Maisons-Alfort, 12 July 2012

The Director General

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

on the development of TRVs by the respiratory route for cadmium and its compounds

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 July 12, 2012 shall prevail.

1. BACKGROUND AND PURPOSE OF THE REQUEST

In 2004, within the framework of the first French National Environment & Health Action Plan (PNSE1) (2004-2008) and the French Cancer Plan (2003-2007), AFSSET began specific work on developing toxicity reference values (TRVs) and enhancing French expertise in this area. Therefore, in accordance with its missions, AFSSET proposed to its scientific partners that a national programme on TRVs be established, investigating reprotoxic chemicals as a first step.

This approach was then extended to the field of chemical carcinogens, which led to the development in 2007 of a method for establishing TRVs based on carcinogenic effects. A pilot phase was conducted to validate implementation of the proposed method. Benzene, cadmium, ethanol, naphthalene and vinyl chloride were selected as the substances to be studied during this pilot phase. This Opinion concerns the TRVs on cadmium.

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 existing knowledge or assumptions made about the substances' mechanisms of action.

“Threshold dose” TRVs are established for substances that cause, above a certain dose, damage whose severity is proportional to the absorbed dose, while “non-threshold dose” TRVs are established for substances for which there is a probability, however small, that

even a single molecule entering the body will cause harmful effects for the organism. Threshold TRVs are usually expressed as acceptable or tolerable daily doses or concentrations [Acceptable Daily Intake (ADI), Tolerable Daily Intake (TDI), Tolerable Concentration in Air (TCA), etc.], or reference doses or concentrations [Reference Dose (RfD) or Reference Concentration (RfC)]. Non-threshold TRVs are generally expressed as excess risk per unit [Excess Risk per Unit (ERU), Drinking Water Unit Risk (DWUR), Inhalation Unit Risk (IUR), Reference Concentration (RC), etc.].

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, or a linear extrapolation to the origin derived from the critical dose for non-threshold TRVs.

TRVs[1] are established 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 French standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)".

The Agency entrusted examination of this expert appraisal to the Expert Committee (CES) on Assessment of the risks related to chemical substances. The CES mandated the Working Group on Toxicity reference values to conduct this expert appraisal. Their work was submitted at regular intervals to the CES. The report produced by the Working Group takes account of the observations and additional information provided by the CES members. The report entitled "Cadmium and its compounds: Development of TRVs by the respiratory route based on chronic toxicity" was validated by the CES on 8 December 2011.

3. ANALYSIS AND CONCLUSIONS OF THE CES

Cadmium is a heavy metal used in industry that is found mainly as an oxide, chloride, sulphate, nitrate or sulphide. Cadmium oxide (CdO) may be present in the atmosphere in the form of dust or fumes. Cadmium is also found in cigarette smoke in the form of minute particles of cadmium oxide, which settle mainly in the pulmonary alveoli.

While workers are most often exposed to cadmium and its compounds by inhalation, the general public is also exposed, orally for the most part (through food and drinking water), but also by the respiratory route, especially smokers (one cigarette contains 2 µg of cadmium, on average).

[1] Method of establishing toxicity reference values for carcinogenic chemicals, ANSES Scientific Edition, March 2010

Cadmium is widely distributed in the body, but particularly affects the kidneys and liver. Animals and humans seem to show comparable distribution patterns. Cadmium accumulation in the liver and kidneys has been reported in several publications describing results from autopsies on deceased individuals (from accidental or other causes) (ATSDR, 2008)[2].

Accidental inhalation of high concentrations of cadmium fumes has led to severe irritation of the respiratory tract and resulted in workplace fatalities (ATSDR, 2008). Similarly, cadmium has been shown to be toxic orally from a single ingestion of massive doses.

In the general population, the health effects of cadmium following repeated exposure are known mainly through studies conducted on inhabitants of areas contaminated by cadmium, whose exposure was estimated by measuring blood and urinary cadmium levels (ATSDR, 2008).

In humans, prolonged exposure induces kidney damage, brittle bones, reproductive disorders and an increased risk of cancer that has led to its being listed as "Carcinogenic to humans" (Group 1) by the International Agency for Research on Cancer (IARC).

