

The Director General

Maisons-Alfort, 1 July 2014

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

regarding the establishment of a carcinogenic TRV by inhalation for
benzene
CAS No.71-43-2

ANSES undertakes independent and pluralistic scientific expert assessments.

ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.

It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are made public. This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 1 July 2014 shall prevail.

On 30 March 2009, ANSES issued an internal request to establish a toxicity reference value (TRV) for the carcinogenic effects by inhalation of benzene (CAS No.71-43-2).

1. BACKGROUND AND PURPOSE OF THE REQUEST

Since 2004, ANSES has been working to establish toxicological reference values (TRVs) and develop related methodologies. Its expert assessments first focused on reprotoxic chemical substances as a priority and then on carcinogenic chemical substances from 2007. In this context, a method for establishing carcinogenic TRVs was implemented as part of a pilot phase launched in 2008. Benzene, cadmium, ethanol, naphthalene and vinyl chloride were selected as study substances for this pilot phase. At the end of this pilot phase, the carcinogenic TRVs proposed for these substances were submitted for validation to the Expert Committee on the "Assessment of the risks related to chemical substances". This Opinion deals with TRVs for benzene.

A TRV is a generic term encompassing all of the types of toxicological indicators that are used to establish a relationship between a dose and an effect (toxic with a threshold effect) or between a dose and a likelihood of effect (toxic without a threshold effect). TRVs are specific to an exposure time (acute, subchronic or chronic), an exposure route (oral or respiratory) and a type of effect (reprotoxic, carcinogenic, etc.). The establishment of TRVs differs based on knowledge or assumptions of substances' mechanisms of action.

"Threshold" TRVs are used for substances that, above a certain dose, cause damage whose severity is proportional to the absorbed dose, while "non-threshold" TRVs are used for substances for which there is likelihood, even very small, that a single molecule penetrating the body will cause harmful effects to that body.

In practice, the establishment of a TRV includes the following stages:

- analysis of the available data,
- choice of the critical effect,
- identification of the establishment assumption, with or without a dose threshold, based on the substance's mode of action,
- choice of a study of good scientific quality enabling the establishment of a dose-response relationship,
- choice or establishment of a critical dose based on experimental doses and/or epidemiological data; for any critical dose obtained in animals, adjustment of this dose to humans,
- application of uncertainty factors to the critical dose to take into account uncertainties for threshold TRVs or linear extrapolation from the origin using the critical dose for non-threshold TRVs.

The establishment of TRVs adheres to a highly structured and stringent approach that involves collective expert appraisals relying on expert judgement (AFSSET, 2010).

2. ORGANISATION OF THE EXPERT APPRAISAL

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

It falls within the sphere of competence of the Expert Committee (CES) on "Assessment of the risks related to chemical substances". ANSES entrusted the expert appraisal to the working group on "Toxicological Reference Values II". The methodological and scientific aspects of the work were presented to the CES on 12 September and 10 October 2013. They were adopted by the CES on "Assessment of the risks related to chemical substances" in its meeting of 10 October 2013.

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 on ANSES's website (www.anses.fr).

3. ANALYSIS AND CONCLUSIONS OF THE CES

There are many data in the scientific literature on the toxicity of benzene to humans. Both animal and human studies have been taken into account to characterise the toxicological profile of this substance.

Carcinogenicity of benzene

The epidemiological data available to date are sufficient to establish that there is a cause-effect relationship between human exposure to benzene and the onset of cancers. Benzene has been recognised as a **human carcinogen** by the various international organisations that have assessed its toxicity (IARC, 2012; Health Canada, 2007; US EPA¹, 2003, etc.). Benzene is classified by Health Canada and RIVM² in group I "carcinogenic to humans", by the US EPA in group A "known

¹ US EPA: US Environmental Protection Agency

² RIVM: Rijksinstituut voor Volksgezondheid en Milieu (Dutch National Institute for Public Health and the Environment)

human carcinogen”, by the IARC³ in group 1 “carcinogenic to humans” (sufficient evidence in animals and humans) and by the European Commission in carcinogen category 1A (H 350) “substance known to be carcinogenic to humans”.

Genotoxicity of benzene

Benzene is metabolised in the liver by cytochromes P450 2E1 and CYP2F1 into phenol, hydroquinone and catechol. Metabolic intermediates (muconaldehyde) after opening of the benzene ring can also give trans-trans-muconic acid. Benzene metabolites play a dominant role in the onset of toxic and carcinogenic effects. Indeed, the available data show that benzene metabolites have direct genotoxic action.

Benzene is considered a clastogenic and aneugenic agent in humans, causing aneuploidy, polyploidy, the formation of micronuclei and chromosome deletions, translocations and rearrangements (Health Canada, 2007). Most cytogenetic studies, in which the blood lymphocytes of exposed workers were studied, show an increased number of structural (chromatid and/or chromosome breakage) and/or numerical chromosomal aberrations in peripheral lymphocytes stimulated by mitogens. *In vitro* and *in vivo* studies confirm the clastogenic effects of benzene. Chromosomal aberrations are regularly found in the bone marrow cells of people occupationally exposed to benzene. These chromosomal aberrations are believed to be responsible for leukaemia in people exposed to benzene. The significant increase in sister chromatid exchanges in bone marrow cells and in the lymphocytes of animals exposed to benzene is additional evidence of benzene's genotoxic potential.

