The Director General

Maisons-Alfort, 10 November 2011

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

on "the development of a chronic oral toxicity reference value for chloral hydrate"

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES's public health mission involves ensuring environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with 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.

AFSSET issued an internal request on 25 February 2010 in order to prepare an oral toxicity reference value for chloral hydrate.

1. BACKGROUND AND PURPOSE OF THE REQUEST

On 22 December 2006 the French Director General for Health (DGS), the Director General for Pollution and Risk Prevention (DGPR) and the Director for Water and Biodiversity made a formal request to the Agency for **"Assessment of the health risks associated with the presence of chemical and/or biological hazards in the water, air and surfaces of regulated swimming pools".** While this request was being addressed by the working group on "Assessment of the health risks associated with water quality and swimming pool water treatment products and processes", the establishment of toxicity reference values (TRVs) for chloral hydrate was considered.

Chloral hydrate is one of the main chlorinated by-products of disinfection found in drinking water and swimming pool water, where it is generally found at the highest concentrations. It has been found in French swimming pool water at concentrations of between 96.5 and 430 µg/L (De Laat, 2009).[1]

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

[1] De Laat J., Berne F., Brunet R. *et al.* (2009). Sous-produits de chloration formés lors de la désinfection des eaux de piscines. Etude bibliographique. *Eur. J. Water Quality;* 40 (2): 109-28.

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. The establishment of a TRV is therefore defined as follows:

TRV= Critical dose/UF

where: *Critical dose = NOAEC, LOAEC or BMDL*
UF = overall Uncertainty Factor applied

In practice, establishing a TRV involves the following four steps:

- choice of the critical effect;
- choice of a good quality scientific study generally enabling establishment of a dose-response relationship;
- choice or development of a critical dose from experimental doses and/or epidemiological data;
- application of uncertainty factors to the critical dose to take uncertainties into account.

TRVs are established[2] according to a highly structured and rigorous approach involving collective assessments by groups of specialists.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with the French Standard NF X 50-110 “Quality in Expertise Activities - General Requirements of Competence for Expert Appraisals (May 2003)" to ensure compliance with the following points: competence, independence, transparency and traceability.

In February 2010 the Agency entrusted this expert appraisal to the “Toxicity reference values” working group, reporting to the Expert Committee (CES) on Assessment of the risks related to chemical substances.

The methodological and scientific aspects of the work were regularly submitted to the CES between 4 November 2010 and 12 May 2011. The work was adopted by the CES on Assessment of the risks related to chemical substances at its meeting of 12 May 2011.

The scientific aspects of this Opinion are based on the final report from this collective expert appraisal, entitled "Chloral hydrate. Preparation of oral TRVs based on chronic toxicity", dated June 2011.

The report was approved by the CES at its meeting of 10 June 2011.

[2] AFSSET, 2007. Establishment of a toxicity reference value for reprotoxic substances

3. ANALYSIS AND CONCLUSIONS OF THE CES

Choral hydrate is a non-volatile, highly soluble chemical. The primary route of exposure is ingestion and a TRV has therefore been established for the oral route.

Analysis and assessment of the choices for establishing the TRV

The primary route of exposure is ingestion and this will be the only route considered for the proposed VTR.

- *Choice of the critical effect*

Although this product has long been used in medicine, no chronic toxicity study on human exposure to chloral hydrate has been published. In animals and by the oral route, several subchronic and chronic studies (including carcinogenicity studies) have confirmed that the liver is the main target organ for chloral hydrate toxicity.

Given the carcinogenicity results, the cancer effect cannot be used for establishing the TRV. Moreover, in 2004, the IARC concluded that chloral hydrate is "not classifiable as to its carcinogenicity to humans" (Group 3) (IARC Monograph Volume 84, 2004).
The experts therefore proposed establishing a threshold dose TRV based on liver toxicity.

- *Choice of the key study*

Among the various published studies, the experts selected the one by **George *et al.* (2000)**[3] as the key study. Its quality was regarded as satisfactory and the effect studied (liver toxicity) was regarded as relevant for assessing the chronic toxicity of chloral hydrate in animals.

In this study, male B6C3F1 mice were exposed via drinking water to doses of 0, 13.5, 65 and 146 mg/kg bw/d for 104 weeks. The authors describe liver toxicity, expressed by the formation of proliferative cellular lesions at the first dose tested. George *et al*. refer to these lesions as preneoplastic altered foci of cells (AFC), but they have also been called "hyperplastic nodules". They correspond to groups of cells that modulate certain enzyme activities. Despite the term used by the authors, their histological features give no indication as to whether or not they may develop into tumours.

