ANSES Opinion
Request No 2018-SA-0152

The Director General

Maisons-Alfort, 29 September 2020

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

on the development of acute and chronic TRVs by the respiratory route for xylenes (mixed xylene CAS No.1330-20-7, meta-xylene CAS No. 108-38-3, ortho-xylene CAS No. 95-47-6, para-xylene CAS No. 106-42-3)

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 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 29 September 2020 shall prevail.

On 2 July 2018, ANSES received a formal request from the Directorate General for Health (DGS) and the Directorate General for Risk Prevention (DGPR) to assess the relevance of a potential cumulative risk for toluene, xylenes and benzene and to establish, to the extent possible, a TRV for this mixture.

1. BACKGROUND AND PURPOSE OF THE REQUEST

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 threshold 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 a dose-response relationship to be established;
- 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;
- for a non-threshold TRV, conducting a linear extrapolation to the origin in order to determine an excess risk per unit.

TRVs are formulated according to a highly structured and rigorous approach involving collective assessments by groups of specialists.

The Provence-Alpes-Côte d'Azur Regional Health Agency (ARS) questioned the DGS about a specific situation concerning exposure to a gaseous mixture of toluene, xylenes and benzene within a school in Marseille located near a former industrial site. ANSES decided to add ethylbenzene as a substance in the mixture, as it is often measured together with toluene, xylenes and benzene.

The Agency also decided to handle this request in two stages by:

1. Responding specifically to the question of a potential cumulative effect of the mixture of the following chemicals: BTEX (Benzene – Toluene – Ethylbenzene – Xylenes),
2. Proposing a general methodology for developing TRVs for substance mixtures.

ANSES already has a toxicological profile and TRVs for benzene, ethylbenzene and toluene (ANSES, 2013; 2016 and 2017b), but not for xylenes. This opinion therefore only concerns xylenes.

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)".

ANSES entrusted examination of this request to the Expert Committee (CES) on "Health reference values", between October 2019 and July 2020. Several CES experts were appointed as rapporteurs, and the methodological and scientific aspects of their expert appraisal work were regularly submitted to the CES. This work was therefore conducted by a group of experts with complementary skills. The CES validated this opinion on 2 July 2020.

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

Xylenes (or dimethylbenzenes) are monocyclic aromatic hydrocarbons belonging to the family of volatile organic compounds. There are three isomers, which differ in the position of their second side chain (ortho- or o-xylene, meta- or m-xylene and para- or p-xylene). All three isomers are present in petroleum and several of its lighter distillation cuts. The general population is therefore liable to be exposed to the mixture of the three isomers.
Mixed xylene contains varying percentages of the three isomers, with m-xylene always in the majority (40-70%). In addition to the xylene isomers, it also contains 6 to 15% ethylbenzene.

Summary of the toxicological data

Toxicokinetics

In humans, xylenes are readily absorbed by inhalation (60-65%, regardless of the isomer considered) and are mainly distributed in adipose tissue. In animals, xylenes concentrate in lipid-rich tissues such as fat and brain and in well-perfused organs such as the liver and kidneys.

In humans, around 95% of absorbed xylenes are metabolised in the liver. The main metabolic pathway consists in the oxidation of a methyl group to produce methyl-benzyl alcohols and the corresponding methylbenzoic acids which, when conjugated with glycine, form methylhippuric acids. Irrespective of the isomer considered, the quantity of urinary methylhippuric acid accounts for more than 90% of the xylene absorbed. Metabolism in laboratory animals is qualitatively similar to that observed in humans.

Xylenes are mainly eliminated in urine in metabolised form. To a lesser extent, they are also exhaled unchanged. Two distinct elimination phases have been identified: the first one is rapid (half-life of one hour), while the second is slower (half-life of 20 hours) and corresponds to the release of xylenes distributed in adipose tissue.