Moreover, it has been observed that exposure to benzene in humans causes types of chromosomal aberrations found in certain forms of leukaemia, such as acute myeloid leukaemia and myelodysplastic syndromes (Smith and Zhang, 1998; IARC, 2012).

In its updated monograph on benzene, IARC sets out the mechanisms of genotoxic action involved for each type of leukaemia (IARC, 2012). Regarding acute myeloid leukaemia, the two main mechanisms of action are as follows:

- centromere breaks, causing so-called unbalanced chromosomal aberrations (loss of various parts of the long arm or loss of the whole chromosome (5q-/-5 or 7q-/-7)). This same type of aberration is observed after therapy with alkylating agents, which are direct genotoxic compounds without a dose threshold;
- inhibition of topoisomerase II, causing so-called balanced chromosomal aberrations through translocations or inversions of non-homologous chromosomes (t(21q22), t(15;17) and inv(16)).

³ IARC: International Agency for Research on Cancer

Establishment of a non-threshold TRV for carcinogenic effects

Choice of the establishment assumption

ANSES took into account the fact that benzene and its metabolites have genotoxic effects (chromosomal aberrations, gene mutations, etc.) some of which (unbalanced chromosomal aberrations) have a non-threshold dose-response relationship.

According to the decision tree proposed by the methodological guide "Method of establishing toxicity reference values for carcinogenic chemical substances" (AFSSET, 2010), even though the mechanism of the carcinogenic effect is not entirely clear, one of the modes of action of this substance (and/or its metabolites), i.e. the production of unbalanced chromosomal aberrations, led to the choice of a **non-threshold assumption**.

Choice of the critical effect

Epidemiological studies provide significant evidence of a causal relationship between exposure to benzene and certain types of leukaemia (acute myeloblastic leukaemia, acute lymphoblastic leukaemia and acute myeloid leukaemia).

ANSES therefore chose increased incidence of leukaemia as the critical effect.

Choice of the key study

Rinsky *et al.* (1981, 1987) were the first to undertake detailed studies, in three facilities in Ohio (USA), in a cohort of 748 male workers who had been occupationally exposed to benzene from 1940 to 1949 and were monitored until the end of 1981.

The Pliofilm cohort in Ohio is a valid database for the assessment of cancer risk in humans resulting from benzene exposure. Indeed, this cohort had the lowest workplace exposure to other potentially carcinogenic substances that could influence the assessment of risk related to benzene. Furthermore, the Pliofilm workers were exposed to a wider range of estimated benzene concentrations than the workers involved in other cohort studies.

Richardson (Richardson, 2008) re-analysed the data for the Pliofilm cohort as they had been definitively established in 1996 by Rinsky *et al.* (Rinsky *et al.*, 2002). Exposure levels at each workstation, each year and for each plant were taken from this publication (Rinsky *et al.*, 2002). Annual exposure was calculated by Richardson by multiplying the length of employment in a job by the exposure rate for that job. For each employee, cumulative exposure was obtained by adding up annual exposure rates throughout the length of employment in the plant. The aim of the study was to analyse variation in the risk of leukaemia with age at first exposure and the time between last exposure and onset of the illness.

Thus, for cumulative benzene exposure of 10 ppm-years, in the ten years after the end of exposure, the relative risk (RR) was 1.19 with a 95% confidence interval (CI_{95%}) ranging from 1.10 to 1.29.

The author specifies that due to the small number of leukaemia cases (n=17), analyses by type of leukaemia were not conducted and that the use of mortality data does not allow for assessment of whether benzene influences disease incidence or prognosis (reducing survival time).

TRV calculation

The carcinogenic TRV for benzene corresponds to the potency factor (PF) which is equal to the relative risk minus 1 divided by the exposure level and the conversion factor (from ppm-year to $\mu\text{g}\cdot\text{m}^{-3}$ with the coefficient explained below).

The PF is therefore obtained with the following formula:

$$\text{PF} = \frac{\text{RR}_{/\text{ppm}\cdot\text{year}} - 1}{\text{Conversion factor} \times \text{exposure}_{(\text{ppm}\cdot\text{year into } \mu\text{g}\cdot\text{m}^{-3})}}$$

where:

- RR: Upper limit of the confidence interval for the relative risk calculated by the author (Richardson, 2008), i.e. 1.29
- Conversion factor: 1 ppm of benzene with occupational exposure is equal to $1.096 \text{ mg}\cdot\text{m}^{-3}$ of benzene with continuous exposure
- Exposure associated with the RR in ppm i.e. 10 ppm-years

The respiratory TRV for the carcinogenic effects of benzene is $2.6 \cdot 10^{-5} (\mu\text{g}\cdot\text{m}^{-3})^{-1}$.

