factor of 75:**

- Inter-species variability (UF_A): 2.5. The dose adjustment made enabled a human equivalent concentration to be calculated, using the PBPK model of Covington *et al.* (2006). To account for toxicodynamic variability and residual uncertainties, an additional uncertainty factor was set at 2.5 according to WHO recommendations (WHO, 2005) and based on ANSES practices (ANSES, 2017).

- Inter-individual variability (UF_H): 10. Because there were no scientific data available to reduce the default value, the value of 10 was used.

- Inadequacy of the data (UF_D): 3. Although the main mechanism of TCE's nephrotoxicity is related to metabolites from the glutathione conjugation pathway, such as DCVC and its metabolites, doubts remain as to the existence of a mechanism of action involving formic acid due to the formation of TCOH or TCA from TCE. Thus, as a precaution, the CES proposes applying a UF_D of 3.

- Proposed chronic TRV and confidence level

TRV = $BMC_{10\%}L_{90\%\ HEC}$ / UF = **0.58 ppm, or 3.2 mg·m^{-3}**

The overall confidence level **moderate/high** was assigned to this TRV based on the following four criteria: nature and quality of the data (high), choice of the critical effect and the mode of action (moderate), choice of the key study (high) and choice of the critical dose (moderate).

- Proposed subchronic TRV and confidence level

Only the ATSDR proposed a subchronic TRV, namely the US EPA's chronic TRV (which was not retained by the CES, see the section entitled "Analysis of the existing chronic TRVs").

The results of the kinetic modelling of TCE indicate that the blood DCVC concentration reaches an equilibrium state after around two weeks of continuous exposure to the $BMCL_{ADJ\ HEC}$ and that a longer exposure time does not a priori generate a higher blood DCVC concentration. This result supports the ATSDR's recent approach to adopt the chronic TRV as a subchronic TRV. Indeed, continuous exposure to the $BMCL_{ADJ\ HEC}$ between 21 days and 90 days generates a stable blood concentration of DCVC in humans. Intensified renal effects are therefore unlikely.

The CES experts therefore selected the chronic TRV as the subchronic TRV, based on the study by Maltoni *et al.* (1988), with renal effects (renal tubular cell karyocytomegaly) as the critical effect.
The overall confidence level assigned to this TRV is the same as that of the chronic TRV, i.e. **moderate/high**.

- **Carcinogenic TRV**

  - Choice of the critical effect

In humans, TCE is associated with the occurrence of various cancers, mainly kidney and hepatobiliary cancers, and non-Hodgkin lymphomas (NHL).
A significant association between the incidence of kidney cancer and TCE exposure has been observed in numerous epidemiological studies, whether in occupational cohort studies (Zhao *et al.,* 2005; Raaschou-Nielsen *et al*., 2003; Henschler *et al*., 1995), case-control studies (Moore *et al*., 2011; Charbotel *et al*., 2006; Brüning *et al*., 2003; Pesch *et al.,* 2000; Dosemeci *et al.,* 1999; Vamvakas *et al.,* 1998) or meta-analyses (Karami *et al.*, 2012; Scott and Jinot, 2011; Kelsch et al., 2010).
The IARC (2012) and NTP (2015) concluded that the level of evidence regarding the association between NHL and TCE exposure was limited. Epidemiological studies also provide limited evidence of a causal association between TCE exposure and hepatobiliary cancers.

In animals, studies show that TCE is a multi-site carcinogen (increased tumours of the liver, kidneys, lungs and testicles).

The CES therefore selected kidney cancer as the critical effect.

  - Choice of the assumption according to which it is established

Some authors believe that there is a threshold below which no renal impairment is expected. However, the experts emphasise firstly the complexity and existing uncertainties regarding TCE's genotoxic mechanism of action, and secondly the formation of proven genotoxic metabolites (mainly in the kidney). Some studies underline the current difficulty of determining which metabolites are responsible for the genotoxic effects of TCE and their relevance in humans (Caldwell and Keshava, 2006). All these data therefore demand the exercise of great caution and it is not possible to draw any conclusions as to the presence of an exposure dose below which there is no genotoxic effect.

In 2014, ECHA produced a synopsis of the approaches used by international institutions and agencies (WHO, EU, AGS, SCOEL, US EPA, ANSES[2]) to propose a carcinogenic TRV or DNEL[3]. Considering the formation of several genotoxic metabolites and the lack of an adequate explanation for a non-genotoxic mode of action of TCE, all these expert groups, with the exception of the SCOEL (2009), believed that a no-threshold approach was the most appropriate for estimating the cancer risk.
The CES therefore adopted a **non-threshold approach** for establishing a carcinogenic TRV by the respiratory route for TCE.

