ANSES Opinion
Request No. 2021-MPEX-0005
Related Request No. 2021-MPEX-0006

The Director General

Maisons-Alfort, 8 July 2024

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

on the updating of short-, medium- and long-term TRVs by the respiratory route for benzene (CAS No. 71-43-2)

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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 8 July 2024 shall prevail.

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 (short-, medium-, long-term) and route (oral, respiratory, dermal) 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. On the basis of current knowledge and by default, it is generally considered that for non-carcinogenic effects, toxicity is only expressed above a threshold dose (ANSES, publication pending).

In practice, establishing a TRV involves the following steps:

- identifying the target organ(s) and critical effect on the basis of the toxicological profile;
- identifying the assumption on which it is based: with or without a threshold dose, depending on the substance's mode of action;

- selecting one or more key studies of good scientific quality and greatest relevance from among the epidemiological or toxicological studies, in order to establish a dose-response relationship;
- defining a point of departure (PoD) for humans or animals on the basis of this study/these studies;
- applying time and allometric adjustments if necessary;
- for a threshold TRV, applying uncertainty factors (UFs) to this PoD so as to derive a TRV that is applicable to the entire population;
- for a no-threshold TRV, determining the slope and/or concentrations/doses associated with several different risk levels;
- setting a confidence level.

The nature of the TRV (short-, medium- or long-term) is defined by the duration of exposure, which in turn is determined by the duration of exposure in toxicological studies and also by health risk assessment (HRA) needs. As a reminder, concerning TRVs, in line with the scenarios generally considered when assessing health risks in humans, ANSES distinguishes between three types of exposure duration:

- short-term exposure, from 1 to 14 days;
- medium-term exposure, from 15 to 364 days;
- long-term exposure, more than 365 days.

TRVs are used to protect the entire population, including sensitive population groups such as children, from the effects of a substance following short-, medium- or long-term exposure.

They are established according to a structured and rigorous approach involving collective assessments by groups of specialists.

As part of its ongoing work on reference values, ANSES has already conducted several expert appraisals on reference values for benzene in the general population:

- In 2008, ANSES selected the TRVs from the ATSDR and WHO to propose four indoor air quality guideline values (IAQGs):
  - an IAQG of 29 $\mu g \cdot m^{-3}$ for threshold effects associated with short-term exposure;
  - an IAQG of 19 $\mu g \cdot m^{-3}$ for threshold effects associated with medium-term exposure;
  - an IAQG of 10 $\mu g \cdot m^{-3}$ for threshold effects associated with long-term exposure;
  - a long-term, no-threshold IAQG corresponding to an excess risk per unit (ERU) of 6.10^{-6} $(\mu g/m^3)^{-1}$ to protect against the carcinogenic effects of benzene.
- In 2014, a long-term no-threshold TRV corresponding to a ERU of $2.6.10^{-5}$ $(\mu g/m^3)^{-1}$ was established based on the study by Richardson *et al.* (2008) to protect against the carcinogenic effects of benzene.

The purpose of the current expert appraisal is therefore to update the inhalation TRVs proposed in 2014. This update is also in relation to:

- the formal request of 2 July 2018 from the Directorate General for Health (DGS) and Directorate General for Risk Prevention (DGPR) to assess the relevance of a potential cumulative risk for benzene, toluene, ethylbenzene and xylenes (BTEX) and, as far as

possible, to establish a TRV for this mixture. As part of this work, an analysis of new toxicological data on the four substances was carried out (ANSES, 2022);

- the update of the indoor air quality guideline values (IAQGs) for benzene (Request No. 2021-MPEX-0006), which forms part of the Agency's ongoing work.

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 on "Health Reference Values" (CES VSR). ANSES entrusted the expert appraisal to the Working Group on "Benzene TRVs". The methodological and scientific aspects of the work were presented to the CES on 28/05/2021, 23/09/2021, 18/11/2021, 13/05/2022, 22/09/2022, 20/10/2022, 09/11/2023 and 14/12/2023. The work was adopted by the CES VSR at its meeting on 26 January 2024.

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 website: https://dpi.sante.gouv.fr/.

3. ANALYSIS AND CONCLUSIONS OF THE CES

3.1. Summary of the toxicological data

The summary of the toxicological data was drawn up based on expert appraisal reports by ANSES (ANSES, 2008; ANSES, 2014) and summary reports by internationally recognised organisations (ATSDR, 2007; ATSDR, 2015; OEHHA, 2014; IARC, 2018; Health Canada, 2023), supplemented by a literature search covering the 2021-2023 period.

As inhalation is the main route of human exposure to benzene, the updated TRVs specifically concern this route of exposure. As a result, only data on toxicity following inhalation exposure are presented in this document.

3.1.1. Toxicokinetics

Benzene is rapidly absorbed after inhalation exposure. In humans exposed by inhalation to 47 and 110 ppm (150 to 351 $mg \cdot m^{-3}$, respectively) of benzene for three to four hours, the absorption rate measured in the first five minutes after exposure was between 70 and 80%. After one hour of exposure, the absorption rate fell to between 20% and 60% (Srbova *et al.*, 1950). Data on workers indicate that benzene is also absorbed through the skin (ATSDR, 2007).

Due to its high lipophilicity, benzene is then distributed throughout the body and tends to accumulate in lipid-rich tissues (ATSDR, 2007). High levels of benzene have been observed

in human adipose tissue and bone marrow after inhalation. Benzene also crosses the blood-brain barrier and can be found in the brain (Winek and Collom, 1971). In addition, it crosses the placental barrier and is found in umbilical cord blood at concentrations equal to or greater than those found in maternal blood (Dowty *et al.*, 1976).

