ANSES Opinion
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Related Request no. 2013-SA-0136

The Director General

Maisons-Alfort, 14 October 2016

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

on the development of TRVs by the respiratory route for ethylbenzene (CAS No 100-41-1)

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).

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 14 October 2016 shall prevail.

In 2016, ANSES issued an internal request to formulate toxicity reference values (TRVs) for ethylbenzene, 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 ethylbenzene, 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.

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).

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 French Standard NF X 50-110 "Quality in Expert Appraisals – 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 work were presented to the CES between October 2014 and May 2016. It was adopted by the CES "Substances" at its meeting on 12 May 2016.

The work was also submitted regularly to the CES on "Assessment of risk 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. ANALYSIS AND CONCLUSIONS OF THE CES

Sources of ethylbenzene

Ethylbenzene is mainly used as a feedstock and solvent in the chemical industry.
Sources of exposure to ethylbenzene in the environment are related to the combustion process of organic materials, the application of paints, varnishes and lacquers, and its natural presence in crude oil.

Toxicological profile

The toxicological profile was prepared primarily on the basis of the reports by the ATSDR[1] and Health Canada (ATSDR, 2010; Health Canada, 2014). An additional literature search was also conducted on the period [2012-2014][2] to identify relevant recent data that would not have been taken into account in the two reports mentioned above.

[1] US Agency for Toxic Substances and Disease Registry

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

- Toxicokinetics

Ethylbenzene is readily absorbed by the respiratory route. Animal studies indicate that ethylbenzene is distributed throughout the body, following absorption by the respiratory and dermal routes. Ethylbenzene is metabolised in the liver by cytochrome P450s. It is first hydroxylated to form 1-phenylethanol, then a series of oxidations leads to the successive formation of 2-hydroxyacetophenone, 1-phenyl-1,2-ethanediol, mandelic acid and phenylglyoxylic acid. This is the main metabolic pathway in humans following inhalation exposure. Ethylbenzene is rapidly metabolised before being eliminated from the body, primarily in the form of urinary metabolites. The metabolism of ethylbenzene varies according to the species, sex and route of exposure.

- Acute toxicity

In humans, inhalation exposure from 2000 ppm[3] of ethylbenzene causes respiratory effects (irritation of the throat and nose, feeling of chest tightness) and neurological disorders (non-specific central nervous system depression, dizziness).

In animals, acute inhalation exposure to ethylbenzene can cause death; deaths were observed in rats at concentration levels of 4000 ppm for 4h, while exposure to 2400 ppm of ethylbenzene for 6h/d for 4 days resulted in 100% mortality in rats or mice. Depressive effects on the central nervous system (CNS) have been observed following acute exposure to ethylbenzene concentrations of around 2000 ppm. A study in rats indicated moderate activation of the motor nervous system at lower doses (of around 400 ppm). Ototoxic effects are manifested from 400 ppm by a change in the hearing threshold and damage to the cochlear morphology. Ethylbenzene is also responsible for respiratory effects (ranging from irritation to lung congestion), hepatic effects (increase in liver weight, induction of hepatic enzymes, changes in the ultrastructure of hepatocytes) and minimal renal effects (increase in kidney weight, induction of microsomal kidney enzymes).

- Irritation and sensitisation

Ethylbenzene is irritating to the ocular and respiratory tract epithelia. Such effects have been observed in both humans and animals. No data have been reported that support possible respiratory or skin sensitisation.

- Chronic toxicity

A few epidemiological studies have been identified that demonstrate respiratory, haematological and general neurotoxic effects or ototoxicity (hearing loss) in humans. Nevertheless, co-exposure to other substances has been observed and this greatly limits the extent to which these effects can be attributed to ethylbenzene alone.

In animals, subchronic inhalation exposure to ethylbenzene concentrations of the order of 50 to 600 ppm is responsible for an increase in kidney weight (rats and mice) and the induction of microsomal kidney enzymes (rats). These effects are similar to those observed following acute exposure. Histopathological changes to the kidneys, including changes induced by α_2-microglobulin, have also been observed in male rats exposed to 750 ppm of ethylbenzene for 4 weeks. Chronic exposure of male and female rats is reflected by more severe kidney effects, including an increase in the severity of chronic progressive nephropathy (significant from 75 ppm in female rats), renal tubular hyperplasia (significant at 750 ppm in male rats) and an increase in the incidence of renal tubular adenomas and carcinomas (significant at 750 ppm in rats of both sexes).

