The Director General

Maisons-Alfort, 26 October 2017

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

on the development of TRVs by the respiratory route for toluene (CAS no 108-88-3)

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 26 October 2017 shall prevail.

In 2017, ANSES issued an internal request to formulate toxicity reference values (TRVs) for toluene, in connection with the internal request relating to the establishment of indoor air quality guidelines (IAQGs).

1. BACKGROUND AND PURPOSE OF THE REQUEST

Since 2004, ANSES has been formulating indoor air quality guidelines (IAQGs) for pollutants of interest in indoor air, including toluene, which is frequently screened for in measurement campaigns in France. To do this, a toxicological profile was prepared and IAQGs were proposed. Because the approach for establishing IAQGs is similar to that for TRVs, ANSES decided to capitalise on this work by also proposing TRVs by inhalation for this substance and revising its chronic TRV by inhalation (ANSES, 2011).

Toluene has been the subject of several studies by ANSES, including the development of a reprotoxic TRV, an Occupational Exposure Limit (OEL) value and a TRV for chronic effects as well as the drafting of a toxicological profile in the framework of the assessment of the risks for reprotoxic substances and/or endocrine disruptors (AFSSET, 2008; AFSSET, 2009; ANSES, 2011; ANSES, 2014a,b).

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 hypothesis is to consider a monotonic relationship between exposure, or dose, and effect, or response. 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, 2016). There are two types of TRV: with and without a threshold. In the present case, toluene can be considered as a substance whose effects are expressed above a threshold dose.

In practice, establishing a threshold 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 establishment of a critical dose from experimental doses and/or epidemiological data;
- adjustments and the application of uncertainty factors to the critical dose to take uncertainties into account.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with the French standard NF X 50-110 "Quality in Expertise – General Requirements of Competence for Expert Appraisals (May 2003)".

The expert appraisal falls within the sphere of competence of the Expert Committee (CES) on "Characterisation of substance hazards and toxicity reference values" (hereinafter referred to as the CES "Substances"). The methodological and scientific aspects of the Group's work were submitted to the CES between 14 January 2016 and 22 June 2017. It was adopted by the CES "Substances" at its meeting on 22 June 2017.

The work was also submitted regularly to the CES on "Assessment of the risks related to air environments", which validates work on IAQGs.

ANSES analyses the links of interest declared by the experts prior to their appointment and throughout the work, in order to avoid potential conflicts of interest with regard to the matters dealt with as part of the expert appraisal.

The experts' declarations of interests are made public *via* the ANSES website (www.anses.fr).

3. THE CES'S ANALYSIS AND CONCLUSIONS

Sources of toluene

Toluene is used in the following products: fuels, grease and lubricants, antifreeze products, biocides (disinfectants and antiparasitics for animals), products for the treatment of non-metallic surfaces, of leather and textiles, inks, polishes and wax dyes, glues and sealants.

Toluene has also been identified as being potentially emitted by products used in construction and decoration. The use of some consumer products (glues, paints, adhesives, etc.) can be a source of toluene emissions in the indoor air of buildings.

Toxicological profile

The toxicological profile was prepared primarily on the basis of the reports by the ANSES studies previously mentioned (AFSSET, 2008; AFSSET, 2009; ANSES, 2011; ANSES, 2014a,b). An additional literature search was also conducted on the period [2011-2016][1] to identify relevant

[1] Detailed information about this additional literature search is available in the Annex of the TRV report accompanying this Opinion.

recent data concerning the health effects of toluene by inhalation that would not have been taken into account in the reports mentioned above.

- Toxicokinetics

In humans, toluene is readily absorbed by inhalation. In animals, the rate of absorption by inhalation (about 90%) varies with the level of ventilation. Toluene is distributed in the adipose tissue but is also found in many organs. In humans, as in animals, toluene is predominantly eliminated in urine, primarily in the form of metabolites, and in a lesser proportion by the pulmonary route in unchanged form. Approximately 7 to 20% of inhaled toluene is thus eliminated unchanged in expired air, while 60 to 80% is metabolised in the liver to form benzyl alcohol, and then benzoic acid followed by conjugation with glycine giving hippuric acid. Hippuric acid is eliminated in urine, 65% in the first 4 hours and 80% within 20 hours. Its elimination is total within 24 hours (its elimination half-life is about 3 hours).

A PBPK model[2] was recently published for toluene (Marchand *et al.,* 2015). The body is modelled in five compartments (lung tissue, richly perfused tissues, weakly perfused tissues, adipose tissue and liver tissue) for which the accumulation of toluene can be assessed by blood flow (partition coefficient).