The metabolic pathway for benzene is similar in humans and small rodents. It does not appear to be influenced by the route of absorption. Benzene is mainly metabolised in the liver, but also in other tissues where it has accumulated, particularly in bone marrow. Benzene metabolism involves several stages. The first consists of oxidation to benzene oxide and benzene oxepin (formation in equilibrium). This stage is catalysed by cytochrome P450 2E1 (CYP450 2E1) (Lindstrom *et al.*, 1997). Several pathways are then involved in the metabolism of benzene oxide: the predominant one is a non-enzymatic rearrangement leading to the formation of phenol (Jerina *et al.*, 1968). The phenol is then oxidised in the presence of CYP450 2E1 to catechol and hydroquinone, which are oxidised to 1,2 and 1,4- benzoquinone, respectively. In bone marrow, this reaction is catalysed by myeloperoxidase (MPO) (Nebert *et al.*, 2002). Catechol and hydroquinone can be metabolised via CYP450 2E1 to 1,2,3-benzenetriol. Benzene oxide can also be metabolised to trans-, trans-muconic or S-phenylmercapturic acid.

The data available for humans following inhalation exposure have shown that exhalation is the main route of elimination for non-metabolised benzene. Absorbed benzene is also excreted via urine in the form of phenols (phenol, catechol, hydroquinone, trihydroxybenzene in free, sulpho- and glucuroconjugated forms), muconic acid and S-phenylmercapturic acid, as well as in unchanged form. Some of these urinary metabolites, like urinary benzene, can be used as biomarkers of benzene exposure.

3.1.2.Acute toxicity

A few older case reports in humans stated that inhalation of benzene at a concentration of 20,000 ppm (64,980 $mg \cdot m^{-3}$) for 5 to 10 minutes was generally fatal (Flury *et al.*, 1928; Gerarde, 1960). Death following exposure to benzene is often attributed to asphyxiation, respiratory arrest or depression of the central nervous system. In mild forms of poisoning, excitation followed by speech problems, headaches, dizziness, insomnia, nausea, paraesthesia of the hands and feet and fatigue have been reported. These symptoms were generally observed at concentrations of between 300 and 3000 ppm (975 and 9750 $mg \cdot m^{-3}$) (Cronin, 1924; Flury, 1928; Midzenski *et al.*, 1992).

3.1.3.Irritation

High concentrations of benzene in the air cause irritation of the mucous membranes of the eyes, nose and respiratory tract (ATSDR, 2007). Liquid benzene is irritating to the skin in the event of prolonged or repeated contact. In the study by Yin *et al.* (1987), 300 workers exposed for over a year to 33 ppm of benzene (105 $mg \cdot m^{-3}$) for men and 59 ppm (188 $mg \cdot m^{-3}$) for women complained of eye irritation.

3.1.4.Subchronic and chronic toxicity

- Haematological effects

Numerous epidemiological studies of workers exposed to different concentrations of benzene over the medium or long term have revealed a number of haematological effects. Bone marrow damage is one of the first signs of chronic benzene toxicity. Most of the blood-related effects

(thrombocytopaenia, granulocytopaenia, lymphopaenia, anaemia, pancytopaenia and leukaemia) were associated with inhalation exposure.

Aplastic anaemia is one of the most severe effects induced by benzene inhalation. It occurs when bone marrow function is impaired and stem cell maturation is affected. Aplastic anaemia can progress to myelodysplastic syndrome and then to leukaemia (Aksoy, 1980). The presence of chromosomal abnormalities may be associated with the occurrence of myeloproliferative syndrome, the transition from aplastic anaemia to myelodysplastic syndrome and the development of leukaemia.

- Immunological effects

Exposure to benzene impairs humoral and cellular immunity (IARC, 2018). Initially, it was shown that benzene impairs humoral immunity by inducing changes in blood concentrations of immunoglobulins. A reduction in IgG and IgA immunoglobulins was observed in several recent studies. Benzene also impairs cellular immunity (ATSDR, 2007). Cases of lymphopaenia were reported in a series of studies of workers exposed to benzene in different industries.

- Respiratory effects

The results of studies into the respiratory effects of benzene are equivocal. Some studies have shown positive associations between exposure to benzene and reduced lung function, particularly in children (Wichmann *et al.*, 2009; Martins *et al.*, 2012; Morales *et al.*, 2015; Charpin *et al.*, 2009; Zhou *et al.*, 2013, according to Health Canada, 2023), while others have found no association between benzene exposure and children's respiratory health (Aguilera *et al.*, 2013; Ferrero *et al.*, 2017). Recently, Liu *et al.* carried out a meta-analysis of 15 epidemiological studies on the link between exposure to benzene in indoor and outdoor air and the onset of respiratory symptoms (Liu *et al.*, 2022). This study reported a relative meta-risk of 1.08 ($CI_{95\%}$: 1.02-1.14) for a 1 $\mu g \cdot m^{-3}$ increase in the atmospheric benzene concentration.

3.1.5.Reprotoxicity and developmental toxicity

Studies conducted in the workplace suggest that benzene reduces female fertility. In particular, studies have revealed menstrual cycle disruptions in women occupationally exposed to benzene.

Some studies also report a decrease in male fertility related to the duration of exposure to benzene (significant decrease in sperm count and motility, significant increase in percentages of morphologically abnormal sperm and sperm DNA fragmentation) (ATSDR, 2015).

Studies indicate a significant association between maternal exposure to benzene and the occurrence of premature births (Wilhelm *et al.*, 2011; Estarlich *et al.*, 2016; Dos Santos *et al.*, 2019) and reduced birth weight (Zahran *et al.*, 2012). No association has been reported between *in utero* environmental exposure to benzene and the occurrence of birth defects (Vinceti *et al.*, 2016; Janitz *et al.*, 2018), cognitive effects or the child's psychomotor development at 15 months (Lertxundi *et al.*, 2015).