In 2009 EFSA proposed a Tolerable Weekly Intake of 2.5 µg/kg bw/wk according to a "Benchmark Dose" (BMD) approach modelling the dose/effect relationship between urinary cadmium and excretion of urinary beta 2-microglobulin (EFSA, 2009)[3].

This Opinion relates to the establishment of TRVs for cadmium and its compounds in connection with repeated exposure through inhalation.

1. *TRV for carcinogenic effects*

- *Choice of the critical effect*

The relationship between cadmium exposure and lung and prostate cancer has been studied in humans in six cohorts in Europe and the USA (with overlapping populations), and in one cohort in China. Epidemiological studies on cancer risks associated with exposure to cadmium have mainly been conducted in occupational settings (inhalation exposures). In the work environment, workers are mainly exposed to cadmium oxide by inhalation. Workers are also exposed to other carcinogenic metals such as nickel, chromium and arsenic. In addition, the smoking habits of workers must also be taken into account. The experts gave priority to animal studies as the most suitable for producing the TRV because they are independent of any confounding factor.

Animals exposed to cadmium compounds by various routes have developed tumours of the lung. Malignant lung tumours have been observed in rats exposed to low doses of cadmium for short periods of time. Local tumours have been observed in rats and mice exposed to cadmium by injection. From these studies the IARC concluded that there was a sufficient level of evidence of the carcinogenicity of cadmium in animals.

Based on the results of genotoxicity studies, cadmium is considered to be an indirect genotoxic agent. The experts considered that the genotoxic action of cadmium is primarily through the inhibition of antioxidants, causing an increased production of intracellular reactive oxygen species such as H_2O_2 and through interactions on metal binding and sites in proteins involved in DNA transcription, replication and repair.

[2] ATSDR (Agency for Toxic Substances and Disease Registry) 2008. Toxicological Profile for Cadmium. 512 p.
[3] The EFSA Journal (2009) 980, 1-139 Cadmium in food. Scientific Opinion of the Panel on contaminants in the Food Chain

The experts selected the increased incidence of lung tumours in animals as the critical effect for establishing a threshold TRV for carcinogenic effects.

- *Choice of the study*

Inhalation studies carried out in rats indicate a relationship between exposure to cadmium derivatives and the onset of lung cancers.
In the study by Takenaka *et al.* (2003)[4], selected as the key study, male Wistar rats were exposed to aerosolised cadmium chloride at concentrations of 12.5 – 25.0 and 50.0 µg/m^3 23 hours a day, 7 days a week, for 18 months. The animals were observed for 13 months after exposure was discontinued. The relationship between cadmium exposure and the onset of adenocarcinomas and epidermoid and mucoepidermoid carcinomas was clearly established at the lowest concentration studied (See Table 1). A dose-response relationship between exposure to $CdCl_2$ and lung cancer was demonstrated in this study (Takenaka *et al.*, 1983).

Table 1: Dose-response relationship (Takenaka *et al*., 1983).

Lesions	**Cadmium Concentrations (µg.m^{-3})**			
	0	**12.5**	**25**	**50**
Pulmonary adenocarcinoma	0/38	4/39	15/38	14/35
Pulmonary epidermoid carcinoma	0/38	2/39	4/38	7/35
Pulmonary mucoepidermoid carcinoma	0/38	0/39	0/38	3/35
Total pulmonary carcinomas	0/38	6/39	20/38	25/35

- *Choice of the critical dose*

The data available in the study by Takenaka *et al.* (1983)[4] were modelled using the US EPA's Benchmark Dose software, BMDS 2.1.1. The key study showed a significant dose-response relationship between an increased incidence of lung tumours and the daily dose of exposure to cadmium (See Table 1).
The aim of the approach is to estimate the dose that corresponds to a defined level of response or a defined percentage of additional response compared to a control. This level or percentage is called the Benchmark Response (BMR). The BMDL, i.e., the lower limit of the confidence interval of the BMD, is considered to be the benchmark dose. The experimental data were fitted by the models developed by the US EPA for dichotomous data (gamma, logistic, multistage, probit, Weibull models, etc.).