PBPK[1] models have been developed for rats (Tardif *et al.*, 1991, 1992, 1993 as cited in US EPA 2003) and humans (Tardif *et al.*, 1993, 1995; Haddad *et al.*, 1999 as cited in US EPA 2003) and can be used to predict blood and tissue concentrations of m-xylene following inhalation exposure. An adapted version of the model, published in 2015, has also modelled the urinary concentration of m-methylhippuric acid for interpreting biomonitoring data (Marchand *et al.*, 2015).

Acute toxicity

The main effects following acute exposure to xylenes are neurological effects, and eye and respiratory irritant effects.

In the body of human data available, subjective neurological effects such as headache, nausea, dizziness, vomiting, vertigo and cognitive impairment have been reported by volunteers during acute exposure under controlled conditions at concentrations of between 50 and 690 ppm. However, in tests investigating the nervous system, no effects on objectively measured parameters have been reported for acute concentrations below 200 ppm (ATSDR, 2007).

The results obtained in animals confirm the neurotoxic effects described in humans. Various neurological tests have thus shown:

- decreased operant responses in rats exposed to 113 ppm of mixed xylene,
- impaired responses to electrical discharge in rats and mice exposed to 230 – 320 ppm of o-xylene,
- decreased axonal transport in rats exposed to 800 ppm of p-xylene,
- decreased motor coordination in the rotarod test in rats exposed to higher concentrations of around 1000-2000 ppm of mixed xylene or of each of the three isomers (ATSDR, 2007).

A recent study (Armenta-Reséndi *et al.*, 2019) conducted in male Wistar rats showed that exposure to m-xylene for 30 minutes induced a dose-dependent anxiolytic and antinociceptive effect, learning disorders and a decrease in social interactions from 4000 ppm, as well as impaired motor coordination at 8000 ppm.

Ototoxic effects have also been reported from acute exposure to 1400 ppm of mixed xylene, through altered auditory thresholds (ATSDR, 2007).

Irritation

In controlled studies in volunteers exposed to concentrations of between 50-690 ppm of xylenes, irritation to the eyes and respiratory tract has been consistently reported (Carpenter *et al.*, 1975; Dudek *et al.*, 1990; Gamberale *et al.*, 1978; Nelson *et al.*, 1943; NIOSH 1981 as cited in ATSDR

[1] Physiological-Based PharmacoKinetic

2007). Exposure of volunteers to 50 ppm of m-xylene for 2 hours induced statistically significant increases in perceived eye and nasal irritation (men and women) and sensations of throat and respiratory tract irritation in women (Ernstgård *et al.*, 2002). In this study, respiratory symptoms measured objectively by spirometry corroborated the perceived symptoms of respiratory irritation: a spirometric examination conducted three hours after cessation of exposure showed statistically significant, albeit small, changes in women exposed to m-xylene compared to controls; forced vital capacity[2] (FVC) was 2.81% lower than the pre-treatment measurement (0.06% reduction during exposure to clean air, $p<0.01$), and the Tiffeneau index (FEV1/FVC ratio) was 1.09% higher (0.34% increase during exposure to clean air, $p<0.03$).
In animals, acute respiratory exposure to xylenes induced decreased lung surfactant from 300 ppm and a 50% decrease in respiratory rate in mice exposed for 6 minutes to concentrations of between 1361 and 2700 ppm (ATSDR, 2007).

- Sensitisation

Xylene is not a respiratory sensitiser. Several recent studies dedicated to assessing indoor air quality have focused on the impact of environmental exposure to pollutant mixtures including xylenes on the occurrence of asthma-like effects (Bolden *et al.*, 2015). Because exposure to xylenes is closely correlated with exposure to other pollutants, distinguishing xylene-induced effects from those resulting from exposure to other pollutants is complex and considerably limits the possibility of attributing these effects to xylenes alone.