In animals, embryotoxic and foetotoxic effects, such as reduced body weight and foetal organ weight, have been observed in studies in which pregnant females were exposed by inhalation during gestation. Impairment of haematopoiesis was also observed in the foetus and offspring of pregnant mice exposed to low concentrations of benzene (≤ 20 ppm) (Keller and Snyder, 1986, 1988). No studies have shown that benzene is teratogenic, even at concentrations that induce maternal and foetal toxicity.

3.1.6. Genotoxicity

Benzene has the main characteristics of a carcinogen. In particular, there is strong evidence, including in humans, that benzene is metabolically activated to electrophilic metabolites, induces oxidative stress and associated oxidative damage to DNA, is genotoxic, immunosuppressive and causes haematotoxicity (IARC, 2018). In addition, there is evidence from experimental studies that benzene alters DNA repair, causing genomic instability by inhibiting topoisomerase II, which is involved in DNA replication. In *in vitro* studies on human cells and in mice, benzene metabolites, in particular 1,4-benzoquinone and hydroquinone, have been shown to directly inhibit topoisomerase II.

In studies on workers, benzene induced oxidative DNA damage, single- and double-strand DNA breaks, gene duplications, chromosomal aberrations and micronuclei. The specific cytogenetic changes induced include aneuploidy, translocations and various other structural changes to chromosomes. These conclusions, based on epidemiological studies, are largely confirmed by *in vitro* and *in vivo* studies. The formation of DNA adducts in bone marrow, chromosomal aberrations and micronuclei have been shown in animals following exposure to benzene. Similarly, after treatment of human cells *in vitro*, benzene or its metabolites induced DNA adducts and damage, as well as chromosomal aberrations (IARC, 2018).

The available data show that it is the metabolites of benzene that lead to genotoxic effects. It is possible that each metabolite produces its own effects, which would explain the differences observed in the results of the various tests carried out (ANSES, 2014).

3.1.7. Carcinogenicity

Benzene has been classified as a human carcinogen (Group 1) by the IARC since 1979, on the basis of sufficient evidence in humans and animals that it causes leukaemia. This assessment was confirmed specifically for acute myeloid leukaemia (AML) and/or acute non-lymphocytic leukaemia (ANLL) in adults, in the IARC monographs published in 2012 and then in 2018[1]. In 2012, the IARC also concluded that there were positive associations with the risks of acute lymphocytic leukaemia (ALL), chronic lymphocytic leukaemia (CLL), multiple myeloma (MM) and non-Hodgkin's lymphoma (NHL)[2]. In addition, the IARC concluded in 2018 that there were positive associations with the risk of chronic myeloid leukaemia (CML), lung cancer and AML in children.

The vast majority of the available studies have been carried out in the workplace. The first occupational cohort studies carried out on workers in the rubber and petroleum industries revealed excess mortality from leukaemia and other malignant diseases of the lymphatic and

[1] In 2018, the IARC clarified that ANLL was included in the AML category due to changes in classification (WHO, 2017)
[2] In 2018, the IARC clarified that ALL, CLL and MM were included in the NHL category (WHO, 2017)

haematopoietic tissues (Rinsky *et al.*, 2002; Glass *et al.*, 2003; Richardson, 2008; Hayes *et al.*, 1997)

The most recent studies confirm the excess mortality risk found previously, particularly for AML and ANLL (Stenejhem *et al.*, 2015; Rhomberg *et al.*, 2016; Linet *et al.*, 2019). These studies have refined the assessment of benzene exposure and report positive results at relatively low levels of exposure compared with previous studies.

Two meta-analyses and meta-regressions on the risk of AML associated with occupational exposure to benzene were recently conducted (IARC, 2018; Scholten *et al.*, 2022).

Thirteen studies on AML incidence and/or mortality in workers were included in the meta-analysis published in the latest IARC monograph (IARC, 2018). A statistically significant meta-RR of 1.54 ($CI_{95\%}$: 1.16-2.05) was obtained. A meta-regression analysis based on six occupational cohort studies showed that the relationship between benzene exposure and the logarithm of the relative risk (RR) was well described by a linear model. The slope was moderately sensitive to the inclusion in the model of a cohort study of workers in the chlorinated rubber sector, which had the highest exposure estimates.

The study by Scholten *et al.* (2022) sought to assess the dose-response relationship for the AML risk by fitting linear and spline-based Bayesian meta-regression models (Scholten *et al.*, 2022). The linear meta-regression model with intercept provided the best prediction of the AML risk after cross-validation.

Data on leukaemia in children are generally based on studies using exposure to road traffic as an indicator of benzene exposure. Two recent meta-analyses produced different results. Filippini *et al.* studied for the first time the shape of the relationship between road traffic density or exposure to atmospheric pollutants (benzene, NO_2) and the risk of childhood leukaemia (Filippini *et al.*, 2019). In the case of benzene, no threshold of exposure was observed, particularly for AML. The association of benzene with AML was considerably stronger than with ALL. On the other hand, the meta-analysis by Gong *et al.* (2019) showed no association between "low" or "high" exposure to benzene and the risk of childhood leukaemia. A non-significant association between moderate exposure to benzene and the risk of childhood leukaemia was reported. The exposure levels corresponding to the three exposure classes "low, moderate, high" were not provided in the study (Gong *et al.*, 2019).

3.1.8. Sensitive population groups

Several population groups can be regarded as sensitive to benzene. The parameters that can modify a population group's sensitivity to benzene are its genetic make-up, age, health and nutritional status. Variability in human susceptibility to benzene is mainly due to genetic polymorphism associated with metabolism (ANSES, 2014).

In mice, ethanol can also increase the severity of benzene-induced anaemia, lymphopaenia and reduced bone marrow cells, and lead to a transient increase in peripheral blood erythroblasts and atypical cell morphology (Baarson *et al.*, 1982).

Gender differences in susceptibility to the toxic effects of benzene have been demonstrated in animals, but not in studies of workers (Kenyon *et al.*, 1998; Brown *et al.*, 1998).