The model that offered the best fit with the experimental data was selected, using the AIC measure[5]. The log-probit model was therefore selected to estimate the lower limit of the 95% confidence interval of a dose corresponding to a 10% increase in response (increased incidence of lung tumours) compared to the unexposed group. The $BMD_{10\%}$ and $BMD_{10\%}L_{95\%}$ were calculated because the 10% threshold is generally used in studies of carcinogenicity. The **$BMD_{10\%}$** was equal to **8.9 µg.m^{-3},** the **$BMD_{10\%}L_{95\%}$** equalled **6.37 µg.m^{-3}.**

[4] Takenaka S, Oldiges H, Konig H, *et al.* 1983. Carcinogenicity of cadmium chloride aerosols in W rats. J Natl Cancer Inst 70:367-373.

[5] The Akaike Information Criterion is a measure for selecting the most suitable model for determination of the BMD or BMC, the model with the lowest AIC being used.

- *Time and dose adjustments*

Time adjustment
In the study by Takenaka *et al.* (1983), the animals were exposed for 23 hours a day, 7 days a week, for 18 out of 24 months[6]. The time-adjusted $BMD_{10\%}L_{95\%}$ was 6.37µg.m^{-3} x 23/24 x 18/24, **or 4.58 µg.m^{-3}.**

Dose adjustment and interspecies variability
For the respiratory route, the US EPA has developed various dose adjustments that are based on physicochemical properties of the inhaled substance (particles or gas) and the site where the critical effects are observed (respiratory or extra-respiratory), which has led to different equations (US EPA, 1994). The objective of this adjustment is to reduce the value of the uncertainty on interspecies variability in order to determine a human equivalent concentration or human equivalent dose (HEC or HED). Calculation of the human equivalent concentration was performed using Multiple-Path Particle Dosimetry Model (MPPD2)[7] software. It yielded a value of **7.8 µg/m^3.**

- *Choice of uncertainty factors*

To account for toxicodynamic variability and residual uncertainties when transposing from animals to humans, an uncertainty factor was established at 2.5 according to the recommendations in the reference document "Toxicity reference values for carcinogenic substances. Establishing TRVs based on carcinogenic effects" (ANSES, 2010)[8]. The value of the uncertainty factor for interspecies variability is therefore 2.5.

A final value of 10 was chosen by default for intra-species variability (ANSES 2010).

- *Calculation of the TRV*

Where a TRV = $BMD_{10\%}$ $L_{95\%}$ equivalent in humans/(UF_A (2.5) * UF_H (10))

TRV = 0.3 µg.m^{-3}

2. *TRV for non-carcinogenic chronic effects*

- *Choice of the critical effect*

The kidney is the primary target organ, following prolonged exposure to cadmium by inhalation (ATSDR, 2008). Among the effects observed in workers were:

- tubular proteinuria (increased excretion of low-molecular-weight proteins),

[6] The standard exposure time of animals during a carcinogenicity study is 24 months.

[7] The original version of the Multiple-Path Particle Dosimetry Model (MPPD2) was jointly developed by the Chemical Industry Institute of Toxicology (CIIT, currently The Hamner Institutes for Health Sciences) and the Dutch National Institute for Public Health and the Environment (RIVM). The latest version of MPPD2 may be downloaded for free from Applied Research Associates, Inc., 4300 San Mateo Blvd. NE, Suite A-220, Albuquerque, NM 87110; 505-882-8074; http://www.ara.com/products/MPPD2.htm).

[8] Method of establishing toxicity reference values for carcinogenic chemicals, ANSES Scientific Edition, March 2010

- decreased resorption of other solutes (resulting in increased excretion of enzymes such as β-N-Acetylglucosaminidase (NAG), amino acids, glucose, calcium, inorganic phosphate,
- increased glomerular permeability (increased albumin excretion),
- decreased glomerular filtration.

The precursor sign accompanying renal toxicity associated with cadmium is an increase in low-molecular-weight proteins in urine, including β2-microglobulin, selected for establishment of the TRV.