- Acute toxicity

In animals as in humans, toluene has relatively low acute toxicity, irrespective of the route of exposure. Effects (inebriation, consciousness disorders) by its action on the central nervous system (CNS) and eye irritation effects have been observed.
Effects have been reported for acute exposure to toluene at a concentration of 100 ppm (380 $mg.m^{-3}$). These included headache, dizziness, a feeling of intoxication, irritation of the upper respiratory tract and the eyes, fatigue, drowsiness, and irritation of the throat. Psychometric tests highlighted a decrease in manual dexterity; a decrease in colour discrimination and in visual perception has also been observed at a concentration of 100 ppm.
A study using a controlled exposure chamber showed the effects of exposure to toluene at 200 ppm (760 $mg.m^{-3}$) on visual discrimination, more particularly in the presence of a distractor related to the orientation of the subject studied, based on behavioural tests (Kobald *et al*., 2015).
In animals, a lethal concentration LC_{50} of 5320 ppm (2022 $mg.m^{-3}$) has been reported in mice (ATSDR, 2015).

- Chronic toxicity

Neurological effects have been demonstrated in humans and rodents by the respiratory route during chronic exposure. Neurological effects are reported at dose levels lower than for the effects on fertility or development. In the workplace, severe neurotoxic effects characterised by disorders in behaviour, hearing and colour vision have been observed (Zavalic *et al*., 1998).

<u>Ototoxicity</u>
A limited number of studies on workers suggest that a loss of hearing may appear in concentrations higher than 50 ppm (Chang *et al*., 2006). The risk of hearing loss in low frequencies among workers exposed to noise and/or to toluene was demonstrated among workers co-exposed to noise and to toluene compared to a group exposed to noise alone. The authors add that these losses were comparable in three sub-groups (noise + toluene) exposed to the same level of noise but at concentrations of 33, 107.6 and 164.6 ppm.

<u>Alteration of colour vision</u>
Impaired colour vision is regarded as one of the possible neurological effects of toluene. It can be detected using tests for colour vision such as the Lanthony D-15 test, which provides a Colour Confusion Index (CCI). Several studies on colour vision among workers have shown that CCI

[2] PBPK model: Physiological-Based PharmacoKinetic model

increased significantly among employees exposed to toluene at an average concentration of 32 ppm (i.e. 123 $mg.m^{-3}$) (Zavalic *et al*., 1998; Cavalleri *et al*., 2000). Other studies show alterations in colour vision among workers chronically exposed to mixtures of solvents including toluene; however, the concomitant exposure to several solvents makes it difficult to interpret the results and conclude as to the implication of toluene in the occurrence of these effects (Campagna *et al.,* 2001).

Neurobehavioural toxicity

Neurobehavioural effects (reduced psychomotor performance or vigilance) are observed in controlled studies in healthy volunteers for acute exposure to toluene at concentrations above 40 ppm (Andersen *et al*., 1983; Baelum *et al*., 1985) and in animals for repeated exposure to concentrations greater than 1000 ppm (Bowen *et al*., 2007; Duncan *et al*., 2012).
Among workers, the majority of studies relating to chronic exposure to toluene at concentrations lower than 50 ppm show no degradation of performance in neuropsychological or psychomotor tests (Neubert *et al*., 2001; Gericke *et al*., 2001; Seeber *et al*., 2004 and 2005). For exposures of the order of 70-100 ppm, the majority of studies on workers show significant alterations at the neurobehavioural and psychomotor levels (Foo *et al*., 1990; Kang *et al*., 2005).

In conclusion, the experts of the CES consider that among the neurobehavioural effects studied in humans and animals, the neurosensory effects (visual discrimination, colour vision) associated with chronic exposure are the most sensitive.

- Toxicity to reproduction and development

In humans, effects on reproduction have been reported but at high levels of exposure or in studies with a methodological bias that limits their interpretation (Ng *et al*., 1992).
In animals, exposure to toluene by inhalation has reprotoxic effects, on both fertility and development. It has also been shown that toluene could lead to a decrease in plasma testosterone levels in male foetuses (Tsukahara *et al*., 2009).