In animals, liver effects observed after acute exposure have also been reported for subchronic exposure: a significant increase in relative liver weight (rats, mice, rabbits, guinea pigs and monkeys, at concentrations of around 250 to 1000 ppm), induction of microsomal liver enzymes

[3] 1 ppm = 4.34 $mg.m^{-3}$

(rats exposed to 50 ppm) and changes in the ultrastructure of hepatocytes (rats exposed to 50 ppm). Changes in liver histology have also been observed with subchronic and chronic inhalation exposure to ethylbenzene. In rats and mice of both sexes, subchronic exposure induces changes in liver histology manifested in particular by hepatocyte hypertrophy (ethylbenzene concentrations of the order of 250 ppm to 2200 ppm). Chronic exposure induces hepatocellular changes (from 75 ppm) as well as hepatocyte hypertrophy and necrosis (750 ppm). These effects are observed only in mice, and not in rats.

No general symptoms of neurotoxicity have been observed in animals following inhalation exposure to ethylbenzene for subchronic or chronic durations. The most sensitive neurological effect induced by exposure to ethylbenzene is ototoxicity, characterised by a deterioration in hearing thresholds and changes to the cochlear morphology, already observed in a situation of acute exposure (from 400 ppm in male rats) and also reported following subchronic exposure from 200 ppm in male rats.

- Genotoxicity

The results of *in vitro* mutagenicity tests indicate that ethylbenzene has no mutagenic effect on bacterial cells, yeasts (with and without metabolic activation) or non-human mammalian cells, with the exception of one positive result in an L5178Y mouse lymphoma cell mutation assay, but at concentrations inducing significant cytotoxicity. Concerning the other *in vitro* tests in mammalian cells, excluding studies testing ethylbenzene concentrations that are cytotoxic or very close, few results were positive. Ethylbenzene exhibits no mutagenic or clastogenic activity in *in vivo* tests; negative results have been obtained in chromosomal aberration tests in rat bone marrow and micronucleus tests in mice.
As a whole, the results of the *in vivo* and *in vitro* genotoxicity studies suggest that ethylbenzene has no genotoxic effect.

- Carcinogenicity

Ethylbenzene has been classified in Group D by the US EPA[4] as a substance "not classifiable as to human carcinogenicity" (US EPA, 1991). Nevertheless, that assessment was conducted before publication of the results of a two-year inhalation carcinogenicity study in rodents by the National Toxicology Program (NTP, 1999). This study showed an increase in the incidence of tumours of the kidney tubules in rats, and an increased incidence of alveolar/bronchiolar tumours and hepatocellular tumours in mice. In 2000, the International Agency for Research on Cancer (IARC) concluded that ethylbenzene was possibly carcinogenic to humans (classification in Group 2B), on the basis of levels of evidence that were sufficient in animals and insufficient in humans. In 2002, ethylbenzene was classified in Group A3 "confirmed animal carcinogen with unknown relevance to humans" by the ACGIH[5] (equivalent to Group 2B of the IARC). Ethylbenzene has not been classified as a carcinogen by the European Union according to its harmonised classification under Regulation (EC) No 1272/2008, known as the CLP Regulation.

- Mechanisms of action

Ototoxicity: inhalation exposure of animals to ethylbenzene induces hearing loss caused by the irreversible loss of outer hair cells in the organ of Corti. In addition, organic solvents are known to be neurotoxic and, as well as cochlear toxicity, are suspected of damaging hearing due to impairment of the central nervous system. The molecular mechanisms of the ototoxicity induced by ethylbenzene have not been determined. A recent study suggests that the ototoxicity induced by low concentrations of ethylbenzene could be mediated by nicotinic acetylcholine receptors. On the basis of studies conducted with toluene (structurally similar to ethylbenzene), it has been suggested that an increase in levels of intracellular calcium could be responsible for the loss of outer hair cells exposed to solvents such as ethylbenzene. One other hypothesis is that the outer

[4] United States Environmental Protection Agency
[5] American Conference of Governmental Industrial Hygienists

hair cells may be affected by the formation of free radicals commonly called reactive oxygen species (ROS).