- Subchronic and chronic toxicity

Following exposure by inhalation, the central nervous system (CNS) is the target organ of xylenes, both in humans and in animals.
Repeated exposure to xylenes in the workplace can induce severe neurotoxic effects characterised by behavioural, hearing and colour-vision disorders.
Xylene is one of the solvents covered by Table 84 of the French general regime of occupational diseases (light-headedness or narcotic syndrome that can lead to coma and encephalopathies characterised by impaired cognitive functions).
A cross-sectional study, in which occupational exposure to xylenes accounted for 70% of the exposure to various solvents, reported increases in the prevalence of subjective neurological symptoms (anxiety, memory loss, floating sensations, dizziness) in Chinese workers exposed to an average concentration of 14 ppm for an average of 7 years (Uchida *et al.*, 1993). The relationship with exposure to xylenes alone is difficult to study because of the concomitant exposure to other solvents, poor quantification of exposure and various methodological problems.
Several recent studies investigating the association between combined exposure to noise and different organic solvent mixtures in the workplace and hearing impairment were identified in the literature for the period 2012-2019 (Metwally *et al.*, 2012; Hughes *et al.*, 2013; Unlu *et al*., 2014; Juárez-Pérez *et al.*, 2014; Staudt *et al*., 2019). The implication of xylenes alone in the hearing impairment observed in these studies is limited by the fact that the workers were exposed to other solvents known to be ototoxic.
A recent study exploring the effects of exposure to xylenes (mean: 36.5 $mg.m^{-3}$, range: 8 to 217 $mg.m^{-3}$) on the hearing ability of histological laboratory workers compared to a control group (students) demonstrated impaired hearing in workers compared to controls at 16 hours post-exposure (Fuente *et al.*, 2013). However, concomitant exposure to ethylbenzene (a more potent ototoxin than p-xylene in animal studies and found in mixed xylene) was not measured, and the assessment of the intensity of past exposure to xylene was based on a survey.

[2] Forced Vital Capacity is the volume of gas exhaled during an exhalation performed as hard, quickly and completely as possible from a full inspiration.

A study from the literature showed colour vision impairments in shipyard painters, exposed mainly to xylenes (Lee *et al.*, 2013). Compared to the controls, the colour confusion index (CCI) among painters, measured by the Lanthony D-15 hue test, was significantly higher. However, only urinary methylhippuric acid was assayed as a biomarker and used in the statistical analyses (there was no assaying of biomarkers from other solvents to which the painters were exposed). The observed effects cannot therefore be attributed to xylenes alone. Moreover, the tests were carried out in the morning before the painters started their shifts, i.e. only one night since cessation of the last exposure, which is not enough to be able to distinguish between acute and chronic effects on colour vision.

In animals exposed to xylenes by inhalation for subchronic periods, neurobehavioural effects were observed. At higher concentrations, xylenes, especially the para-isomer, are also ototoxic.

In the only available chronic study, in which rats were exposed to 1096 ppm of o-xylene for 8 h/d for 1 year, potential effects on the nervous system were not investigated (ATSDR, 2007). In contrast, several subchronic studies investigating the neurotoxicity of m-xylene in rats were conducted by the same team (Korsak *et al.*, 1992, 1994; Gralewicz *et al.*, 1995; Gralewicz and Wiaderna, 2001).

In a subchronic study in male Wistar rats exposed for 6 h/d, 5 d/week, for 3 months to 0, 50 or 100 ppm of m-xylene, Korsak *et al.* (1994) found a decrease in motor coordination (dose-dependent decrease in performance in the rotarod test, statistically significant only at 100 ppm) and an increase in sensitivity to thermal pain (statistically significant decrease in latency time in the hot-plate test at 50 and 100 ppm). In contrast, Gralewicz *et al.* (2001) observed a decrease in thermal pain experienced in a hot-plate test performed 50 days after stopping 4-week (6 hr/d, 5 d/week) exposure to 100 ppm of m-xylene and 24 hours after inducing electric shock stress (investigation of stress modulation of the heat-induced pain response).

In the various studies conducted by this team, the other neurological effects observed in male rats exposed to 100 ppm of m-xylene were decreased performance in the rotarod test and decreased motor activity at exposure for 6 h/d, 5 d/week for 6 months (Korsak *et al.*, 1992), decreased spatial learning abilities (star maze) at 6 h/d, 5 d/week for 3 months (Gralewicz *et al.*, 1995), and decreased learning and memory abilities (passive and active avoidance test) at 6 h/d, 5 d/week for 4 weeks. Some neurological damage persisted for 5 to 9 weeks after cessation of exposure (Gralewicz and Wiaderna, 2001).