- Genotoxicity

Most *in vivo* and *in vitro* genotoxicity studies show no genotoxic effects for toluene.
In vitro, toluene did not induce gene mutation of bacteria in several studies with or without metabolic activation, sister chromatid exchange, chromosomal aberration on human lymphocytes with or without metabolic activation, and micronuclei on human lymphocytes, again with or without metabolic activation.
According to the International Agency for Research on Cancer (IARC), the US EPA[3] and the NTP[4], toluene is not mutagenic or genotoxic in animals. The results on human cells are equivocal (NTP, 1990; IARC, 1999).
Toluene has been examined by the European Union, which has not classified it as a genotoxic compound.

In conclusion, the experts of the CES consider on the basis of the *in vivo* and *in vitro* studies that toluene is not genotoxic in the current state of knowledge.

- Carcinogenicity

The IARC (1999) has not classified toluene as a carcinogen (not classifiable as to its carcinogenicity to humans).

Toluene has also been classified by the US EPA (Group D: *not classifiable as to human carcinogenicity*) (US EPA, 2005). Toluene has not been classified as a carcinogen by the

[3] US EPA: United States Environmental Protection Agency
[4] NTP: National Toxicology Program

European Union according to its harmonised classification under Regulation (EC) No 1272/2008, known as the CLP Regulation.

In conclusion, the experts of the CES consider that toluene is not carcinogenic in the current state of knowledge.

- Mechanisms of action

The functional alterations of the CNS observed following acute or chronic exposure are related to a mechanism of action that includes an alteration of the membrane and the membrane channels, direct damage to nerve cells by oxidative stress and/or apoptosis, and alteration of neurotransmitter synthesis, liberation and degradation, and the way they are bound to receptors of the hypothalamo-pituitary axis.

The mechanism of action of toluene that causes reprotoxic effects is unknown. In light of all the data available on the metabolism and effects in animals and humans, the experts consider that the reprotoxic effects of toluene observed in animals could also occur in humans.

Development of acute and chronic TRVs by inhalation

1. Acute TRV by inhalation

Choice of the critical effect

Studies in humans suggest that the central nervous system (CNS) is the primary target organ for the toxicity of toluene. Acute exposure by inhalation of toluene causes headaches, tremors, locomotor disorders, dizziness, nausea, and degraded performance observed during neurobehavioral tests at concentrations between 40 and 200 ppm, reversible after the cessation of the exposure.

In animals, the reported effects are also an impairment of the CNS, with hearing losses, ataxia and tremors from 250 ppm, as well as impaired locomotor performance at concentrations from 100 ppm.

Animal studies also suggest an effect on foetal development, in the event of exposure to toluene during gestation. These effects are observed for concentrations greater than 1000 ppm and predominantly between 1000 and 3000 ppm, in the form of delayed foetal growth and skeletal development. These are mostly associated with maternal toxicity.

In humans, the effects of toluene on foetal development in the event of exposure during pregnancy are described in children where the mother has intentionally breathed in a large quantity of toluene during pregnancy (substance abuse behaviour, with exposure concentrations from 4000 to 12,000 ppm).

The CES has therefore chosen the neurological effects as the critical effect.

Analysis of the guideline values and toxicity reference values

An analysis was carried out of the guideline values and TRVs by inhalation proposed by the main nationally or internationally recognised agencies and institutions.

In 2009, ANSES established an acute reprotoxic TRV by inhalation of 5 $mg.m^{-3}$ based on the decrease in the weight of the offspring (F1 generation) found in the study by Roberts *et al.* (2003). Since the publication of this TRV, other elements have been taken into consideration. Access to raw data that had not been brought to the knowledge of the Agency at the time has enabled us to observe that the study by Roberts *et al*. (2003) has certain statistical limitations. For instance, the

statistical unit considered does not seem to be the litter but the weight of the individual offspring, contrary to what is stated in the article. In addition, knowledge acquired more recently on toluene enables us to form a different opinion about the nature of the reprotoxic effect observed. The decrease in weight of the F1 and F2 generations could be linked to maternal toxicity and not to a direct developmental effect of the substance.

Accordingly, the experts consider that the acute reprotoxic TRV by inhalation of toluene (ANSES, 2009) is no longer considered relevant: it is withdrawn.

An indoor air quality guideline as a result of short-term exposure of 4 ppm (15 $mg.m^{-3}$) has been developed by Health Canada (Residential Indoor Air Quality Guideline, 2011).

