The Director General

Maisons-Alfort, France, 10 August 2009

OPINION

of the French Agency for Environmental and Occupational Health Safety

Relating to establishing chronic HTVs for ingestion of chloronitrobenzene (ortho, meta and para isomers)

Afsset Solicited Request No "070057"

The mission of the French Agency for Environmental and Occupational Health Safety (Afsset) is to assist in the areas of environmental and occupational health safety and assess potential health risks.

It provides the competent authorities with all information required on these risks, as well as the expertise and technical support needed to draft legislative and statutory provisions and implement risk management strategies (Article L. 1336-1 of the French Public Health Code).

Presentation of the question

Afsset received a solicited request from the Directorate General for Health (DGS) on 12 November 2007 to establish human toxicity values (HTV) for ortho, meta and para isomers of chloronitrobenzene (CNB), following the contamination of the Alsace groundwater to the north of Mulhouse by organic products, including the 3 isomers of chloronitrobenzene, originating from two manufacturing companies.

At the same time, the DGS solicited the French Food Safety Agency (Afssa) to establish drinking water limit values for these compounds from the HTVs set by Afsset.

Context

Chloronitrobenzene (CNB) is used as an intermediate in chemical synthesis. Its natural occurrence is not known. Its presence in the environment is accidental, particularly in water. Furthermore, it can be formed from nitrobenzenes during the chlorination of drinking water. It has also been identified in various species of fish close to rivers polluted by this compound. The hypothesis in which humans are mainly exposed to this compound and not to its degradation products cannot be dismissed with the available data. Human exposure can therefore be considered as plausible by ingestion of contaminated water, by inhalation of vapours or aerosols (time spent in baths and showers, degassing of groundwater) and by the CNB vapours and/or aerosols contained in drinking water (baths, showers) passing through the skin. Due to its

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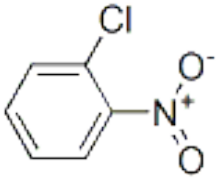
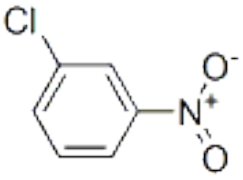
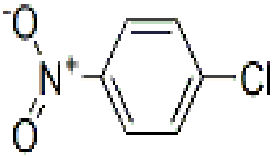
physico-chemical properties, the main exposure route for humans, excluding occupational exposure, appears to be ingestion (direct or indirect ingestion of contaminated water).

The chemical structure of CNB consists of a chlorine atom in the ortho, meta or para position. CNB can therefore occur in the form of 3 different isomers, each one having different toxicokinetic and toxic properties (see table). Setting a specific HTV for each of the isomers has therefore been considered.

Table: Identification of the CNB

CAS number	88-73-3	121-73-3	100-00-5
Name	Ortho-chloronitrobenzene	Meta-chloronitrobenzene	Para-chloronitrobenzene
Empirical formula	$C_6H_4ClNO_2$	$C_6H_4ClNO_2$	$C_6H_4ClNO_2$
Structural formula			

Organisation of the expert appraisal

Afsset referred the validation of the HTVs for ortho-, meta- and para- isomers of chloronitrobenzene (CNB) to the Committee of Specialised Experts (CES) "Assessment of risks linked to chemical substances". For this work, three experts from the working group "Human Toxicity Values and Carcinogenic Substances" and two experts from the CES "Assessment of the risks linked to chemical substances" were nominated as rapporteurs.

The work was presented several times to the working group "Human Toxicity Values" for comments, on 19 December 2008, 13 February 2009 and 10 April 2009. The reports "Establishing a chronic HTV for ingestion based on carcinogenic and non-carcinogenic effects of ortho-chloronitrobenzene (CASRN 88-73-3)", "Establishing a chronic HTV for ingestion based on carcinogenic and non-carcinogenic effects of meta-chloronitrobenzene (CASRN 121-73-3)" and "Establishing a chronic HTV for ingestion based on carcinogenic and non-carcinogenic effects of para-chloronitrobenzene (CASRN 100-00-5)" were submitted to the CES "Assessment of health risks linked to chemical substances" on 20 March 2008 and validated on 28 May 2008.

This work was carried out by a group of experts with complementary competences in compliance with the French NF X 50-110 Standard "Quality in Expert Appraisal Activities" with the aim of covering the following points: competence, independence, transparency and traceability.