Young children are likely to be more susceptible than adults to benzene inhalation, as the respiratory rate adjusted for body weight and the percentage of absorption are greater in young children than in adults. However, the available studies in humans and animals have not shown any age-related susceptibility.

3.2. Proposed short-term TRV by the respiratory route

3.2.1. Choice of the critical effect

Numerous animal studies have shown that haematological effects are the best documented effects following short-term exposure to benzene, as well as following medium- or long-term exposure (lymphopaenia, leukopaenia, anaemia, reduction in the number of haematopoietic stem cells, etc.). These effects occur at the lowest doses. These changes have also been observed in humans in medium- and long-term epidemiological studies.

The CES therefore selected haematological effects as the critical effect.

3.2.2. Choice of the assumption according to which it is established

For most **non-carcinogenic effects**, it is generally considered, on the basis of current knowledge and by default, that toxicity is only expressed above a threshold dose. **The CES VSR therefore considered that the haematological effect associated with short-term exposure resulted from a threshold dose mechanism**.

3.2.3. Analysis of the existing TRVs

Two short-term respiratory TRVs are available: the ATSDR's 2007 TRV of 29 $\mu g \cdot m^{-3}$ and the OEHHA's 2014 TRV of 27 $\mu g \cdot m^{-3}$. In both cases, the critical effect was a haematotoxic effect observed in mice.

The CES decided **not to select the OEHHA's TRV**. It considered that there were limitations in the critical effect selected by the OEHHA, which was based on a significant reduction in erythroid precursor cells in 2-day-old neonates, with no change in the numbers of total circulating nucleated red blood cells in foetuses or polychromatophilic nucleated red blood cells in neonates at 5 ppm (16 $mg \cdot m^{-3}$). Time and allometric adjustments were made to arrive at a $LOAEC^{3}_{ADJ\ HEC}$ (human equivalent concentration). In addition, the uncertainty factors applied by the OEHHA to the $LOAEC_{ADJ\ HEC}$ differed considerably from the values recommended by ANSES in this case, i.e. an interspecies factor of 2 for the residual uncertainty on the kinetic component ($UF_{A\text{-}TK}$); a default interspecies factor of $\sqrt{10}$ for the dynamic component ($UF_{A\text{-}TD}$); a default intraspecies factor of 10 for the kinetic component ($UF_{H\text{-}TK}$) (according to the OEHHA, a number of toxicokinetic studies and studies of the association between genetic polymorphisms in metabolising enzymes and chronic benzene poisoning suggest that the toxicokinetic variation in adults can be accommodated by the default factor of 10), a default intraspecies factor of $\sqrt{10}$ for the dynamic component (UF_D), and a factor of $\sqrt{10}$ for the use of a LOAEC instead of a NOAEC[4] (UF_L).

The key study selected by the ATSDR, considering the persistent effects on granulopoietic stem cells in adult mice exposed *in utero*, is of good quality. The TRV development process is described very well and complies with ANSES's recommendations on TRV establishment methods (ANSES, publication pending). The ATSDR applied a total uncertainty factor of 300 to the $LOAEC_{ADJ\ HEC}$: a factor of 3 to account for interspecies variability (UF_A), 10 for intraspecies variability (UF_H) and 10 for the use of a LOAEC instead of a NOAEC (UF_L).

[3] Lowest Observed Adverse Effect Concentration
[4] No Observed Adverse Effect Concentration

The CES adopted the ATSDR's short-term respiratory TRV of 29 µg·m^{-3}, considering it to be of good quality, although the uncertainty factors applied differed from the values recommended by ANSES. However, applying the uncertainty factors recommended by ANSES would result in an overall uncertainty factor identical to that of ATSDR.

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

3.3. Proposed medium-term TRV by the respiratory route

3.3.1. Choice of the critical effect

Numerous animal studies (short-, medium- and long-term exposure) have shown that haematological effects are the best documented effects following medium-term exposure to benzene, as well as following short- or long-term exposure (lymphopaenia, leukopaenia, anaemia, reduction in the number of haematopoietic stem cells, etc.). These effects occur at the lowest doses. These changes have also been observed in humans in medium- and long-term epidemiological studies.

The CES therefore selected haematological effects as the critical effect.

3.3.2. Choice of the assumption according to which it is established

For most **non-carcinogenic effects**, it is generally considered, on the basis of current knowledge and by default, that toxicity is only expressed above a threshold dose. **The CES VSR therefore considered that the haematological effects associated with medium-term exposure resulted from a threshold dose mechanism**.

3.3.3. Analysis of the existing TRVs

A medium-term respiratory TRV is available, the ATSDR's 2007 TRV of 19 µg·m^{-3}. This value was calculated from the 1987 study by Rosenthal and Snyder on male mice. They were exposed by inhalation to 32.5, 97.2 and 325 mg·m^{-3} of benzene, 6 hours a day, 5 days a week for 20 exposure days. The ATSDR proposed a LOAEC of 32.5 mg·m^{-3} for the delayed *in vitro* alloreactivity of splenic lymphocytes from exposed mice (same type of critical effect as for the short-term TRV). Time and allometric adjustments were made to arrive at a $LOAEC_{ADJ\ HEC}$ of 5.8 mg·m^{-3}. A total uncertainty factor of 300 was applied to this $LOAEC_{HEC}$: a $UF_{H\text{-}TD}$ of 3 to take account of differences in toxicodynamics between animals and humans, with differences in toxicokinetics taken into account by allometric adjustment, an UF_A of 10 for intraspecies variations and an UF_L of 10 for the use of a LOAEC.

The CES adopted the ATSDR's medium-term respiratory TRV of 2007, considering it to be of good quality, although the uncertainty factors applied differed from the values recommended by ANSES. However, applying these uncertainty factors recommended by ANSES would result in a total uncertainty factor identical to that of ATSDR.