- *Choice of the critical dose*

This TRV was established in two steps:

- a meta-analysis conducted by EFSA[9] identified a relationship between urinary cadmium concentration and nephrotoxicity (5% increase in tubular damage in the general population, measured by increased β2-microglobulin) (EFSA, 2009);
- a pharmacokinetic model was then used to determine the corresponding atmospheric concentrations of cadmium.

The critical internal dose, i.e., urinary cadmium concentration expressed in µg/g of creatinine, was estimated on the basis of studies in which exposure was mainly due to environmental factors, using a population composed of 93.5% Asian subjects (a population with greater sensitivity to cadmium toxicity). This meta-analysis combining 35 different studies enabled identification, by modelling, of the relationship between β2-microglobulin concentration and urinary cadmium concentration, for a target urinary cadmium concentration equal to 4 µg per gram of creatinine. EFSA then applied a calculated safety factor of 3.9 to allow for the uncertainty associated with the use of summary and not individual data (EFSA, 2009). A $BMD_{5\%}L_{95\%}$ was selected by ANSES experts for establishing the TRV, giving a target urinary cadmium concentration of **1.0 µg/g creatinine**[10].

Using the previously calculated internal dose, the atmospheric concentrations of cadmium were estimated using a physiologically-based pharmacokinetic (PBPK) model. Thus, daily exposure to an atmospheric concentration of cadmium oxide at 0.45 µg/m^3 would result in a urinary cadmium concentration of 1 µg/g creatinine, assuming that air was the sole source of cadmium.
This value was determined by taking an exhaled air volume of 20 m^3 and a urinary creatinine level of 1.5 g/d.

- *Choice of uncertainty factors, time and dose adjustment*

Considering that the critical dose established by EFSA was based, first, on a precursor effect, and second, on average values of 165 groups derived from 35 studies (or over 30,000 cases), the Working Group did not consider it useful to apply an additional uncertainty factor (UF_H). It should be noted that the majority of subjects in the study population being of Asian descent also produced a protective value for the less sensitive Caucasian populations.

- *Calculation of the TRV*

TRV= 0.45 µg.m^{-3}

[9] The EFSA Journal (2009) 980, 1-139 Cadmium in food. Scientific Opinion of the Panel on contaminants in the Food Chain
[10] Unlike the ATSDR, which has elected to use the urinary cadmium concentration associated with a 10% increase in proteinuria as the internal dose.

4. CONCLUSIONS AND RECOMMENDATIONS OF THE COLLECTIVE EXPERT ASSESSMENT ON TRVS FOR CADMIUM AND ITS COMPOUNDS

The Working Group established two chronic TRVs for cadmium by the respiratory route: one based on a study of carcinogenicity in animals, the other based on renal toxicity in humans.

Critical effect	Critical dose	Uncertainty factor	TRV
Combined incidence of lung tumours in rats Takenaka *et al.*, 1983	$BMD_{10\%}L_{95\%}$ = 6.37 µg.m^{-3} Time adjustment $BMD_{10\%}L_{95\% ADJ}$ = 4.58 µg.m^{-3} Dose adjustment $BMD_{10\%}L_{95\%}$ equivalent in humans = 7.8 µg.m^{-3}	UF = 25 UF_A=2.5 UF_H 10	**TRV = 0.3 µg.m^{-3}**

Critical effect	Critical dose	TRV
5% increase in tubular damage in the general population EFSA, 2009	Urinary cadmium $BMD_{5\%}L_{95\%}$ = 1 µg/g creatinine PBPK modelling Cadmium intake through food not considered	**TRV= 0.45 µg.m^{-3}**

The experts noted that in the study by Takenaka *et al.* (1983), the animals were exposed to $CdCl_2$ only by inhalation. The source of the cadmium was not fully identified in the EFSA study.

The experts consider that the TRVs established can be applied to cadmium and its compounds.

5. AGENCY 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 on the development of toxicity reference values for cadmium and adopts these TRVs.

The Director General

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KEY WORDS

Cadmium, toxicity reference values, critical dose, uncertainty factors, general population.