There are two acute TRVs by inhalation available: a Reference Exposure Level of 37 $mg.m^{-3}$ from the OEHHA (2003) and a Minimum Risk Level of 3.8 $mg.m^{-3}$ from the ATSDR (2000). The different values established by international agencies for acute exposure are based on the data from the study by Andersen *et al*. (1983). The authors observed effects on the CNS of 16 volunteers exposed to toluene at nominal concentrations of 10, 40 and 100 ppm, 8 hours per day for 4 days. At 100 ppm, the exposed subjects showed signs of fatigue, a feeling of intoxication, drowsiness, and irritation of the eyes, nose and throat. No symptoms were observed at 10 ppm or 40 ppm. The study was considered to be of good quality but the effects on health, such as irritation, were collected by subjective questionnaire; no clinical observations were reported.

A time adjustment was made by applying Haber's Law to determine the applicable dose following continuous exposure, considering the conditions of exposure in the study.

On completion of the analysis of the acute toxicity data available for humans and animals, the effects on the CNS observed at the lowest doses were chosen as the critical effect.
The experts of the CES did not wish to retain the existing values and decided to establish an acute TRV by inhalation.

Establishing the acute TRV

- Choice of the key study and critical dose

Updating the bibliography as far as 2016 brought to light a new controlled exposure study involving exposure to toluene and the occurrence of acute effects on the CNS: Kobald *et al*. (2015). This study was considered to be of good quality. It presents a rigorous experimental plan (psychometric test) as well as a good quality statistical analysis of the data. The results obtained by the authors are consistent with those published previously.

The study by Kobald *et al*. (2015) was carried out on 33 healthy volunteers divided randomly into two groups (the history of the subjects is not given; the average age is 25 years). The control group was composed of 16 individuals, of whom nine were women, and the group exposed to toluene of 17 individuals, of whom ten were women. The subjects were exposed to toluene in an exposure chamber at a single concentration of 200 ppm for 40 minutes. Behavioural tests were carried out outside the exposure chamber; they consisted of tasks involving attention and visual discrimination. The results were quantified objectively by electro-encephalographic and electro-oculographic measurements. The results showed an effect of toluene on visual discrimination, more particularly under Luminance Orientation Bilateral (LOB) conditions; this measures visual discrimination in the presence of a distractor related to the orientation of the object. The resulting LOAEC was 200 ppm.
The experts of the CES chose the study by Kobald *et al*. (2015) as the key study. It shows an alteration of visual discrimination observed at 200 ppm (LOAEC). This concentration was selected as the critical dose.

- Time adjustment

In the study by Kobald *et al*. (2015), the individuals were exposed for 40 minutes. To establish an acute TRV that can be applied over 24 hours, a time adjustment for continuous exposure over 24 hours is made using a PBPK model developed by the team of R. Tardif to identify the blood concentration corresponding to the exposure dose of 200 ppm for a duration of 24 hours (Marchand *et al*., 2015). Considering the exposure value of 1440 minutes (24 hours), an adjusted concentration of 323 $mg.m^{-3}$ (86 ppm) was calculated.

- Allometric adjustment

As the study was conducted in humans, no allometric adjustment was performed.

- Choice of uncertainty factors

The TRV was calculated on the basis of the adjusted concentration using the PBPK model of 323 $mg.m^{-3}$ (86 ppm), applying the following uncertainty factors (UFs):

- **Inter-individual variability (UF_H): 5**

The value used by default for this factor is 10, divided into two components, a toxicokinetic value of 3.16 ($UF_{H\,TK}$) and a toxicodynamic value ($UF_{H\,TD}$) of 3.16.

This value may be replaced by Chemical-Specific Adjustment Factors (CSAFs – WHO-IPCS, 2005). These CSAFs are based on knowledge of the chemical's toxicokinetics (in terms of human variability). This CSAF is calculated using the ratio between the 95th percentile of the value of the

toxicokinetic parameter within the population and the 50th percentile of this same toxicokinetic parameter.

In order to refine the toxicokinetic component, the CES chose to apply an HK_{AF} (*human kinetic adjustment factor*) to take human toxicokinetic variability into account (WHO-IPCS, 2005).

Mörk *et al*. (2014) calculated **Chemical-Specific Adjustment Factors** for the human kinetic component for toluene, styrene and methylene chloride. A factor ($UF_{H\text{-}TK}$ or HK_{AF}) of 1.7 was determined for the entire population.

The CES decided to apply this value to replace the default factor of 3.16.

The factor UF_H is therefore equal to $UF_{H\text{-}TD}$ x $UF_{H\text{-}TK}$ = 3.16 x 1.7 = **5.3, rounded to 5**

- **Use of a LOAEC (UF_L): 3**

The guidelines for developing TRVs recommend applying a safety factor of 3 or 10 when using a LOAEC. The CES proposes a factor of 3 because the LOAEC resulting from the study by Kobald *et al.* (2015) is lower than that of other studies, taking into account the time of exposure, suggesting that the chosen effect is very sensitive.

