Opinion
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The Director General

Maisons-Alfort, 4 August 2017

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

on the development of TRVs by the respiratory route for decamethylcyclopentasiloxane (D5) (CAS No. 541-02-6)

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 the necessary information concerning these risks as well as the requisite expertise 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 4 August 2017 shall prevail.

On 3 February 2012, ANSES received a formal request from the Directorate General for Labour (DGT) to produce occupational exposure limits (OELs). Among the substances on the 2012 work programme was decamethylcyclopentasiloxane (D5).

1. BACKGROUND AND PURPOSE OF THE REQUEST

On 3 February 2012, the DGT asked ANSES to conduct an expert appraisal with a view to producing an OEL for decamethylcyclopentasiloxane (D5). To do this, a toxicological profile was prepared. Because D5 is used in a very broad range of applications (cosmetic raw material, biocides, perchloroethylene substitute for dry cleaning in laundries, etc.), ANSES decided to capitalise on the work performed by also proposing a TRV by inhalation for this compound.

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 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 (ANSES, 2015).

In practice, establishing a threshold 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 establishment of a critical dose from experimental doses and/or epidemiological data;
- adjustments and the application of uncertainty factors to the critical dose to take uncertainties into account.

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 (CES) on "Characterisation of substance hazards and toxicity reference values" (hereinafter referred to as the CES "Substances"). The methodological and scientific aspects of the work were presented to the CES between June 2014 and November 2015. It was adopted by the CES "Substances" at its meeting on 12 November 2015.

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 ANSES website (www.anses.fr).

3. ANALYSIS AND CONCLUSIONS OF THE CES

Most of the data used to draw up the toxicological profile for D5 come from animal studies.

Several effects have been observed in the many toxicity studies carried out on D5. The following critical effects were analysed and discussed:

- **Hepatic toxicity**: an effect on the liver was the most frequently observed effect in studies conducted on D5. Indeed, in all the repeated toxicity studies (oral and inhalation), at least an increase in liver weight was observed. This increase in liver weight (reflecting the hepatic toxicity of a compound) was associated with a doubling of γ-GT in females in the subchronic study by Burns-Naas *et al.* (1998b). However, the CES considers that the lack of reproducibility of this increase in γ-GT (whose basal level in rats is normally very low) and the fact that this effect is not associated with other changes in biochemical or histopathological parameters in this same study support an adaptive liver effect, and not toxicity.
- **Pulmonary vascular mineralisation**: this effect was observed in the two-generation study (Siddiqui *et al.*, 2007), but is not detailed by the authors. It is likely that what is referred to as mineralisation is actually calcification. The fragmentary information on this effect as described in the study means that it cannot be retained as a critical effect of D5 toxicity.

- **Alveolar histiocytosis**: alveolar histiocytosis is an infiltration of lung tissue by monocyte-macrophage lineage cells. This histiocytosis differs from Langerhans cell histiocytosis, which is a multisystem disease related to the accumulation of Langerhans cells in tissues, which may be attributable to tobacco and is found in young human subjects. This alveolar histiocytosis is also observed in humans following exposure to the herbicidal active substance paraquat, bituminous products and paraffin oils. Histiocytosis has also been found in various animal studies: according to Élies (thesis, 2009), it has been reported in controls in carcinogenicity studies in which the C57BL/6 mouse is the model used. According to Boorman (1990) and Mohr (1992), alveolar histiocytosis may be an exacerbation of lesions found in older rats due to the presence of material in the lungs. The fragmentary information on this effect as described in the study by Siddiqui *et al.*, 2007 means that it cannot be retained as a critical effect of D5 toxicity.
- **Carcinogenicity**: adenocarcinomas of the uterine endometrium were observed in a chronic D5 toxicity study (Jean *et al.*, 2015). Despite statistical significance at the highest dose, no dose-response relationship appears, meaning that it is not possible to affirm the existence of a causal relationship associated with exposure to D5. Moreover, the mechanism of action has not been fully elucidated. Although it is conceivable that the onset of endometrial adenocarcinomas is related to the ageing of rats, it is still not possible to conclude that this effect cannot be transposed to humans. However, a genotoxic effect of D5 can be ruled out, as the various tests performed, *in vitro* or *in vivo*, were all negative.
- **Lung inflammation**: lung inflammation was also a consistent effect reported in the repeated toxicity studies available on D5. Despite the predominance of this effect, dose-response relationships are difficult to establish in the available studies. Inflammatory effects are mainly observed at high doses, at which animals would no longer be exposed only to vapours, but also to aerosols. It may therefore be possible that this inflammation is the body's physiological reaction to the entry of a foreign body, and is therefore not specific to D5. However, in the subchronic exposure study by Burns-Naas *et al.* (1998b), an increase in this inflammatory phenomenon was observed at concentrations at which D5 was still in vapour form. Moreover, this effect was not reversible after one month.