Neurotoxicity (central nervous system): *in vivo* studies in animals at cellular level indicate that changes in levels of dopamine and other biochemical changes in the brain, as well as the electrical activity in the brain, could be involved in ethylbenzene's toxicity to the central nervous system. *In vitro* studies of the mechanism of toxicity have stressed the effect of ethylbenzene on cell membranes, in particular that of the astrocytes. The results of several studies suggest that changes in the structure and integrity of the cell membrane after distribution of ethylbenzene in the lipid bilayer could constitute a mechanism of neurotoxicity.

Renal effects (non-carcinogenic): the mechanisms responsible for renal toxicity, in particular the aggravation of the chronic progressive nephropathy observed in rats connected with chronic exposure to ethylbenzene, have not been elucidated.

Carcinogenic effects: the mechanisms behind the formation of kidney tumours observed in rats and liver tumours observed in mice are unknown. The results of genotoxicity studies seem to rule out the hypothesis of a genotoxic mechanism.

- Extrapolation from animals to humans

Studies conducted *in vivo* or *in vitro* on the cellular processes seem to indicate that the mechanisms in humans and animals are similar. There are some inter-species differences, in particular with regard to the metabolism of ethylbenzene. The rat appears to be the most suitable animal model for studying the mechanisms of toxicity of ethylbenzene with regard to assessing its effects on human health, because firstly, it is the species whose metabolism is closest to that of humans (the main oxidation pathway is the same as in humans, through CYP450 2E1, and the metabolites are common to both species) and secondly, rats appear to be the most sensitive species.

The rat is therefore the best animal model available for studying the potential ototoxic effects of ethylbenzene in humans from a metabolic perspective. In addition, the same histopathological signature (loss of the outer hair cells first affecting the third row of outer hair cells) of the cochleotoxic effects induced by exposure to aromatic solvents such as ethylbenzene is present in humans and animals. Moreover, the cochleotoxic effects observed in humans following exposure to other structurally similar aromatic solvents, such as toluene and styrene (presence of a benzene ring and a short aliphatic chain), are comparable to those of ethylbenzene. It can therefore be concluded from the available data that it is relevant to transpose to humans the ototoxic effects observed in rats for exposure to ethylbenzene.

With regard to the kidney effects, the transposability to humans of the chronic progressive nephropathy observed in rats is a matter of debate in the scientific community. This age-related disease manifests spontaneously in laboratory rats. It is characterised by a range of changes including dilation of the renal tubules, interstitial fibrosis and infiltration of inflammatory mononuclear cells, foci of tubular regeneration, and transitional hyperplasia of the epithelium of the renal papilla. While these clinical signs are similar to those observed in humans, they are not specific to a precise renal pathology. Some scientists consider it to be a species-specific effect, as exposed mice do not exhibit kidney damage. The available data are insufficient to enable any conclusion to be drawn regarding the plausibility of transposing to humans the aggravation of the chronic progressive nephropathy observed in female rats.

Development of the TRV by inhalation

Choice of the critical effect

The auditory system is the target organ that is most sensitive to ethylbenzene for subacute and subchronic exposure. Significant irreversible loss of the outer hair cells in the organ of Corti has been observed in animals. This effect was identified from 400 ppm following subacute exposure (8h/d, 5 days) and from 200 ppm following subchronic exposure (6h/d, 6d/wk, 13 weeks). This cochlear damage is accompanied by a significant increase in the hearing threshold in the medium frequency zone, which has also been identified at higher doses. Similarities in the type and chronology of the damage to the auditory system are observed for subacute and subchronic exposure: loss of outer hair cells first affecting the third row of outer hair cells (histopathological signature), increase in hearing thresholds (decline in hearing) and then losses of the inner hair cells. In addition, the destruction of the hair cells is irreversible; the decline in hearing induced by the cochleotoxicity of ethylbenzene is therefore persistent.