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

3.4. Proposed long-term TRV by the respiratory route

3.4.1. Choice of the critical effect

The main non-carcinogenic effects identified for inhalation exposure to benzene are haematological, immunological, respiratory and reprotoxic. Haematotoxicity and immunotoxicity are well-documented effects of occupational exposure to benzene and are those occurring at the lowest doses. Bone marrow damage is one of the first signs of chronic benzene toxicity. Most of the blood-related effects (anaemia, thrombocytopaenia, granulocytopaenia, lymphopaenia, pancytopaenia and leukaemia) were associated with inhalation exposure.

The CES therefore selected haematological effects as the critical effect.

3.4.2. Choice of the assumption according to which it is established

For most **non-carcinogenic effects**, it is generally considered, on the basis of current knowledge and by default, that toxicity is only expressed above a threshold dose. **The CES VSR therefore considered by default that the haematological effects resulted from a threshold dose mechanism**.

3.4.3. Analysis of the existing TRVs

Four long-term respiratory TRVs are available: US EPA, 2003; ATSDR, 2007; OEHHA, 2014 and TCEQ, 2015. These four TRVs were developed on the basis of epidemiological studies in the workplace, considering the same critical effect: the reduction in the number of lymphocytes.

The TRVs by the US EPA (2003) and TCEQ (2015) were developed from the 1996 Rothman *et al.* study, while those by the ATSDR (2007) and OEHHA (2014) were based on the Lan *et al.* study of 2004. These four TRVs are based on the use of a BMCL[5] that takes the entire dose-response relationship into account.

The two studies, by Lan *et al.* (2004) and Rothman *et al.* (1996), are of equal quality. The Lan *et al.* study has the following advantages: it was conducted on a larger number of individuals, i.e. 240 exposed subjects compared with 44 in the Rothman *et al.* (1996) study. In addition, three exposure groups were formed: < 1 ppm, from 1 to < 10 ppm and ≥ 10 ppm (< 3.19 $mg \cdot m^{-3}$, from 3.19 to 31.9 $mg \cdot m^{-3}$ and ≥ 31.9 $mg \cdot m^{-3}$). Lastly, the benzene exposure concentrations in the Lan *et al.* study were lower. The study by Lan *et al.* (2004) therefore appears to be the most suitable for assessing the dose-response relationship for benzene and proposing a long-term TRV.

The main differences between the ATSDR and OEHHA values, which were both based on the Lan *et al.* study, relate to the choice of benchmark response (BMR) – 0.25sd[6] for the ATSDR versus 0.5sd for the OEHHA – and the uncertainty factors applied, which led the OEHHA to adopt an overall uncertainty factor 20 times higher than that of the ATSDR.

The ATSDR justified its selection of a BMR of 0.25sd by the fact that the resulting $BMCL_{0.25sd}$ (0.1 ppm) was lower than the LOAEC provided in the 2014 study by Lan *et al*. The choice of a BMR of 0.5sd was not justified by the OEHHA. The $BMCL_{0.5SD}$ calculated from this BMR is

[5] Lower limit of the confidence interval of the benchmark concentration (BMC)
[6] BMR of 0.25 or 0.5 standard deviations.

close to the LOAEC in the study (0.476 ppm versus 0.54 ppm, i.e. 1.5 $mg \cdot m^{-3}$ versus 1.7 $mg \cdot m^{-3}$).

With regard to the choice of uncertainty factors, the OEHHA applied an intraspecies factor (UF_H) of 60, considering the genetic polymorphisms in enzymes involved in benzene metabolism and the toxicokinetics of benzene in infants and children, and a factor of 3.16 ($\sqrt{10}$) to make allowance for the fact that the average duration of exposure is only 6.1 years (≤ 12% of life expectancy) (UF_S). The ATSDR only considered a UF_H of 10. The ATSDR did not apply a UF_S, considering that its TRV was applicable for exposure durations of more than one year.

The CES believes that the ATSDR's development of the TRV, and in particular its justification for the choice of BMR and the application of uncertainty factors, is consistent with ANSES's principles (ANSES, publication pending), unlike the OEHHA's choice of interspecies and intraspecies uncertainty factors.

The CES therefore adopted the ATSDR's TRV as the long-term respiratory TRV (ATSDR, 2007).

The overall confidence level of this TRV was estimated to be moderate-high*, based on the following five criteria: nature and quality of the data (high), choice of the critical effect and the mode of action (high), choice of the key study (high), choice of the critical dose (high), and the adjustments and choice of default uncertainty factors (low).*

3.5. Development of a carcinogenic TRV by the respiratory route

3.5.1. Choice of the critical effect

Benzene has been classified in Group 1 (known human carcinogen) by the IARC (IARC, 1979, 1982, 2012, 2018). This classification is based on a sufficient level of evidence for benzene's carcinogenicity to humans. There is a causal relationship between exposure to benzene and the development of ANLL, particularly AML in adults.

For the other haematological cancers (NHL, CLL, MM, CML and childhood AML) and lung cancer, although positive associations have been reported, the IARC considered that the level of evidence of a causal association with benzene exposure was limited.

The CES therefore selected acute myeloid leukaemia (AML) as the critical effect.

3.5.2. Choice of the assumption according to which it is established

The mechanisms of benzene's carcinogenic action and the dose-response relationships associated with these mechanisms are complex and have not been fully elucidated. The mechanism of action is generally based on a linear dose-response relationship, although the toxicokinetics and shape of the dose-response relationship at low environmental concentrations are still poorly understood.

Several agencies have developed assumptions considering that there is no concentration threshold below which there is no risk of carcinogenic effect (US EPA, 2003; ANSES, 2014; TCEQ, 2015). Other bodies and authors consider that the carcinogenicity of benzene results from modes of action for which there is a threshold and make the assumption, for the development of occupational exposure limits, that there is a concentration threshold below which the risk of benzene-related cancer would be zero (DECOS, 2014; ECHA, 2018; North *et al.*, 2020; North *et al.*, 2021). They believe that, overall, the genotoxicity data for benzene

support an indirect genotoxic mode of action (e.g. inhibition of topoisomerase II, generation of oxidative stress, etc.), while there is no evidence to support a direct genotoxic mode of action.