The ototoxicity of the three xylene isomers was compared in male rats exposed for 6 h/d, 5 d/week for 3 months (Gagnaire *et al.*, 2001) or 6 h/d, 5 d/week for 3 weeks (Maguin *et al.*, 2006). No ototoxic effects were associated with subchronic exposure to o-xylene or m-xylene up to a concentration of 1800 ppm. On the other hand, exposure to p-xylene induced an ototoxic effect at 1800 ppm for 3 weeks and from 900 ppm for 3 months. In a study conducted in 23-day-old weaned rats exposed for 14 h/d, 7 d/week for 6 weeks to 0, 800, 1000 and 1200 ppm of mixed xylene, increased auditory thresholds determined by tonal audiometry and auditory evoked potential measurements were observed at 800 ppm and above (Pryor *et al.*,1987 as cited in ATSDR, 2007).

- Reprotoxicity and effects on development

The available human data on the potential effects on fertility or development of occupational exposure to xylenes are inconclusive, in view of their divergent results and methodological biases that limit their interpretation.

Several recent epidemiological studies (McCanlies *et al.*, 2012; Von Ehrenstein *et al*., 2014; Talbott *et al.*, 2015; Stingone *et al*., 2017; Yousefian *et al.*, 2018; Dellfratte *et al.*, 2019) have examined exposure to pollutant mixtures including xylenes with regard to neurodevelopmental effects (effects on cognitive function, mainly autistic syndrome). However, the design of these studies, the way they characterised exposure and the co-exposure to other neurotoxic agents limit attribution of the observed effects to xylenes alone.

In a one-generation reproductive toxicity study conducted by inhalation in rats, no effects on fertility, reproductive organ weights or reproductive parameters were observed up to the maximum tested concentration of 500 ppm (Biodynamics, 1983 cited in OEHHA, 2012).
Xylenes are foetotoxic in animals (decreased foetal weight, increased skeletal variations) at high concentrations (500 ppm), sometimes in the absence of maternal toxicity. Comparative studies have shown no qualitative differences in the developmental toxicity of the different xylene isomers (Saillenfait *et al.*, 2003).
Neurodevelopmental deficits (decreased motor coordination and impaired performance in learning and memory tests) have been observed in the offspring of female rats exposed during gestation to 500 ppm of mixed xylene (Hass *et al.*, 1993, 1995 and 1997 as cited in OEHHA, 2012).
In Europe, according to the harmonised classification currently in force, xylenes are not classified as reprotoxic compounds. However, they are included in the Community rolling action plan (CoRAP) and among the concerns listed by Germany, the Member State responsible for their assessment, the need for a classification as Repr. 2 H361 (suspected of damaging fertility or the unborn child) should be clarified.

- Genotoxicity

A large number of *in vitro* tests with or without metabolic activation indicate that neither mixed xylene nor any of the three isomers are mutagenic in bacteria, yeast or mammalian cells, nor are they clastogenic in mammalian cells.

The limited human data show no increase in sister chromatid exchanges or chromosomal aberrations in peripheral blood lymphocytes of workers exposed to xylenes by inhalation. The absence of any genotoxic effect *in vivo* is supported by negative results from animal chromosomal aberration or micronucleus assays by the oral or intraperitoneal route (ATSDR, 2007).

According to the International Agency for Research on Cancer (IARC) and the NTP[3], xylenes are not genotoxic (NTP, 1986; IARC, 1999). Xylenes have been examined by the European Union, which did not classify them as genotoxic compounds (CLP, 2008).