An overall uncertainty factor of 15 was thus used to determine the TRV.

Critical effect	Critical dose	UF	Acute TRV by inhalation
Neurological effects (visual discrimination and electro-encephalographic measurement) *Kobald et al. (2015): controlled study in humans (healthy volunteers)*	**LOAEC = 752 mg.m^{-3}** (200 ppm) <u>Time adjustment (PBPK model, dose equivalence adjusted over 24 hours):</u> $LOAEC_{ADJ}$ = 323.4 mg.m^{-3} (86 ppm)	**15** UF_H = 5 UF_L = 3	**TRV = 21 mg.m^{-3}** (6 ppm)

<u>Confidence level:</u>

An overall confidence level was assigned to this TRV based on the following criteria:

- Level of confidence in the nature and quality of the data: **High**

- Level of confidence in the choice of the critical effect and the mode of action: **High**

- Level of confidence in the choice of the key study: **High.**

This study was considered to be of good quality. It presents a rigorous experimental plan (psychometric test) as well as a good quality statistical analysis of the data. The results obtained by the authors are consistent with those published previously.

- Level of confidence in the choice of the critical dose: **Moderate**

The data available for acute exposure to toluene in humans show a lack of effect for concentrations of less than 100 ppm. However, this study was carried out with a single dose of 200 ppm.

Thus, in the current state of knowledge, the overall confidence level for this TRV is **high**.

2. Chronic TRV by inhalation

In the framework of the establishment of a chronic TRV by inhalation carried out in 2011 by ANSES and based on the neurotoxic effects of toluene, the existing TRVs proposed by Health Canada (1992), the ATSDR (2000), the RIVM (2001), the OEHHA (2003) and the US EPA (2005) were not retained. A chronic TRV of 3 $mg.m^{-3}$ was established. This TRV was updated in parallel with the expert appraisal concerning IAQGs using the PBPK model developed by the team of R. Tardif, which allows for extrapolations and a reduction of the UFs (Marchand *et al*., 2015).

Choice of the key study and critical dose

Updating the bibliography as far as 2016 did not bring to light any new studies in humans associating chronic exposure to toluene and the occurrence of effects, calling into question ANSES's choice of the work by Zavalic *et al*. (1998) as the key study for establishing its chronic TRV in 2011. That study reported atmospheric concentrations of toluene and quantified the alteration of colour vision in exposed workers when compared to a control group. It showed a significant increase in the Colour Confusion Index (CCI) at a concentration of 132 ppm (500 $mg.m^{-3}$). The level of exposure of the study's E1 group was therefore accepted as a NOAEC (32 ppm, 123 $mg.m^{-3}$).

The choice of this critical dose associated with neurotoxic effects observed in humans is supported by the doses of the same order of magnitude found in other studies.

The experts of the CES therefore confirm the choice of the study by Zavalic *et al*. (1998) as the key study, with a critical dose of 123 $mg.m^{-3}$ (32 ppm).

Time adjustment

In the study by Zavalic *et al*. (1998), the workers were exposed for 8 hours per day, 5 days a week. To establish a chronic TRV, a time adjustment for continuous exposure was made using a PBPK model developed by the team of R. Tardif (Marchand *et al*., 2015).

The use of the PBPK model makes it possible to determine the blood concentration corresponding to the exposure dose of 123 $mg.m^{-3}$ (32 ppm). For a longer period of exposure, the exposure dose corresponding to the blood concentration was calculated at 96 $mg.m^{-3}$ (25 ppm).

Allometric adjustment

As the study was conducted in humans, no allometric adjustment was performed.

Choice of uncertainty factors

The TRV was calculated on the basis of the adjusted concentration of 96 $mg.m^{-3}$ (25 ppm), applying the following uncertainty factors:

- **Inter-individual variability (UF_H): 5**

The value used by default for this factor is 10, divided into two components, a toxicokinetic value of 3.16 ($UF_{H\,TK}$) and a toxicodynamic value ($UF_{H\,TD}$) of 3.16.

This value may be replaced by Chemical-Specific Adjustment Factors (CSAFs – WHO-IPCS, 2005). These CSAFs are based on knowledge of the chemical's toxicokinetics (in terms of human variability). This CSAF is calculated using the ratio between the 95th percentile of the value of the

toxicokinetic parameter within the population and the 50th percentile of this same toxicokinetic parameter.