The AGS proposed considering a non-threshold but sublinear dose-response relationship for the risk of kidney cancers induced by TCE, based on mechanistic data. Indeed, high occupational exposure including exposure peaks leads to cytotoxic effects responsible for renal tubular lesions that may contribute decisively to the onset of cancer by initiating cell proliferation. The risk of kidney cancer is considered to be lower below exposure levels resulting in a cytotoxic response. A linear relationship would therefore overestimate the risk for low levels of exposure.
Although the AGS approach has the advantage of staying close to epidemiological reality and takes mechanistic hypotheses into account, the studies used to justify the inflection point of the curve have limitations, and their use is questionable. For this reason, **the CES used a linear non-threshold approach to establish its carcinogenic TRV, which has the advantage of being more protective.**

- Analysis of the existing TRVs

Five agencies proposed carcinogenic TRVs: Health Canada (1993), OEHHA (2009), WHO (2000), US EPA (2011) and ECHA (2014).
The TRVs proposed by Health Canada, OEHHA and the WHO are based on tumours observed in animals (hepatocellular or pulmonary tumours in male mice and pulmonary tumours and malignant lymphomas in female mice, Leydig cell tumours in rats). These TRVs were not retained by the CES on "Health Reference Values" because:

- they are not based on kidney cancer;
- the mechanism of action of the hepatic and pulmonary tumours observed in mice is not transposable to humans;
- regarding the lymphomas observed in the study by Henschler *et al*. (1980), the methodological limitations (two doses tested, insufficient number of animals) make it difficult to interpret the results;
- the Leydig cell tumours in the study by Maltoni *et al.* (1988) are not relevant effects for establishing the TRV because they are species and genus specific. Indeed, no testicular cancer in humans has been found (US EPA, 2011). However, epidemiological studies have shown prostate cancer in men, and breast and uterine cancer in women. In animals, experimental studies have shown prostate and testicular cancers in males, and cancers of the uterus, ovaries, mammary glands and genital tract in females. Only the study by Maltoni *et al.* (1988) showed an increase in Leydig cell tumours in rats following respiratory exposure for 104 weeks.

In 2014, ECHA proposed a DNEL for the general population based on the AGS's approach. The AGS's objective was to propose an occupational exposure limit value for TCE as an example in a methodological guide. The AGS's approach has the advantage of staying close to epidemiological reality and taking mechanistic hypotheses into account. However, ECHA's DNEL was not chosen

[2] WHO: World Health Organisation; EU: European Union; SCOEL: Scientific Committee on Occupational Exposure Limits; AGS: *Ausschuss für Gefahrstoffe* (German Committee on Hazardous Substances); US EPA: United States Environmental Protection Agency

[3] Derived no effect level

by the CES on "Health Reference Values" because the model proposed by the AGS has many limitations:

- The study used by the AGS to estimate the exposure level (Roller, 2005) was not published, resulting in a lack of transparency in understanding the approach followed to calculate the 40-year cumulative dose of 3000 ppm-years, used to calculate the starting point at 75 ppm.
- The studies used, by Selden *et al.* (1993) and Green *et al.* (2004), to justify the inflection point of the curve at 6 ppm, have several limitations (doubts by the authors about the significance of the results, no analysis of co-exposures, inadequate description of exposure levels, financial support by an industry association).
- Their use by the AGS is questionable.
    - In their study, Green *et al.* (2004) considered that the increase in N-acetyl-β-D-glucosaminidase (NAG) and in urinary albumin, although significant, does not constitute an adverse effect, and therefore concluded as to an absence of effect below 250 ppm. On the contrary, the AGS considers that the increase in these markers, although not correlated to urinary TCA levels and therefore to TCE exposure, constitutes a critical effect.
    - NAG is not the most specific and earliest renal marker that could be chosen, as noted by Green *et al*. As for urinary albumin levels, this does not directly indicate proximal tubular impairment.
    - Considering this critical effect, the AGS took the average exposure of exposed workers in the study by Green *et al.* (32 ppm) as the LOAEC, which poorly reflects the absence of a dose-response relationship between 0.5 and 252 ppm.
    - In the study by Selden *et al.*, the AGS only considered the 23 individuals with the lowest exposure levels out of the 29 exposed, without providing any explanation for the exclusion of the six highest values. It was noted that drawing on the same study, the SCOEL selected the 25 highest values.