Development of a chronic TRV by inhalation

Analysis of the existing TRVs

There is currently no TRV available for D5.

Choice of the critical effect

From the analysis of the many studies on D5, it appears that all the effects assessed are questionable and none clearly stands out as a critical effect. Nevertheless, the widespread use of this compound in many products (cosmetics, biocides, etc.) justifies the establishment of a TRV. The CES experts therefore decided, by default, to select the lung inflammation effects as the critical effect for establishing the TRV. In addition, a TRV on these inflammatory effects would protect against occurrence of the adenocarcinomas observed in the chronic exposure study.

Choice of the key study

Lung inflammation was observed in many studies. The study by Burns-Naas *et al.* (1998b) is the only one for which a dose-response relationship appeared to emerge, and for which a Benchmark Concentration (BMC) could be established. This was a 13-week study conducted in Fischer 344 rats at the following doses: 0; 28.6; 49.2; 87.7 and 233 ppm (0; 432; 743; 1324; 3518 $mg \cdot m^{-3}$)[1]. In addition, this study followed the OECD 413 guideline. However, at the highest concentration, the CES notes that D5 was no longer only in vapour form, but also in aerosol form.

Choice of the critical dose

The experimental data established on lung inflammation were modelled with mathematical models used by the PROAST software (PROAST version 38), developed by the RIVM[2], in order to establish a BMC.

The aim of this approach is to estimate the concentration 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).

When determining the BMCL (lower limit of the confidence interval of the BMC), several mathematical models were tested. The maximum likelihood method was used to fit the model to the data. The confidence level associated with the BMCL is 95% (ANSES, 2015).

In the case of D5, the model best fitted to the experimental data was the Gamma model.

The critical dose comes from the establishment of a BMC. The parameters used are an excess risk of 10%. The values selected were as follows:

- $BMC_{10\%}$: 82.3 ppm
- $BMC_{10\%}L_{95\%}$: 59.1 ppm

Dose adjustment

The aim is to reduce the value of the uncertainty about interspecies variability in order to determine a human equivalent concentration (HEC). For the respiratory route, the US EPA[3] has developed various dose adjustments that are made according to the physico-chemical properties of the inhaled substance (particles or gas, highly soluble or relatively insoluble in water) and the site where the critical effects are observed (respiratory or extra-respiratory), leading to different equations (US EPA, 1994).

According to the recommendations of the US EPA (1994), D5 should be regarded as a Category 3 gas (systemic toxicity). The dose adjustment applied by default for a Category 3 gas is as follows:

$$BMC_{10\%}L_{95\%\ HEC} = BMC_{10\%}L_{95\%}\ animal \times (Hb/g)_{Rat}/(Hb/g)_{Human}$$

Where (Hb/g): blood/air partition coefficient of D5
HEC: human equivalent concentration

[1] 1 ppm = 15.1 $mg \cdot m^{-3}$

[2] RIVM: Rijksinstituut voor Volksgezondheid en Milieu

[3] US EPA: United States Environmental Protection Agency

According to the data available in the literature, the blood/air partition coefficients of D5 are 0.55 in rats and 0.5 in humans (M. Andersen, personal communication, currently being published). However, because the $(Hb/g)_{Rat}/(Hb/g)_{Human}$ ratio is greater than 1 (1.1), the US EPA proposes retaining the default value of 1, which is more protective.