This opinion is based on the notes from the collective expert appraisal of the CES "Assessment of risks linked to chemical substances" and on the overall report "Establishing HTVs for ingestion of ortho, meta and para isomers of chloronitrobenzene".

HTV of ortho-chloronitrobenzene (CASRN 88-73-3)

The European Union has not classified ortho-chloronitrobenzene (o-CNB) with regards to its carcinogenicity. In 1996, the IARC classified o-CNB in group 3 (not classifiable with regards to its human carcinogenicity).
Since then, new subchronic and chronic toxicity and carcinogenicity studies have been carried out, clearly showing carcinogenic effects of o-CNB in animals at the hepatic level (Matsumoto *et al.* 2006[1]). The mechanism causing these hepatic tumours is based on a genotoxic mechanism of action.
Furthermore, o-CNB exhibits hepatic and hematological toxicity during subchronic exposures via ingestion and inhalation in rats and mice.

In accordance with the conclusions of the collective expert appraisal report, and in order to protect against the effects of o-CNB by ingestion, Afsset suggests two HTVs:

- A chronic threshold dose HTV based on the hepatotoxic effects,
- A non-threshold dose HTV based on the hepatic carcinogenic effects (genotoxic mechanism of action).

Type of HTV	Critical effect	Critical dose	UF*	HTV
Threshold dose, ingestion	Hepatic toxicity in the female BDF1 mouse Matsumoto *et al.* (2006)[1]	NOAEL = 14 $mg.kg^{-1}.d^{-1}$ Allometric adjustment $NOAEL_{aj}$** = 2 $mg.kg^{-1}.d^{-1}$	25 UF_A (toxicodynamic component) =2.5 UF_H = 10	**HTV = 80 $\mu g.kg^{-1}.d^{-1}$**
Non-threshold, ingestion	Hepatocarcinomas and hepatoblastomas in the female BDF1 mouse Matsumoto *et al.* (2006)[1]	$BMD_{10}L_{95}$*** = 11.7 $mg.kg^{-1}.d^{-1}$ Allometric adjustment $BMD_{10}L_{95aj}$** = 1.7 $mg.kg^{-1}.d^{-1}$	-	After linear extrapolation of the original: **HTV = 6.10^{-5} ($\mu g.kg^{-1}.d^{-1}$)$^{-1}$** 0.017 $\mu g.kg^{-1}.d^{-1}$ for a risk of 10^{-6} 0.17 $\mu g.kg^{-1}.d^{-1}$ for a risk of 10^{-5} 1.7 $\mu g.kg^{-1}.d^{-1}$ for a risk of 10^{-4}

* UF: Overall uncertainty factor (applied), UF_A: inter-species variability; UF_H: individual variability
** $NOAEL_{aj}$ / $BMD_{10}L_{95aj}$: NOAEL / $BMD_{10}L_{95}$ adjusted (allometric adjustment)
*** $BMD_{10}L_{95}$: lower limit of the 95% confidence interval of the benchmark dose corresponding to an increase in the response in comparison with the non-exposed group of 10%

[1] Matsumoto, M., Umeda, Y., Senoh, H., Suzuki, M., Kano, H., Katagiri, T., Aiso, S., Yamazaki, K., Arito, H., Nagano, K., Yamamoto, S., and Matsushima, T. (2006). Two-year feed study of carcinogenicity and chronic toxicity of ortho-chloronitrobenzene in rats and mice. *J. Toxicol. Sci.* 31(3), 247-264.

HTV of meta-chloronitrobenzene (CASRN 121-73-3)

m-CNB has not been classified by the European Union. In 1996, the IARC classified m-CNB in group 3 (compound not classifiable with regards to its human carcinogenicity).
No carcinogenicity data has been found in the literature for meta-chloronitrobenzene (m-CNB). It is not possible to conclude any carcinogenic potential taking into account the few available animal and human data.

In accordance with the conclusions of the collective expert appraisal, Afsset considers that, taking into account the lack of human and animal toxicological data, it is not possible to establish an HTV whatever the type of effect for m-CNB. Neither is it conceivable to establish an HTV by structural similarity with the other chloronitrobenzenes as the genotoxicity values are different and the chronic toxicity profiles are different between o-CNB (essentially hepatotoxic) and p-CNB (essentially hematotoxic), and unknown for m-CNB.