The US EPA's excess risk per unit (ERU) by the respiratory route, based on renal cancers observed in the study by Charbotel and adjusted for the potential risk of multi-site tumours (NHL, liver tumours), had been analysed by the experts of the TRV WG and the CES on "Assessment of the risks related to chemical substances" (2010-2013 mandate) in 2013, and had not been selected at that time (ANSES, 2013). As part of its recent work, the CES on "Health reference values" re-analysed this ERU by inhalation, and the source data. It concluded that the main limitations identified in 2013 could be removed. In particular, the fact that the ERU is based on an exposure estimate representing aggregate exposure (respiratory route and some dermal penetration due to the deposition of solvent vapours on the skin) means that this ERU cannot be rejected (see Table 46 of the report). However, the CES did not retain the combined ERU for kidney cancer, non-Hodgkin lymphoma and hepatobiliary cancers proposed by the US EPA, insofar as the only tumours for which there is sufficient epidemiological evidence of an excess risk associated with exposure to TCE and for which the dose-response relationship is well characterised are renal tumours. **The CES therefore recommends the use of the US EPA's TRV based on kidney cancers.**

The US EPA also recommends the use of an uncertainty factor (age-dependent adjustment factor - ADAF) specific to children when calculating the risk. The CES considers that there is no evidence that children are particularly sensitive to the carcinogenic effects of TCE. Therefore, **no child-specific uncertainty factors need to be used**.

- Proposed carcinogenic TRV and confidence level

ERU kidney cancer = $5.49 \cdot 10^{3}$ $(ppm)^{-1}$, or 10^{-6} $(\mu g \cdot m^{-3})^{-1}$

The overall confidence level **moderate/high** was assigned to this TRV based on the following four criteria: nature and quality of the data (high), choice of the critical effect and the mode of action (high), choice of the key study (moderate) and choice of the critical dose (high).

The report was validated by the majority of the experts present (14 out of the 15 experts present). One expert chose to abstain: "The expert appraisal conducted by the CES is comprehensive and of good quality; however, it would have been interesting to be able to propose a usable value for acute exposure. The approach was discussed during the various stages of the creation of the dossier, but no consensus was reached because of the weaknesses of the available data."

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions and recommendations of the CES on "Health reference values" on the proposed toxicity reference values by the respiratory route for trichloroethylene.

As a reminder, when dealing with TRVs and in line with the scenarios generally taken into account when assessing health risks in humans, ANSES distinguishes between three types of exposure duration:

- Acute exposure, from 1 to 14 days;
- Subchronic exposure, from 15 to 364 days;
- Chronic exposure, for 365 or more days.

Concerning the non-threshold carcinogenic TRV, the CES wishes to draw attention to the fact that the US EPA's ERU was retained, without further adjustment for the occurrence of other cancers, and without any recommendations to use a child-specific uncertainty factor (ADAF) for the risk calculation.

Table 1: TRVs by the respiratory route for trichloroethylene

Type of TRV	Organisation	Critical effect (key study)	Critical concentration	UF	TRV
Acute TRV	The CES was unable to identify quantitative data in the literature on short-term inhalation exposure of sufficient quality to establish an acute TRV, despite the existence of neurological effects. The CES is therefore not proposing an acute TRV.				
Subchronic TRV	ANSES	Adoption of the chronic TRV			3.2 $mg \cdot m^{-3}$ (0.58 ppm) Confidence level moderate/high
Chronic TRV	ANSES	Renal effect observed in male rats Maltoni *et al.* (1988)	NOAEC = 100 ppm LOAEC = 300 ppm $BMC_{10\%}L_{90\%}$ = 238 ppm <u>Temporal adjustment</u> $BMC_{10\%}L_{90\%\ ADJ}$ = 238 x 5/7 x 7/24 = 49.6 ppm <u>Allometric adjustment</u> $BMC_{10\%}L_{90\%\ ADJ}$ = 43.7 ppm (DCVC)	75 UF_A = 2.5 UF_H = 10 UF_D = 3	3.2 $mg \cdot m^{-3}$ (0.58 ppm) Confidence level moderate/high
Carcinogenic TRV	US EPA	Renal carcinoma Charbotel *et al.* (2006)	Linear regression using US survival tables, estimation of "background" incidence rates of kidney cancer, and conversion of data on occupational exposure into environmental exposure → POD = LEC_{01} = 1.82 ppm Linear extrapolation to the origin → $ERU_{kidney\ cancer} = 5.49 \cdot 10^3$ $(ppm)^{-1}$		**10^{-6} $(\mu g \cdot m^{-3})^{-1}$** $5.49 \cdot 10^3$ $(ppm)^{-1}$ Concentrations associated with several levels of risk: **10^{-4}: 100 $\mu g \cdot m^{-3}$** **10^{-5}: 10 $\mu g \cdot m^{-3}$** **10^{-6}: 1 $\mu g \cdot m^{-3}$** Confidence level moderate/high

Dr Roger Genet

KEYWORDS

Valeur toxicologique de référence, VTR, trichloroéthylène, 79-01-6, inhalation, aiguë, subchronique, chronique

Toxicity reference value, TRV, trichloroethylene, 79-01-6, inhalation, acute, subchronic, chronic