- Carcinogenicity

Human data on the carcinogenic potential of xylenes are limited. In a retrospective cohort of 14,457 workers, which investigated the potential link between exposure to different solvents and increased mortality, particularly from haematological malignancies, none of the 108 xylene-exposed workers died from myeloma or non-Hodgkin's lymphoma (ATSDR, 2007). A recent meta-analysis of nine case-control studies investigating a potential link between occupational exposure to aromatic hydrocarbons (benzene, toluene and xylenes) – estimated using job-exposure matrices – and multiple myeloma risk, showed that exposure to each of the solvents was associated with an excess risk of multiple myeloma (De Roos *et al.*, 2018). However, the high correlation of exposure to the three solvents (particularly benzene, a known carcinogen of the haematopoietic system) limits the interpretation of causality, and it is not possible to attribute these effects to xylenes.

No data are available on the carcinogenic effect of xylene by inhalation in animals.

The IARC (1999) and the European Union (CLP, 2008) have not classified xylenes as carcinogenic (not classifiable as to its carcinogenicity to humans).

[3] National Toxicology Program

TRVs

All three isomers have similar toxicokinetic properties and induce toxicological effects of similar nature and potency. The TRVs established by the different authorities therefore relate to mixed xylene, as well as these three isomers.

Acute TRV

Choice of the critical effect

The available data, both in humans and in animals, provide consistent evidence that acute inhalation exposure to xylenes can affect the CNS and respiratory system.
In humans, studies under controlled conditions have shown subjective neurological effects from 50 ppm. In contrast, no effects on objectively measured parameters have been reported in the available corpus of data for acute concentrations of xylenes below 200 ppm. In animals, various neurological tests have objectively demonstrated CNS damage from acute exposure to high concentrations of xylenes.
Of the many studies under controlled conditions in volunteers demonstrating irritation of the respiratory tract, only the study by Ernstgård *et al.* (2002) reported respiratory symptoms measured objectively by spirometry combined with subjective symptoms of irritation. Here, a decrease in FVC was observed in women three hours after cessation of exposure.
In animals, acute respiratory exposure to xylenes induced decreased lung surfactant from 300 ppm and a 50% decrease in respiratory rate in mice exposed for 6 minutes to concentrations of between 1361 and 2700 ppm of xylenes (ATSDR, 2007).
Several mechanistic assumptions underlying the observed pulmonary effects have been advanced (altered lung surfactant, inhibition of lung microsomal enzymes, oxidative stress and inflammation).

Despite the limited extent of the objective effects measured in the study by Ernstgård (2002) and the uncertainties inherent in the spirometry examination, the CES decided to adopt the decrease in FVC (an objectively measured effect) as the critical effect, in view of the large body of evidence supporting an irritant effect of xylenes on the respiratory tract.

Analysis of the existing TRVs/GVs[4]

An acute TRV developed by OEHHA in 1999, a second TRV developed by the ATSDR in 2007 and a guideline value set as part of the INDEX project[5] (EC, 2005) are available.

- Since OEHHA's acute TRV (1999) and the INDEX project's short-term GV (EC, 2005) were based only on subjective symptoms of irritation, the CES did not accept these values.
- The critical effects (i.e. neurological and respiratory effects) for acute exposure identified by the ATSDR (2007) are based on a substantial body of human data under controlled conditions, consistently reporting respiratory tract irritation (breathing difficulties, nose and throat discomfort, decreased respiratory capacity) and neurological disorders (dizziness, headache, short-term memory impairment, increased reaction time).

  The ATSDR selected the study by Ernstgård *et al.* (2002) as the key study, because it provided the lowest LOAEC[6] for the two critical effects identified (i.e., neurological and respiratory effects) among the available body of human and animal data. This study

[4] Guideline values
[5] In 2005, the report of the European INDEX project (EC, 2005), funded by the European Commission's Directorate General for Health and Consumers (DG SANCO), compiled a list of priority chemical pollutants in indoor environments that could be regulated in the future, and proposed indoor air quality guideline values.
[6] Lowest Observed Adverse Effect Concentration

contained a large number of individuals (56 volunteers, 28/sex). In addition to a questionnaire on the symptoms experienced, the protocol included objectively measured parameters (spirometry, blinking, colour vision and nasal wash). However, the fact that only one concentration of m-xylene was tested (50 ppm for two hours) limits the quality of the study. Furthermore, the CES points out that the methodology used for the spirometry examination is not detailed in the publication.

