The Director General

Maisons-Alfort, 29 September 2017

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

on the development of TRVs by the respiratory route for octamethylcyclotetrasiloxane (D4) (CAS No. 556-67-2)

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 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 29 September 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 octamethylcyclotetrasiloxane (D4).

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 octamethylcyclotetrasiloxane (D4). To do this, a toxicological profile was prepared. D4 is used in a very broad range of applications: a raw material in cosmetics, in biocides, a formulation ingredient in plant protection products, etc.

In view of D4's wide-ranging uses and its classification as a Category 2 reprotoxic substance, ANSES decided to capitalise on the work performed by also proposing a toxicity reference value, or 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.

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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 February 2014 and October 2016. It was adopted by the CES "Substances" at its meeting on 20 October 2016.

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 D4 come from animal studies.

Since the concentration limit of the vapour phase for D4 has been identified at 700 ppm (or 8400 mg·m^{-3}), in the majority of studies on D4 this concentration was not exceeded.

Toxicokinetics

According to the available studies, absorption of D4 by inhalation varies between 4 and 13%. For example, in a study of 12 volunteers exposed to D4, absorption during rest periods was 11% and during exercise 6% (Utell *et al.*, 1998). D4 is very poorly absorbed via the skin (<1%).

Concerning metabolism in studies conducted in volunteers and rats, D4 was measured in non-metabolised form in exhaled air, whereas in urine, only metabolised forms were measured (Plotzke *et al.*, 2000; Reddy *et al.*, 2003). The two major metabolites identified in the urine of rats are dimethylsilanediol ($Me_2Si(OH)_2$) and methylsilanetriol ($MeSi(OH)_3$). They account for between 75 and 85% of urinary metabolites (SCCS[1], 2010).

The main pathways of elimination of D4 are firstly exhaled air and secondly the urinary tract.

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[1] SCCS: Scientific Committee on Consumer Safety

Toxicity

Several effects have been observed in the various studies available on D4:

- **Hepatic effects**: effects on the liver are the most frequently observed effects in studies conducted on D4. Indeed, in all the repeated toxicity studies (oral and inhalation), at least an increase in liver weight was observed. In the study by Burns-Naas *et al.* (2002), this increase was associated with a sharp increase in serum gamma-glutamyl transferase (γ-GT) at the highest concentration in males (168%) and at the two highest concentrations in females (330% at 5856 mg·m^{-3} and 975% at 10,776 mg·m^{-3}), as well as a slight increase in alanine aminotransferases (ALT) at the highest concentration in both sexes (males: 26%, females: 15%). However, histopathological lesions were not observed in this study. In its guidance on liver effects, the US EPA[2] (2002) states that an ALT increase should not be considered harmful until they reach two or three times the levels observed in the control group. The US EPA also states that in the absence of histopathological lesions, the serum levels of at least two serum parameters should be significantly increased for them to be likened to liver toxicity. Consequently, the lack of reproducibility of this increase in γ-GT between studies, and the fact that this increase was not associated with other changes in biochemical or histopathological parameters in the study by Burns-Naas *et al.* (2002), would therefore support an adaptive liver effect, and not toxicity;

- **Respiratory effects**: the observed respiratory effects (goblet cell hyperplasia, squamous epithelial hyperplasia, increased incidence of eosinophilic globules in the respiratory tract, etc.) are local non-specific effects related to rat anatomy. Indeed, in rats, the olfactory epithelium is much more developed than in humans, making these effects difficult to transpose to humans;

- **Renal effects**: the effects on the kidney do not show a dose-response relationship, and no serum markers are available to demonstrate impaired function;

- **Reproductive toxicity**: two studies are available for assessing the reproductive effects of D4 (Meeks *et al.*, 2007; Siddiqui *et al.*, 2007). They are highly consistent in their results. The number of corpora lutea was reduced, but this effect is difficult to exploit, as it is difficult to establish a dose-response relationship.

  The selected effects are therefore a decrease in the number of implantation sites and a decrease in the number of viable foetuses, statistically significant at least at the highest dose (8400 mg·m^{-3}), and presenting a dose-response relationship. These effects, which were observed in both studies, cannot be ruled out in humans.

Development of a chronic TRV by inhalation

Analysis of the existing TRVs

There is currently no TRV available for D4.

Choice of the critical effect

Concerning the effects on reproduction, the two parameters for which a statistical significance and a dose-response relationship appear were analysed: the decrease in the number of implantation sites and the decrease in the number of viable foetuses.

Following the establishment of benchmark concentrations (BMCs) based on the studies by Meeks *et al.* (2007) and Siddiqui *et al.* (2007), the decrease in the number of implantation sites appears to

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[2] US EPA: US Environmental Protection Agency

occur at a slightly lower concentration than the decrease in the number of viable foetuses. This parameter was therefore used to establish a TRV (see Table 1).