In order to refine the toxicokinetic component, the CES chose to apply an HK_{AF} (human kinetic adjustment factor) to take human toxicokinetic variability into account (WHO-IPCS, 2005).

Mörk *et al*. (2014) calculated **Chemical-Specific Adjustment Factors** for the human kinetic component for toluene, styrene and methylene chloride. A factor ($UF_{H\text{-}TK}$ or HK_{AF}) of 1.7 was determined for the entire population.

The CES decided to apply this value to replace the default factor of 3.16.

The factor UF_H is therefore equal to $UF_H = UF_{H\text{-}TD} \times UF_{H\text{-}TK} = 3.16 \times 1.7 =$ **5.3, rounded to 5**

An overall uncertainty factor of 5 was thus used to determine the long-term TRV.

Critical effect	Critical dose*	UF	Chronic TRV by inhalation
Neurological effects (alteration of colour vision) *Zavalic et al., (1998): epidemiological study in workers*	**NOAEC = 123 mg.m^{-3}** (32 ppm) Temporal adjustment (use of a PBPK model, equivalence of dose adjusted over the entire lifetime): $NOAEC_{ADJ}$ = 96 mg.m^{-3} (25 ppm)	**5** $UF_H = 5$	**19 mg.m^{-3}** (5 ppm)

Confidence level:

An overall confidence level was assigned to these TRVs based on the following criteria:

- Level of confidence in the nature and quality of the data: **High**.
- Level of confidence in the choice of the critical effect and the mode of action: **High.**
- Level of confidence in the choice of the key study: **Moderate**

  The key study was considered to be of better quality than the other existing studies. However, it presents a few methodological weaknesses (non-homogeneous groups, precluding inter-group comparisons, quantity of alcohol consumption defined on the basis of questionnaires, and measurements carried out in a static position, which is a source of variability).

- Level of confidence in the choice of the critical dose: **High.**

  The choice of this critical dose associated with neurotoxic effects observed in humans is supported by the doses of the same order of magnitude found in other studies.

In the current state of knowledge, the overall confidence level is therefore **high** for the chronic TRV.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions and recommendations of the CES "Substances" on the formulation of acute and chronic toxicity reference values by inhalation for toluene.

The nature of the TRVs (acute, subchronic, chronic) is partly determined by the duration of exposure in the toxicological studies but also by the health risk assessment needs.

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.

Toluene has been the subject of several studies by ANSES, including the development of a reprotoxic TRV in 2008 and a TRV for chronic effects by inhalation in 2011. This expert appraisal has produced an updated chronic TRV for ANSES on the basis of the new data identified on the toxicokinetics of toluene (PBPK model), and proposes an acute TRV. Since the publication of the reprotoxic TRV, other elements have been taken into consideration that led to the experts withdrawing this reprotoxic TRV.

Table 1: TRVs by the respiratory route for toluene (CAS No 100-88-3)

Critical effect ***Key study***	**Critical dose**	**UF**	**TRV**
Neurological effects (visual discrimination and electro-encephalographic measurement) *Kobald et al. (2015): controlled study in humans (healthy volunteers)*	**LOAEC = 752 mg.m^{-3}** (200 ppm) Time adjustment (PBPK model, dose equivalence adjusted over 24 hours): LOAEC $_{ADJ}$ = 323.4 mg.m^{-3} (86 ppm)	**15** UF_H = 5 UF_L = 3	**Acute TRV = 21 mg.m^{-3}** (6 ppm) Confidence level: high
Neurological effects (alteration of colour vision) *Zavalic et al., (1998): epidemiological study in workers*	**NOAEC = 123 mg.m^{-3}** (32 ppm) Time adjustment (use of a PBPK model, equivalence of dose adjusted over the entire lifetime): $NOAEC_{ADJ}$ = 96 mg.m^{-3} (25 ppm)	**5** UF_H = 5	**Chronic TRV = 19 mg.m^{-3}** (5 ppm) Confidence level: high

The use of the PBPK model enabled the adjustment of the acute TRV over a period of 24 hours; it should be noted that this time adjustment can be applied for periods of less than a day. For example, the value of the acute TRV calculated for 1 hour would thus be 40 $mg.m^{-3}$ (11 ppm).

Dr Roger GENET

KEY WORDS

Toluene, toxicity reference value, inhalation, acute, chronic, neurotoxicity