- *Choice of the critical dose*

The experts selected the dose of **13.5 mg/kg bw/d** as the **LOAEL**. It was not possible to calculate a BMD and $BMD_{10}L_{90\%}$ from these studies.

- *Choice of uncertainty factors and allometric adjustment*

The aim is to calculate a Human Equivalent Dose (HED).

$$\text{Human dose equivalent} = \text{Animal dose} \times \left(\frac{\text{Animal weight}}{\text{Human weight}} \right)^{1/4}$$

[3] George, M., Moore, T., Kilburn, S., Olson, G.R. and DeAngelo, A.B. (2000) Carcinogenicity of chloral hydrate administered in drinking water to the male F344/N rat and male B6C3F1 mouse. Toxicol. Pathol., 28: 610-618

In the study by George *et al.* (2000), the average mouse weight is equal to 40.6 g (±1.3 g), while that of humans is estimated at 70 kg. Doses are expressed in mg/kg bw/d.

Therefore, an **$LOAEL_{HED}$ = 2.1 mg/kg bw/d**

- An allometric adjustment was made to take interspecies variability into account, in order to be able to calculate a Human Equivalent Dose (HED), using the previous equation. To take toxicodynamic variability and residual uncertainties into account, an additional uncertainty factor was set at 2.5 as recommended by the reference document for "Establishment of a toxicity reference value for reprotoxic substances" (AFSSET 2007).
  UF_A = 2.5 (interspecies variability)

- To take intraspecies variability into account, a final default value of 10 was chosen:
  UF_H = 10 (intraspecies or interindividual variability)

- Because an LOAEL was used, an additional safety factor was introduced:
  UF_L = 3.

In view of the other toxicity studies, notably that by Poon *et al.* (2002)[4], use of a factor of 3 seems sufficient. As a reminder, the study by Poon *et al.* (2002) is a subchronic study in which a human equivalent critical dose of the same order of magnitude was calculated on the basis of a decrease in aldehyde dehydrogenase activity.

Result of the collective expert appraisal

Following the expert appraisal, the proposed chronic oral TRV for the hepatotoxic effects of chloral hydrate is:

Critical effect	Critical dose*	UF	Chronic oral TRV
Formation of hepatocellular proliferative lesions in mice George *et al*. (2000)	**LOAEL = 13.5 mg/kg/d** <u>Allometric adjustment</u> $LOAEL_{HED}$* = 2.1 mg/kg/d	**75** UF_A 2.5** UF_H 10 UF_L 3	**TRV = 28 µg/kg/d**

*HED: Human Equivalent Dose; **In order to take toxicodynamic variability into account, an uncertainty factor, UF_A, was set at 2.5.

The CES noted that establishing a TRV from the study by Poon *et al.* (2002) conducted in rats leads to an equivalent value.

Moreover, although no organisation has classified chloral hydrate for adverse effects during pregnancy, the CES drew attention to the fact that it is impossible to rule out a reprotoxic effect through a metabolite, TCA, a substance for which a reprotoxic TRV has also been proposed by the Agency (chronic oral TRV of 300 µg/kg bw/d based on an increase in cardiac malformations in rats).

[4] Poon *et al*. (2002) Subchronic toxicity of chloral hydrate on rats: a drinking water study.

4. AGENCY'S CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions and recommendations of the CES on Assessment of the risks related to chemical substances, regarding the preparation of an oral toxicity reference value for chloral hydrate, and adopts the chronic oral TRV of 28 µg/kg/d.

Critical effect	Critical dose*	UF	Chronic oral TRV
Formation of hepatocellular proliferative lesions in mice George *et al*. (2000)	**LOAEL = 13.5 mg/kg/d** <u>Allometric adjustment</u> $LOAEL_{HED}$* = 2.1 mg/kg/d	**75** UF_A 2.5** UF_H 10 UF_L 3	**TRV = 28 µg/kg/d**

*HED: Human Equivalent Dose;

** In order to take toxicodynamic variability into account, an uncertainty factor, UF_A, was set at 2.5.

Produced in six copies,

The Director General

Marc Mortureux

KEY WORDS

Chloral hydrate, toxicity reference values, critical dose, uncertainty factors, health effects, general population.