The effects taken into consideration by the ATSDR in order to set the acute TRV are mostly self-reported neurological symptoms and perceived irritation, however, one objectively measured parameter (i.e. decrease in forced vital capacity) was affected by the treatment. The experts believe that the objectively measured effect should be considered as the critical effect in line with the TRV development methodology used at ANSES.

The uncertainty factors adopted by the ATSDR (10 for the inter-individual variability, UF_H, and 3 to take into account the use of a LOAEC, UF_L) are consistent with the method used by ANSES to develop TRVs (ANSES, 2017).

Despite the limitations noted above and given the lack of new data appropriate for establishing an acute TRV by inhalation, the CES adopts the acute TRV developed by the ATSDR of 8.7 mg.m^{-3} (2 ppm).

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

- **Chronic TRV**
  - Choice of the critical effect

Following exposure by inhalation, the nervous system is the target organ of xylenes, both in humans and in animals.

While chronic occupational exposure to a solvent mixture is associated with neurological effects, the available data cannot be used to establish a relationship with chronic exposure to xylenes alone, due to concomitant exposure to other solvents and various methodological issues.

In animals, the results obtained in experimental studies show neurobehavioural effects in adults (50-100 ppm) and neurodevelopmental effects in the young (500 ppm). At higher concentrations, xylenes, especially the p-xylene isomer, are also ototoxic.

In the study by Korsak *et al.* (1994), two neurological parameters were altered in the treated groups:

- increased sensitivity to thermal pain (statistically significant decrease in latency time in the hot-plate test at 50 and 100 ppm),
- decreased motor coordination (statistically significant increase in the percentage of failures in the rotarod test at 100 ppm).

Apart from the fact that the hot-plate test is ethically questionable and may present experimental biases, the increased sensitivity to thermal pain seems counter-intuitive, in view of xylene's narcotic and anaesthetic properties. Furthermore, in a second study (Gralewicz and Wiaderna, 2001), the hot-plate test did not show any increased response to heat-induced pain. However, this test had different objectives to the study by Korsak *et al.* (1994) (i.e., stress modulation of the heat-induced pain response vs heat-induced pain response) as well as different durations of exposure and lengths of time between cessation of exposure and performing the test, which limits a direct comparison of the results obtained in the two studies.

The rotarod test may also have experimental biases and should preferably be supplemented with other tests in the range of available behavioural tests to assess motor skills. However, convergent

results were observed in another study: decreased performance in the rotarod test and decreased motor activity at 100 ppm for 6 months and at 1000 ppm for 3 months (Korsak *et al.*, 1992). Impaired performance in the rotarod test was also reported for acute exposure (Korsak *et al.*, 1989, 1990, 1993 cited in ATSDR, 2007) and, although not significantly, after *in utero* exposure (Haas *et al.*, 1995).

The CES therefore decided to adopt the decrease in motor coordination shown in the rotarod test as the critical effect.

- Analysis of the existing TRVs/GVs

Three chronic TRVs (RIVM[7], 2001; US EPA, 2003 and ATSDR, 2007) and three GVs (WHO, 1997; INDEX Project[8], 2005 and UBA[9], 2015) were identified for xylenes.

- Because the chronic TRVs from the ATSDR (2007) and OEHHA (2000) and the chronic GV from the INDEX project (EC, 2005) were based only on subjective symptoms, the CES did not accept any of these values.
- The study by Hass and Jakobsen (1993) selected as the key study by the WHO and RIVM had the following methodological limitations: only one concentration tested (200 ppm) and rotarod test not performed "blind", nor on the same day. In addition, in a subsequent study by the same team (Hass *et al.*, 1995), the performance in the rotarod test (blind, same day) of animals exposed *in utero* to 500 ppm of mixed xylene did not differ significantly from that of controls. For the above reasons, the CES did not adopt the value set by the WHO (1997) and the RIVM (2001).
- In the absence of suitable human data and chronic animal studies, the choice of the key study and related studies by the US EPA and UBA appears justified. The study by Korsak *et al*. (1994) is considered to be of good quality: population size (12 animals/test group and 24 controls) was satisfactory for a subchronic study, neurological effects were measured using objective tests, and general toxicity was properly investigated.