In the absence of any human data, the relevance of transposing to humans the ototoxic effects observed in animals following exposure to aromatic solvents was assessed by the ANSES experts. Considering the suitability of the rat model for studying the ototoxic effects induced by aromatic solvents such as ethylbenzene, the available data on the ototoxic mechanisms of aromatic solvents, in particular the existence of a histopathological signature for the cochleotoxic effects related to aromatic solvents, and the fact that the cochleotoxic effects induced by toluene and styrene in humans are comparable to those of ethylbenzene, the ANSES experts concluded that it is relevant to transpose to humans the ototoxic effects observed in rats for subacute and subchronic exposure. In addition, considering the application of Haber's law, these effects are assumed to appear for chronic inhalation exposure, probably at lower ethylbenzene concentration levels than those tested in the studies for acute and subchronic exposure durations.

Concerning the possible carcinogenic effects following chronic exposure, there are currently still major doubts about the mechanism of action and the relevance of transposing to humans the tumours induced in animals by ethylbenzene, a substance classified as possibly carcinogenic (Group 2B) by the IARC. In the current state of knowledge, it is not possible to rule out the carcinogenic potential of the substance for humans. Nevertheless, the results of the genotoxicity studies seem to rule out the hypothesis of a genotoxic mechanism, which suggests the existence of a threshold dose. Formulating a chronic TRV based on ototoxic effects identified for subchronic exposure to 200 ppm may therefore provide protection *a priori* from the development of renal tumours in animals, which are observed for chronic exposure to a concentration of 750 ppm. Thus, the CES selected the ototoxic effects, and more specifically the loss of outer hair cells dependent on the concentration of ethylbenzene, as the critical effect regardless of the duration of exposure (acute, subchronic or chronic).

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 agencies and institutions recognised at national or international level.

→ Acute exposure

Only the ATSDR has proposed a TRV for acute exposure of 5 ppm (ATSDR, 2010). This value was established using data from the study by Cappaert *et al.* (2000). In this study, rats were exposed under different conditions of exposure (concentrations of 0, 300, 400 and 550 ppm, 8 hours per day for 5 days). The critical effect considered is an ototoxic effect, the shift in the auditory threshold reflecting a decline in hearing.

The CES deemed the study by Cappaert *et al.* (2000) to be of good quality and therefore selected it.

Several points relating to the method of establishing this value were discussed in the framework of this expert appraisal:

- A critical analysis of the PBPK[6] models used for calculating an equivalent benchmark concentration (BMC) in humans ($BMCL_{HEC}$) at 154.26 ppm was carried out. These models had good predictive ability (for exposures at various concentrations, a good match between what is measured and what is calculated): their use proved to be relevant in formulating a TRV for acute exposure to ethylbenzene. The different stages of establishing the critical dose were appraised and validated:
  - 1. Transformation of atmospheric exposures into arterial concentrations using the PBPK model in rats;
  - 2. Modelling of the relationship between the increase in ototoxicity and the estimated daily arterial concentration/dose-response curve;
  - 3. Reconstruction of the atmospheric exposure of humans from the arterial concentration.
- The application of an uncertainty factor of 3 for the interspecies variability and 10 for the interindividual variability is consistent with the methods used within ANSES.

Critical effect **Source study**	**Critical dose**	**UF**	**TRV**
Ototoxicity **Shift in the auditory threshold** *Cappaert et al., 2000: 5-day study in rats*	$BMCL_{1SD}$ = 81.10 µmol.L^{-1} Dose adjustment $BMCL_{HEC}$ = 154.26 ppm	**30** $UF_{A\text{-}TD}$ 3 UF_H 10	**MRL = 5 ppm** (22 mg.m^{-3})

MRL: minimum risk level

Confidence level:

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

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

Moderate, because there is no study showing acute ototoxicity in humans.

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

High. Despite the absence of any study in humans demonstrating ototoxicity, there are studies in humans for other organic solvents of very similar structure and resulting in similar effects, which inspires confidence in the choice of critical effect and mode of action. In addition, the rat model has a similar metabolism to that of humans.

- Level of confidence in the choice of the key study:

High. The study was deemed to be of good quality.

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

High. The effect appears at 400 ppm and is not observed at the lower dose (300 ppm).