The CES emphasises the fact that benzene and its metabolites produce direct genotoxic effects. Even if the mechanism of benzene's carcinogenic effect has not been fully elucidated, the possibility that the genotoxicity of benzene is partly linked to a direct mechanism cannot be ruled out. The decision tree proposed by the methodological guide for developing and selecting reference values (ANSES, publication pending) leads to the assumption that there is no threshold for deriving a carcinogenic TRV, when there is insufficient evidence of a threshold of effect.

The CES adopted the assumption of a carcinogenic effect with no threshold dose, in order to develop the TRV.

3.5.3. Analysis of the existing TRVs

Six long-term no-threshold respiratory TRVs are available (EC, 1998; WHO, 2000; RIVM, 2001; US EPA, 2003; OEHHA, 2009; TCEQ, 2015).

All these TRVs are based on cohort studies investigating cancer mortality. Five of these six TRVs are based on all types of leukaemia combined. However, the CES considers that leukaemia as a whole is not an acceptable pathological entity, because:

- the term "leukaemia" refers to a heterogeneous group of diseases that affect different haematopoietic and lymphatic tissues and do not have the same risk factors;
- the level of evidence for benzene's carcinogenicity differs according to the type of leukaemia (IARC, 2018); it is highest for AML.

The TCEQ's 2015 TRV was developed specifically considering acute myeloid and monocytic leukaemia, a subtype of AML, and by applying a life-table approach. The proposal for this TRV was based on a study from the "Pliofilm" cohort, and drew on mortality data from the United States (Crump *et al.*, 1994).

Several major epidemiological studies have been published since the above-mentioned TRVs were issued.

Given these limitations, the CES did not retain the existing values and proposed establishing a long-term no-threshold TRV by the respiratory route.

3.5.4. Establishment of the carcinogenic TRV by the respiratory route

- Choice of the key study

Several epidemiological studies investigating AML mortality or incidence in the workplace are available in the literature. They show considerable variability of results within exposure classes due to the small number of cases of AML, particularly at the lowest exposure levels. Combining the results of several epidemiological studies is therefore beneficial for increasing the robustness and accuracy of AML risk estimates.

Two studies have carried out meta-regressions combining the results of the main epidemiological studies in workers assessing the association between occupational exposure to benzene and the risk of AML: the ones conducted by the IARC in 2018 and Scholten *et al.* in 2022. The ultimate aim of the Scholten *et al.* study was to combine different types of data in order to estimate the risk associated with exposure to benzene (epidemiological studies considering all types of leukaemia or just AML, animal studies and mechanistic studies). **Only**

epidemiological data relating specifically to AML have been taken into account in the remainder of this document.

The studies taken into account in the models used by the IARC and Scholten *et al.* (2022) investigated either incidence or mortality, or combined mortality and incidence for AML, and concerned the same cohorts. With regard to the CAPM+NCI cohort of Chinese workers, the study selected by Scholten *et al.* (Linet *et al.*, 2019) is more recent than the one taken into account by the IARC (Hayes *et al.*, 1997). The study by Linet *et al.* combines mortality and incidence of AML and myelodysplastic syndrome (MDS). The Hayes *et al.* study examined mortality from ANLL, including AML, combined with MDS. The study by Linet *et al.* has the advantage of covering a longer follow-up period (1972-1999 versus 1972-1987 in the Hayes *et al.* study), a larger number of subjects (110,631 versus 74,828 in the study by Hayes *et al.*) and is more precise in the assessment of occupational exposure to benzene, using a calibrated Bayesian hierarchical model based on historical measurements of benzene exposure and industrial documents describing tasks and processes.

The mathematical model used by the IARC was a linear model with intercept, whereas Scholten *et al.* (2022) considered several models: linear model with intercept, linear model with no intercept, linear model with intercept and intercept subtracted, linear model with intercept and interpolation, spline model with intercept and spline model with no intercept. The value of the intercept may reflect environmental background noise or uncertainties in the input data (exposure measurement error, uncontrolled confounding factors). The use of a model with intercept implies that the predicted risk for zero exposure is non-zero, which presents a limitation for deriving TRVs.

Among the models proposed by Scholten *et al.*, the CES believes that the linear model with intercept and intercept subtracted should be preferred for deriving TRVs. Indeed, the linear model with intercept is the model that best fits the data and best predicts the increased risk per unit increase in exposure. Subtracting the intercept from the origin gives a zero predicted risk for a zero exposure concentration.

The CES chose the study by Scholten *et al*. (2022) as the key study. Among the models proposed, the CES chose the linear model with intercept and intercept subtracted for deriving the excess risk per unit (ERU).

- Establishing the ERU

An Excess unit risk (ERU) is the excess risk of an adverse health effect occurring in individuals exposed to an exposure concentration unit over their lifetime or at work, compared with unexposed individuals. The ERU is calculated from the excess lifetime risk (ELR). It corresponds to the slope obtained by linear extrapolation at the origin of the curve representing the ELR when the concentrations in the epidemiological study are higher than the concentrations in the environment.

  - *Approach adopted*

Two approaches have traditionally been used to express the ELR for various levels of exposure. These approaches can be applied using the concentration-risk functions reported in the key epidemiological study:

- a "simple" approach using the probability P of the critical effect occurring in an unexposed population,

- a cumulative-risk approach based on the use of life or incidence tables; this involves subtracting the cumulative lifetime risk of the critical effect in the unexposed population from that in the exposed population.