Confidence level: High

- Choice of the critical effect: high level of confidence (epidemiological studies of good scientific quality; effects consistent with toxicological studies)
- Quality of the key study: high level of confidence (this study meets the selection criteria for the derivation of a TRV. It provides dose-response relationships for the presented relative risks)
- Choice of the critical dose: high level of confidence
- Establishment of the TRV: high level of confidence

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) endorses the conclusions and recommendations of the Expert Committee (CES) on “Assessment of the risks related to chemical substances” concerning the establishment of toxicological reference values by inhalation for benzene.

Critical effect and source study	Establishment method	TRV
Acute leukaemia Richardson (2008)	$CI_{95\%}RR_{10 \text{ ppm-year}} = 1.29$ Upper limit of the confidence interval for the exposure-risk function calculated by Richardson	$PF = 2.6 \cdot 10^{-5} (\mu\text{g}\cdot\text{m}^{-3})^{-1}$ 0.038 $\mu\text{g}\cdot\text{m}^{-3}$ for a risk of 10^{-6} 0.38 $\mu\text{g}\cdot\text{m}^{-3}$ for a risk of 10^{-5} 3.8 $\mu\text{g}\cdot\text{m}^{-3}$ for a risk of 10^{-4} Confidence level: HIGH

Moreover, in light of new studies available on the Pliofilm cohort, the Agency recommends revising the “lifetime” indoor air guideline value (currently set at 2 $\mu\text{g}\cdot\text{m}^{-3}$ for a risk of 10^{-5}).

On 3 February 2012, ANSES received a formal request from the French Ministry of Labour to re-assess the 8-hour Time-Weighted Average (TWA) for benzene (3.25 $\text{mg}\cdot\text{m}^{-3}$) and to propose, if necessary, new occupational exposure values based on health considerations for benzene.

Marc Mortureux

KEYWORDS

Benzene, toxicological reference value, inhalation, cancer

REFERENCES

- French Agency for Environmental and Occupational Health Safety (AFSSET) (2010) Toxicity reference values for carcinogenic substances. Method of establishing TRVs based on carcinogenic effects. Request No.2004/AS16. (AFSSET, Maisons-Alfort) 107p.
- International Agency for Research on Cancer (IARC) (2012) Benzene. Chemical Agents and related occupations. Volume 100F. A review of human carcinogens. p249-294 (IARC, Lyon) Available at <http://monographs.iarc.fr/ENG/Monographs/vol100F/mono100F-24.pdf>
- Eastmond DA, Rupa DS, Hasegawa LS. (1994) Detection of hyperdiploidy and chromosome breakage in interphase human lymphocytes following exposure to the benzene metabolite hydroquinone using multicolor fluorescence in situ hybridization with DNA probes. *Mutat Res.* 1994;322(1):9-20
- Richardson DB. (2008) Temporal variation in the association between benzene and leukemia mortality. *Environ Health Perspect.* 2008;116(3):370-4.
- Rinsky RA, Hornung RW, Silver SR, Tseng CY. (2002) Benzene exposure and hematopoietic mortality: A long-term epidemiologic risk assessment. *Am J Ind Med.* 2002;42(6):474-80.
- RIVM (National Institute of Public Health and the Environment) (2001) Re-evaluation of human toxicological maximum permissible risk level. RIVM report 711701 025. (RVIM, Bilthoven) 68p. Available at <http://www.rivm.nl/bibliotheek/rapporten/711701025.pdf>
- Health Canada (2007) Le benzène dans l'eau potable. Document for public consultation. (Government of Canada, Environment Canada, Health and Social Welfare Canada, Ottawa).
- Smith MT, Zhang L. (1998) Biomarkers of leukemia risk: benzene as a model. *Environ Health Perspect.* 1998;106 Suppl 4:937-46.
- Stillman WS, Varella-Garcia M, Gruntmeir JJ, Irons RD. (1997) The benzene metabolite, hydroquinone, induces dose-dependent hypoploidy in a human cell line. *Leukemia.* 1997;11(9):1540-5.
- US Environmental Protection Agency (US EPA) (2003) Integrated Risk Information System: Benzene (CASRN 71-43-2). (US EPA, Washington) Available at <http://www.epa.gov/iris/subst/0276.htm>
- Zhang B. (1996) (Investigation of health status in workers exposed to low-level benzene). *Zhonghua Zhang L, Rothman N, Wang Y, Hayes RB, Bechtold W, Venkatesh P, et al. (1996b) Interphase cytogenetics of workers exposed to benzene. Environ Health Perspect.* 1996;104 Suppl 6:1325-9.
- Zhang L, Rothman N, Wang Y, Hayes RB, Li G, Dosemeci M, et al. (1998a) Increased aneusomy and long arm deletion of chromosomes 5 and 7 in the lymphocytes of Chinese workers exposed to benzene. *Carcinogenesis.* 1998;19(11):1955-61.
- Zhang L, Wang Y, Shang N, Smith MT. (1998b) Benzene metabolites induce the loss and long arm deletion of chromosomes 5 and 7 in human lymphocytes. *Leuk Res.* 1998;22(2):105-13.