[6] Physiological pharmacokinetics

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

➔Chronic exposure

The ATSDR has proposed a subchronic TRV of 2 ppm, which was established based on the shift in the hearing threshold. Ototoxic effects have been observed in rats following subchronic inhalation exposure to ethylbenzene: shift in the hearing threshold and loss of outer hair cells (Gagnaire *et al.,* 2007). This was a 13-week study conducted in male rats at the following doses: 0, 200, 400, 600 and 800 ppm. A decline in hearing was observed in animals exposed from 400 ppm. Losses of external hair cells were identified, dependant on the concentration of ethylbenzene, with significant losses from 200 ppm and almost complete losses at doses of 600 and 800 ppm.

None of the models tested by the ATSDR could be used to correctly fit the modelled data to the experimental data concerning the loss of external hair cells. Thus, the CES did not select this TRV.

For chronic exposure

The available chronic TRVs by inhalation, proposed by the US EPA (1991), the OEHHA[7] (2000), the RIVM[8] (2001) and the ATSDR (2010), were not adopted because they were not established on the critical effect considered.

Thus, the CES proposed establishing subchronic and chronic TRVs by inhalation.

Establishment of subchronic and chronic TRVs

Choice of the key study

The study by Gagnaire *et al.* (2007), for which ototoxic effects by inhalation were observed in rats following subchronic exposure (13 weeks), was found to be of good quality and was also chosen by the ATSDR for establishing its subchronic TRV. This study was chosen for establishing the subchronic TRV.
In the absence of data on the ototoxicity of ethylbenzene for chronic inhalation exposure, the study by Gagnaire *et al.* (2007) was chosen for establishing the chronic TRV. Indeed, the ototoxic effects observed following subchronic exposure are irreversible lesions and can be regarded as chronic effects.

Choice of the critical dose

The experimental data established in this study on the loss of outer hair cells were modelled by ANSES with mathematical models used by the PROAST software (PROAST version 38.9), developed by the RIVM, in order to establish a BMC.

The aim of the 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). Following the recommendations of the US EPA, the BMR corresponds to an increase of one standard deviation in relation to the mean of the control. An increase of one standard deviation corresponds here to a response level of 50%.

[7] Office of Environmental Health Hazard Assessment
[8] RIVM: Rijksinstituut voor Volksgezondheid en Milieu

When determining the BMCL (lower limit of the confidence interval of the BMC), several mathematical models were tested. The maximum likelihood method was used to fit the model to the data.

In the case of ethylbenzene, the model providing the best fit to the experimental data relating to the loss of hair cells was the Hill model for estimating the lower limit of the 90% confidence interval of a concentration corresponding to a 50% increase in the response compared to the non-exposed group:

- $BMC_{0.5}$ = 136.9 ppm
- $BMC_{0.5}L_{90}$ = 119.7 ppm

On the basis of these results, it was proposed to adopt as the critical concentration the $BMC_{0.5}L_{90}$ established for the "loss of external hair cells" critical effect, **i.e. $BMC_{0.5}L_{90}$ = 119.7 ppm.**

Dose adjustment

The aim is to reduce the value of the uncertainty about interspecies variability in order to determine a human equivalent concentration. For the respiratory route, the US EPA has developed various dose adjustments based on the physico-chemical properties of the inhaled substance (particles or gas, highly soluble or relatively insoluble in water) and the site where the critical effects are observed (respiratory or extra-respiratory), leading to different equations (US EPA, 1994).

According to the recommendations of the US EPA (1994), ethylbenzene should be regarded as a Category 3[9] gas (systemic toxicity). The dose adjustment applied by default for a Category 3 gas is as follows:

$$BMC_{0.5}L_{90\ HEC} = BMC_{0.5}L_{90} \times (Hb/g)_{rat}/(Hb/g)_{human}$$

Where (Hb/g): blood/air partition coefficient of ethylbenzene
HEC: human equivalent concentration

According to the data available in the PBPK models, the blood/air partition coefficient of ethylbenzene for animals is higher than that for humans. Because the $(Hb/g)_{rat}/(Hb/g)_{humans}$ ratio is greater than 1, the US EPA proposes retaining the default value of 1, which is more protective.