The ELR established using the life-table approach is considered more accurate than that obtained using the simple approach. This is because life tables allow for the calculation of probabilities conditional on survival from one age group to the next, taking account of potential competing risks[7] over a lifetime that are different from the health event of interest, i.e. risks linked to diseases or causes of death other than the one of interest. The life-table approach should be favoured when the necessary data (incidence or mortality by age group in France for the critical effect) are available. **The CES therefore adopted the life-table approach.**

Several assumptions need to be made to support the use of a concentration-risk function to calculate an ELR. First of all, it is necessary to ensure that this function and the associated risk are applicable throughout a lifetime – or in any case, at the ages considered in the simple and cumulative-risk approaches. Next, the function obtained from the epidemiological study should be considered applicable to the population targeted by the ELR calculation.

In human studies, lifetime risk is seldom directly observed. Nevertheless, epidemiological analyses of disease risk over shorter time periods can be used to calculate lifetime risk subject to certain assumptions:

1. the exposure-risk (disease or death) relationship is applicable at various ages (if there are not enough epidemiological data to provide age-specific exposure-risk relationships, empirical data can be used and no assumptions are then necessary);
2. the exposure-risk (disease or death) relationship observed in the epidemiological study is applicable to the target population.

The life-table approach includes several successive calculation steps, enabling R0 and RX to be estimated for the calculation of the ELR:

1) R0 is the cumulative conditional lifetime probability of the critical effect occurring in an unexposed population – this is the lifetime baseline risk. A lifetime here corresponds to the range of age groups considered in the life table (from <1 to 84 years). Calculating R0 requires two types of primary data in the unexposed population that must be available by age group: the probability of death from all causes for individuals, and the probability of occurrence of the critical effect, in this case, AML;

2) RX is the cumulative conditional lifetime probability of the critical effect occurring in an exposed population. In addition to the data used and values calculated for R0, the calculation of RX uses the risk reported in an epidemiological study linking a level of exposure to the critical effect (i.e. a concentration-risk function). Exposure can be considered average or cumulative;

3) The ELR is calculated as an extra risk: ELR = (RX-R0)/(1-R0)

[7] A competing risk is a situation or event (other than the one of interest) that fundamentally impacts the probability of occurrence of the health event of interest (= critical effect). In this specific case, death – irrespective of the cause – is considered a competing risk.

According to *Santé Publique France*, the estimated number of new cases of AML in 2018 was 3428, corresponding to a standardised incidence rate of 3.1 per 100,000 person-years in men and 2.3 in women (SPF, 2020). With the exception of rare subtypes, AML is a blood disorder that has an unfavourable prognosis, with an estimated standardised net survival rate of 50% at one year and 27% at five years. Estimates of observed survival are very similar to those of net survival, reflecting the aggressive nature of the disease: patients primarily die from their AML. There is a wide disparity in five-year net survival depending on age at diagnosis: from 69% at 30 years to 6% at 80 years. From 1990 to 2015, there was a steady improvement in standardised net survival (from 1.5 to 10 years), with this improvement being more marked in younger people (SPF, 2020).

Although the meta-regression by Scholten *et al.* (2022) combined mortality and incidence studies, those involving the largest number of subjects were mortality studies.

In view of the critical effect selected (AML), the CES adopted the life-table approach specifically for the use of mortality data, which uses a life table with all-cause mortality data and mortality data for the critical effect.

- *Collection of health data for the lifetime baseline risk*

The ELR was calculated by projecting a concentration-risk function selected from the epidemiological literature onto the baseline risk of the health event in the target population, denoted R_0 for the life-table approach. The target population was the French population (metropolitan France and overseas territories).

The International Classification of Diseases (ICD) codes used for the studied diseases were as follows:

- all-cause mortality: ICD10 code A00-Y89,
- AML mortality: ICD10 code C92.

Numbers of deaths and crude mortality rates in France, for all causes and those associated with the critical effect (Code C92) in an unexposed population, were collected for men and women.

The R_0 data were mortality rates for a disease in France. These were crude rates by age group from <1 year to 84 years, both men and women, for 2015, 2016 and 2017. These rates were obtained from the Epidemiology Centre on Medical Causes of Death (CépiDC - Inserm), which owns the data.

In order to use these data in the life table, the mortality data (CépiDc – Inserm) for 2015, 2016 and 2017 were averaged and weighted based on the numbers of men and women (numbers for France and for each age group).

- *Calculation of the ELR using the life table*

The ELR was calculated as an extra risk and required a preliminary calculation phase using the life table, which is provided in Annex 5 of the report.

The risk was estimated using the equation for the linear model with intercept and intercept subtracted presented in the study by Scholten *et al.* (2022).

In the event that epidemiological data were used for cancer data and in accordance with US EPA guidelines (US EPA, 2005), the excess lifetime risk (ELR) was set at 1% to establish the point of departure (PoD) for linear extrapolation to the origin.

The risk was calculated using a life table for continuous exposure to benzene up to the age of 84. The exposure observed in the epidemiological study was converted into continuous lifetime exposure by multiplying occupational exposure by a factor considering the number of days of exposure per year (365/240 days) and the difference in the amount of air inhaled per day between workers and the general population (20/10 m^3). The PoD was calculated by considering the upper bound of the 95% confidence interval for the β regression coefficient ($\beta_{95\%}$ = 0.0037) in accordance with the US EPA's guidelines and ANSES's practices.

The ERU expressed in $(ppm)^{-1}$ was then converted to $(\mu g \cdot m^{-3})^{-1}$ using the following conversion factor: 1 ppm of benzene is equal to 3190 $\mu g \cdot m^{-3}$ at 25°C.