HTV of para-chloronitrobenzene (CASRN 100-00-5)

In 2004, the European Union classified para-chloronitrobenzene (p-CNB) as a category 3 carcinogen (substance that is of concern because of its possible human carcinogenic effects) and category 3 mutagen (substance that is of concern because of its possible human mutagenic effects). In 1996, the IARC classified p-CNB in group 3 (not classifiable with regards to its human carcinogenicity).
Since then, new subchronic and chronic toxicity and carcinogenicity studies have been carried out, clearly showing carcinogenic effects of p-CNB (Matsumoto *et al.* 2006[2]).
p-CNB is hematotoxic during subchronic exposures by ingestion and inhalation in animals. Signs of hepatic toxicity are also observed in rodents. o-CNB and p-CNB are both responsible for hematotoxic and hepatotoxic effects, however p-CNB would appear more hematotoxic than o-CNB, in comparison with its strong methemoglobinising power.

In accordance with the conclusions of the collective expert appraisal, Afsset proposes two HTVs in order to protect from the effects of p-CNB by ingestion:

- A chronic threshold dose HTV based on the hematotoxic effects,
- A non-threshold dose HTV based on the splenic carcinogenic effects (genotoxic mechanism of action).

Type of HTV	Critical effect	Critical dose	UF*	HTV
Threshold dose, ingestion	Hematotoxicity in the male F344 rat Matsumoto *et al.* (2006)[2]	$BMD_{10}L_{95}$** = 3.2 $mg.kg^{-1}.d^{-1}$ Allometric adjustment $BMD_{10}L_{95ai}$*** = 0.85 $mg.kg^{-1}.d^{-1}$	UF = 25 UF_A (toxicodynamic component) =2.5 UF_H = 10	**HTV = 34 $\mu g.kg^{-1}.d^{-1}$**
Non-threshold, ingestion	Hemangiosarcomas of the spleen in the male F344 rat Matsumoto *et al.* (2006)[2]	$BMD_{10}L_{95}$** = 7.4 $mg.kg^{-1}.d^{-1}$ Allometric adjustment $BMD_{10}L_{95ai}$*** = 1.97 $mg.kg^{-1}.d^{-1}$	-	After linear extrapolation of the original: **HTV = 5.10^{-5} ($\mu g.kg^{-1}.d^{-1}$)$^{-1}$** 0.02 $\mu g.kg^{-1}.d^{-1}$ for a risk of 10^{-6} 0.2 $\mu g.kg^{-1}.d^{-1}$ for a risk of 10^{-5} 2 $\mu g.kg^{-1}.d^{-1}$ for a risk of 10^{-4}

* UF: Overall uncertainty factor (applied), UF_A: inter-species variability; UF_H: individual variability
** $BMD_{10}L_{95}$: lower limit of the 95% confidence interval of the benchmark dose corresponding to an increase in the response in comparison with the non-exposed group of 10%
** $BMD_{10}L_{95aj}$: $MD_{10}L_{95}$ adjusted (allometric adjustment)

[2] Matsumoto M, Aiso S, Senoh H, Yamazaki K, Arito H, Nagano K, Yamamoto S, Matsushima T. Carcinogenicity and chronic toxicity of para-chloronitrobenzene in rats and mice by two-year feeding. *J Environ Pathol Toxicol Oncol*. 2006;25(3):571-84.

Recommendations

Taking into account the lack of knowledge of the carcinogenic mechanisms of action, non-threshold dose HTVs have been established in order to protect from the carcinogenic effects of o-CNB and p-CNB. These HTVs are very protective but their confidence level is weak. The agency therefore recommends initiating research in order to determine the carcinogenic mechanisms of action of o-CNB and p-CNB.

The agency also recommends that, due to the uncertainty over the carcinogenic potential of o-CNB and p-CNB, studies be initiated on the sources and levels of exposure to these substances, both in the general population as well as in the occupational environment, in order to assess the risks.

Furthermore, generally, during an overall risk assessment in a multi-exposure context, it would be advisable to take into account the sum of the risks of the compounds as they have the same target organs and the same mechanisms of toxic action.

The Director General

Martin GUESPEREAU