$$\mathbf{BMC_{0.5}L_{90\ HEC} = 119.7\ ppm}$$

Time adjustment

The animals were exposed for 6 hours per day, 6 days per week for 13 weeks. To take account of the discontinuity of the exposure, a time adjustment was made:

$$\mathbf{BMC_{0.5}L_{90\ HEC\ ADJ} = BMC_{0.5}L_{90\ HEC} \times (6/24) \times (6/7) = 119.7 \times (6/24) \times (6/7) = 25.6\ ppm}$$

Choice of uncertainty factors

The TRVs were calculated from the $BMC_{0.5}L_{90\ HEC\ ADJ}$ using the following uncertainty factors (ANSES, 2015):

- Inter-species variability (UF_A): 2.5

The applied dose adjustment enabled a human equivalent concentration to be calculated, using the previous equation. To take toxicodynamic variability and residual uncertainties into account, an additional uncertainty factor was set at 2.5.

[9] The US EPA has identified three categories of gas based on the solubility and reactivity of the substance considered

- Inter-individual variability (UF_H): 10

Because there were no scientific data available to reduce the default value, the value of 10 was used.

- Subchronic to chronic transposition (UF_S): 3 for formulating the chronic TRV

The duration of the selected key study, regarded in toxicology as "subchronic" (the animals were exposed for 6 days per week for 13 weeks), corresponds to approximately 10% of the life of the animals which, in humans, would correspond to about 7 years of exposure according to convention.

In the framework of the establishment of a chronic TRV, the data are insufficient for determining whether similar effects could appear following chronic exposure to lower concentrations than those tested in the subchronic studies. Therefore, the ANSES experts decided to apply a value of 3 for this factor only in the framework of establishing the chronic TRV.

The overall uncertainty factor is 25 for establishing a subchronic TRV and 75 for the chronic TRV.

Critical effect Source study	Critical dose	UF	TRV
Ototoxic effect Loss of outer hair cells in the organ of Corti *Gagnaire et al., 2007: 13-week study in rats*	$BMC_{0.5}L_{90}$ = 119.7 ppm (Hill model – PROAST 38.9 software) $BMC_{0.5}L_{90\ HEC\ ADJ}$ = 25.6 ppm	25 UF_A 2.5 UF_H 10	**Subchronic TRV** **1 ppm** (4.3 $mg.m^{-3}$)
		75 UF_A 2.5 UF_H 10 UF_S 3	**Chronic TRV** **0.3 ppm** (1.5 $mg.m^{-3}$)

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** for the subchronic TRV
  - **Moderate** for establishing the chronic TRV in the absence of a chronic study
- 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**
- Level of confidence in the choice of the critical dose: **high**

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

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 toxicity reference values for inhalation for ethylbenzene.

As a reminder, when assessing health risks in humans, ANSES distinguishes between three types of exposure duration:

- Acute exposure, from a few hours to a few days;
- Subchronic exposure, from a few days to a few months;
- Chronic exposure, from one or more years to an entire lifetime.

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.

Table 1: TRVs by the respiratory route for ethylbenzene (CAS No 100-41-4)

Critical effect ***Key study***	**Critical dose**	**UF**	**TRV**
Ototoxic effect Loss of outer hair cells in the organ of Corti *Cappaert et al. 2000:* *5-day study in rats*	$BMCL_{HEC}$ = 154.26 ppm after dose adjustment of a $BMCL_{1SD}$ = 81.10 µmol/L	**30** UF_A 3 UF_H 10	**Acute TRV** **5 ppm** (22 $mg.m^{-3}$) Confidence level: high
Ototoxic effect Loss of outer hair cells in the organ of Corti *Gagnaire et al., 2007:* *13-week study in rats*	$BMC_{0.5}L_{90}$ = 119.7 ppm (Hill model – PROAST 38.9 software) $BMC_{0.5}L_{90\ HEC\ ADJ}$ = 25.6 ppm	25 UF_A 2.5 UF_H 10	**Subchronic TRV** **1 ppm** (4.3 $mg.m^{-3}$) Confidence level: high
		75 UF_A 2.5 UF_H 10 UF_S 3	**Chronic TRV** **0.3 ppm** (1.5 $mg.m^{-3}$) Confidence level: high

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KEYWORDS

Ethylbenzene, toxicity reference value, inhalation, acute, subchronic, chronic, ototoxicity