$$BMC_{10\%}L_{95\%\ HEC} = \mathbf{59.1\ ppm}$$

Temporal adjustment

The animals were exposed for 6 hours a day, 5 days a week. To take the discontinuity of the exposure into account, a time adjustment was made:

$$BMC_{10\%}L_{95\%\ HED\ ADJ.} = BMC_{10\%}L_{95\%\ HED} \times (6/24) \times (5/7) = \mathbf{10.55\ ppm}$$

Choice of uncertainty factors

The TRV was calculated from the $BMC_{10\%}L_{95\%\ HEC\ ADJ}$ using the following uncertainty factors (ANSES, 2015):

- Inter-species variability (UF_A): 2.5

The dose adjustment performed enabled a human equivalent concentration to be calculated, using the previous equation. To take toxicodynamic variability and residual uncertainties into account, an additional uncertainty factor was set at 2.5.

- Inter-individual variability (UF_H): 10

Because there were no scientific data available to reduce the default value, the value of 10 was used.

- Subchronic to chronic transposition (UF_S): 1

A UF_S was considered, since the key study used for establishing the TRV was one involving subchronic exposure. However, by expert consensus, for this type of effect, it is considered that a 90-day subchronic exposure study is adequate for establishing a chronic TRV without adding a further uncertainty factor.

- Use of a BMDL, LOAEL/C or NOAEL/C ($UF_{B/L}$): 1

Because establishment of the TRV was based here on a BMCL, this factor does not apply.

- Inadequacy of the data (UF_D): 1

The toxicological data for D5 were considered sufficient for establishing the TRV.

An overall uncertainty factor of **25** was thus used to determine the TRV for D5.

Calculation of the TRV

$$TRV = 0.422\ ppm \text{ or } \mathbf{6.4\ mg \cdot m^{-3}}$$

Confidence level

An overall confidence level was assigned to this chronic TRV by the respiratory route based on the following criteria:

- Level of confidence in the type and quality of the data:

High: The toxicological data are sufficient for assessing this compound.

- Level of confidence in the choice of the critical effect and the mode of action:

Low: This is an effect found in many studies, but for which a dose-response relationship is difficult to demonstrate. In addition, it is an effect found mainly during exposure to aerosols.

- Level of confidence in the choice of the key study:

Moderate: This is a well detailed study that followed the OECD 413 guideline. However, the highest concentration was made up of 40% aerosols, and no longer only vapours.

- Level of confidence in the choice of the critical dose:

Low: Even though it was possible to establish a BMC, the quality of the dose-response relationship is poor. In addition, with regard to the uncertainty factors and adjustments, there were no data that would have made it possible to move away from the default values.

Thus, the overall level of confidence for this TRV is **low**.

The report was validated by a majority of the experts present (14 out of the 18 present). Four experts chose to abstain in light of the data available for characterising the hazardous nature of the substance, the critical effect selected, i.e. lung inflammation in rodents, and the absence of a dose/response for this effect. However, they did not call into question the value of establishing a TRV in order to be able to manage the potential risks of D5, in view of the many uses of this substance by the general population.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions and recommendations of the CES "Substances" on the formulation of toxicity reference values by inhalation for D5.

Critical effect **Key study**	**Critical concentration**	**UF**	**TRV**
Lung inflammation Burns-Naas *et al.*, 1998b: 13-week study in F344 rats	$BMC_{10\%}$ $L_{95\%}$ = 59.1 ppm <u>Dose adjustment</u> $BMC_{10\%}L_{95\%\ HEC}$ = 59.1 ppm <u>Temporal adjustment</u> $BMC_{10\%}L_{95\%\ HED\ ADJ.}$= 10.55 ppm	25 UF_A: 2.5 UF_H: 10	TRV = **6.4 mg·m^{-3}** or 0.422 ppm Confidence level: **low**

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KEYWORDS

Decamethylcyclopentasiloxane, D5, toxicity reference value, inhalation

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