Table 1: PoD, UR and concentrations associated with the various risk levels

PoD[1]	ERU[2]	Concentrations for various risk levels
2 ppm	0.005 $(ppm)^{-1}$	10^{-4}: 0.02 ppm 10^{-5}: 0.002 ppm 10^{-6}: 0.0002 ppm
6380 $\mu g \cdot m^{-3}$	$1.6.10^{-6}$ $(\mu g \cdot m^{-3})^{-1}$	10^{-4}: 60 $\mu g \cdot m^{-3}$ 10^{-5}: 6 $\mu g \cdot m^{-3}$ 10^{-6}: 0.6 $\mu g \cdot m^{-3}$

[1] PoD: calculated with the upper bound of the 95% confidence interval for the β regression coefficient;
[2] UR = 0.01/PoD

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

3.6. Conclusion of the CES

Four TRVs by the respiratory route have been proposed for benzene (Table 2):

- a short-term threshold TRV based on the MRL proposed by the ATSDR in 2007 with a moderate confidence level;
- a medium-term TRV based on the MRL proposed by the ATSDR in 2007 with a moderate confidence level;
- a long-term TRV based on the MRL proposed by the ATSDR in 2007 with a moderate-high confidence level;
- a long-term no-threshold TRV based on the meta-regression carried out by Scholten *et al.* in 2022 with a moderate-high confidence level.

Table 2: Short-, medium- and long-term threshold TRVs by the respiratory route for benzene

Type of RV		Short term	Medium term	Long term
RV	Organisation	ATSDR	ATSDR	ATSDR
	Year	2007	2007	2007
	Name	MRL	MRL	MRL
	Value	**29 µg·m^{-3}**	**19 µg·m^{-3}**	**9.7 µg·m^{-3}**
Target population		General population	General population	General population
Critical effect		Decrease in the proliferative response of B lymphocytes (mitogenic action induced by LPS), decrease in circulating lymphocytes	Delayed *in vitro* alloreactivity of lymphocytes	Decrease in the number of lymphocytes
Key study	Reference	Rozen et al. 1984	Rosenthal and Snyder 1987	Lan et al. 2004
	Study population or species	Male mice	Male mice	Men (workers)
	Exposure (duration, route)	6h/d for 6 consecutive days Respiratory route	6h/d, 5d/w for 20 days Respiratory route	On average 6.1 years Respiratory route
Point of departure (PoD)		LOAEC = 33 mg·m^{-3}	LOAEC = 32.5 mg·m^{-3}	$BMCL_{0,25sd}$ = 0.33 mg·m^{-3}
Time adjustment		$LOAEC_{ADJ}$ = 8.3 mg·m^{-3}	$LOAEC_{ADJ}$ = 5.8 mg·m^{-3}	$BMCL_{0.25sd\ ADJ}$ = 0.1 mg·m^{-3}
Allometric adjustment		$LOAEC_{ADJ\ HEC}$ = 8.3 mg·m^{-3}	$LOAEC_{ADJ\ HEC}$ = 5.8 mg·m^{-3}	-
Uncertainty factors (UF)		300 $UF_{A\text{-}TD}$: 3, UF_H: 10, UF_L: 10	300 $UF_{A\text{-}TD}$: 3, UF_H: 10, UF_L: 10	10 UF_H : 10
Confidence level		**Moderate**	**Moderate**	**Moderate-high**

MRL: Minimal risk level; LPS: Lipopolysaccharides; $UF_{A\text{-}TD}$: Toxicodynamic component of the interspecies uncertainty factor, UF_H: Inter-individual uncertainty factor; UF_L: Uncertainty factor related to use of a LOAEC.

Table 3: Long-term no-threshold TRV by the respiratory route for benzene

RV	Organisation	ANSES
	Year	2024
	Name	Long-term no-threshold TRV
	Value	**ERU = $1.6.10^{-6}$ ($\mu g \cdot m^{-3}$)$^{-1}$** 60 $\mu g \cdot m^{-3}$ for a risk of 10^{-4} 6 $\mu g \cdot m^{-3}$ for a risk of 10^{-5} 0.6 $\mu g \cdot m^{-3}$ for a risk of 10^{-6}
Target population		General population
Critical effect		Acute myeloid leukaemia
Key study	Reference	Scholten et al. 2022
	Species	Men (workers)
	Exposure (route, duration)	Respiratory route
Development		Ln $RR_{UB95\%}$ = $\beta_{UB95\%}$ x [benzene] $\beta_{UB95\%}$ = upper bound of the 95% confidence interval for the β coefficient = 0.0037 (unitless) [benzene] = occupational exposure concentration for benzene (in ppm) Life table with time adjustment Linear model with intercept and intercept subtracted ELR of 1%
Confidence level		**Moderate-High**

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the proposed TRVs developed for benzene and the conclusions of the CES on "Health Reference Values".

The Agency reiterates that a toxicity reference value (TRV) is a toxicological indicator for qualifying or quantifying a risk to human health. TRVs enable the potential health effects of exposure to substances to be assessed. They can be used as part of quantitative health risk assessments (QHRAs) carried out at population level, in a given exposure context, and thus help in the choice of risk management measures. They can also be used to draw up guideline values such as indoor air guidelines (IAQGs): in parallel with this expert appraisal, ANSES also worked on updating the IAQGs for benzene proposed in 2008.

The updated knowledge of benzene's toxicity led ANSES to recommend four respiratory TRVs: a short-term TRV of 29 $\mu g \cdot m^{-3}$ (exposure from 1 to 14 days), a medium-term TRV of 19 $\mu g \cdot m^{-3}$ (from 15 days to less than a year), a long-term TRV of 9.7 $\mu g \cdot m^{-3}$ (more than a year) and a carcinogenic TRV of $1.6.10^{-6}$ $(\mu g \cdot m^{-3})^{-1}$ (exposure lasting more than a year). The short-term and medium-term TRVs were established with a moderate confidence level. The long-term and carcinogenic TRVs were established with a moderate-high confidence level.

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Valeur toxicologique de référence, VTR, benzène, inhalation

Toxicity reference value, TRV, benzene, inhalation