  The US EPA considered that sensitivity to thermal pain should not be selected as the critical effect in view of the divergent results obtained for this same parameter in the study by Gralewicz and Wiaderna (2001). The US EPA therefore selected impaired motor coordination as the critical effect and set a NOAEC[10] of 50 ppm as the point of departure. The CES adopted the same critical effect as the US EPA in view of the converging results for this test in the body of available data.

  The time adjustment to extrapolate the exposure duration used in the study by Korsak *et al.* (1994) to a continuous exposure ($NOAEC_{ADJ}$ = 217 $mg.m^{-3}$ x 6 h/24 h x 5 d/7 d = 38.75 $mg.m^{-3}$) and the allometric adjustment to establish a $NOAEC_{HEC}$ used by the US EPA are consistent with the method used to develop TRVs at ANSES (ANSES, 2017).

  To develop its chronic TRV, the US EPA applied an uncertainty factor of 300 to the $NOAEC_{HEC}$ (3 to account for the toxicodynamic component of interspecies variability, $UF_{A\text{-}TD}$, 10 for interindividual variability, UF_H, 3 to account for subchronic to chronic transposition, UF_S, and 3 to account for the lack of data exploring the chronic toxicity of xylene UF_D). The uncertainty factors used by the US EPA are consistent with the method used by ANSES to develop TRVs (ANSES, 2017); however, it should be noted that this value of 300 is in fact the value of 270 rounded up.

[7] Rijksinstituut voor Volksgezondheid en Milieu
[8] In 2005, the report of the European INDEX project (EC, 2005), funded by the European Commission's Directorate General for Health and Consumers (DG SANCO), compiled a list of priority chemical pollutants in indoor environments that could be regulated in the future, and proposed indoor air quality guideline values.
[9] Umweltbundesamt – German Federal Environment Agency
[10] No Observed Adverse Effect Level

Despite the limitations noted above and given the lack of new data appropriate for establishing a chronic TRV by inhalation, the CES adopts the chronic TRV developed by the US EPA of 0.1 mg.m^{-3} (0.03 ppm).

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

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions and recommendations of the CES "Health reference values" on the development of toxicity reference values by inhalation for xylenes.

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.

Based on the available institutional reports and an additional literature search over the period [2012-2019], this expert appraisal led to an acute TRV and a chronic TRV being selected from among the TRVs/GVs identified.

Type of TRV	Organisation	Critical effect (key study)	Critical concentration	UF	TRV
Acute TRV	ATSDR (2007)	Respiratory effects: decreased forced vital capacity associated with perceived effects of respiratory irritation. *Ernstgård et al., 2002: controlled study in humans (healthy volunteers)*	LOAEC = 50 ppm	30 UF_H = 10 $UF_{B/L}$ = 3	**8.7 mg.m^{-3}** (2 ppm) Confidence level moderate/low
Chronic TRV	US EPA (2003)	Neurological effects: Impaired motor coordination (rotarod test) *Korsak et al., 1994: 90-day study by inhalation (dynamic chamber) in rats, 6 hr/d, 5 d/week*	NOAEC = 217 mg.m^{-3} (50 ppm) <u>Time adjustment</u> $NOAEC_{ADJ}$ = 38.75 mg.m^{-3} <u>Allometric adjustment</u> $NOAEC_{HEC}$ = 38.75 mg.m^{-3}	300 UF_{A-TD} = 3 UF_H = 10 UF_S = 3 UF_D = 3	**0.1 mg.m^{-3}** (0.03 ppm) Confidence level moderate/low

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KEYWORDS

Valeur toxicologique de référence, VTR, xylène, inhalation, respiratoire, irritation, neurotoxicité, aiguë, chronique

KEY WORDS

Toxicity reference value, TRV, xylene, inhalation route, respiratory, irritation, neurotoxicity, acute, chronic