Choice of the key study

Two studies show a decrease in the number of implantation sites in rats with highly comparable results: Siddiqui *et al.* (2007) and Meeks *et al.* (2007).

The exposure doses are identical in these two studies: 0, 840, 3600, 6000 and 8400 mg·m^{-3}. However, the exposure protocols are slightly different. In the study by Siddiqui *et al.* (2007), 30 rats per sex per dose were exposed for 70 days before mating until weaning. In the study by Meeks *et al.* (2007), 20 female rats per dose were exposed before mating and until the 19th day of gestation.

In addition, the study by Siddiqui *et al.* (2007) was conducted according to OPPTS[3] guidelines and Good Laboratory Practice (GLP), which was not the case with the study by Meeks *et al.* (2007).

Based on these observations, the key study selected to establish the TRV for D4 was the study by Siddiqui *et al.* (2007).

The CES considered that it was appropriate to establish a chronic TRV based on a decrease in the number of implantation sites because:
-   this is the effect that occurs at the lowest doses;
-   rats of both sexes were exposed in the study by Siddiqui *et al.* (2007), and the males showed no evidence of D4 toxicity. They will therefore be protected by a chronic TRV based on a decrease in the number of implantation sites;
-   in the study by Siddiqui *et al.* (2007), animals were exposed for 70 days. Although it has been shown that the effect may occur following a shorter period of exposure, such a duration of exposure (as well as the subchronic and chronic studies on the substance) demonstrates that there is no more sensitive effect occurring with longer exposure. This is therefore the most sensitive effect.

Choice of the critical dose

The experimental data, established on the decrease in the number of implantation sites summarised in the table below, were modelled with mathematical models used by the PROAST software (PROAST version 38) developed by the RIVM[4], in order to establish a BMC.

Table 1: Number of implantation sites in the F0 generation (Siddiqui *et al.*, 2007)

Dose (mg·m^{-3})	0	840	3600	6000	8400
Average	14.2	13.7	12.8	11.6	10.4**
Standard deviation	2.9	4.4	3.3	4.6	5

The aim of the 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). It corresponds to an excess risk of 5% (BMR recommended by ANSES and EFSA[5] for quantal data for effects on reproduction).

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[3] OPPTS: Office of Prevention, Pesticides and Toxic Substances (US EPA)
[4] RIVM: Netherlands National Institute for Public Health and the Environment
[5] EFSA: European Food Safety Authority

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.

In the case of D4, the model best fitted to the experimental data was the exponential model.

The values selected were as follows:

- BMC$_{5\%}$: 96 ppm
- BMC$_{5\%}$L$_{95\%}$: 72.9 ppm

Dose adjustment

Physiologically-based pharmacokinetic (PBPK) models have been developed to model the fate of D4 in organisms of different species (rats, humans) for different routes of exposure (oral, respiratory, dermal) (McMullin, 2016).

The approach consists in converting the external exposure dose in animals to an internal dose using a PBPK model (expressed in mg/L).

Thus, for a calculated critical dose (BMD$_{5\%}$L$_{95\%}$ = 72.9 ppm), the associated internal dose of D4 would be 0.6 mg/L.

In humans, the same internal quantity can be expected to cause the same effects.

A human PBPK model was used to predict the corresponding concentrations of D4.

The D4 exposure dose in humans was estimated from the internal dose previously calculated in rats.

Thus, daily exposure to a D4 concentration of 160 ppm, i.e. 1920 mg·m^{-3}, would in humans lead to an internal quantity of substance in the body close to 0.6 mg/L. This value can be regarded as the BMC$_{5\%}$L$_{95\%\ HEC}$.

Temporal adjustment

The animals were exposed for 6 h/day every day for 70 days. However, given the nature of the effect and the experiments conducted in the study by Meeks *et al.* (2007) showing that the effect only develops over very short exposure windows, it was not considered necessary to apply a time adjustment.

Choice of uncertainty factors

The TRV was calculated from the BMC$_{5\%}$L$_{95\%\ HEC}$ using the following uncertainty factors (ANSES, 2015):

- Inter-species variability (UF$_A$): 2.5

A human equivalent concentration was calculated using the PBPK model (McMullin *et al.*, 2016). To account for toxicodynamic variability and residual uncertainties, an additional uncertainty factor was set at 2.5 according to IPCS[6] recommendations (IPCS, 2005) and based on ANSES practices.

- Inter-individual variability (UF$_H$): 4.2

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[6] IPCS: International Programme on Chemical Safety

The value used by default for this factor is 10, divided into two components, a toxicokinetic value of 3.16 and a toxicodynamic value of 3.16.

The PBPK model was used to refine the toxicokinetic component, in accordance with WHO[7] proposals (McMullin *et al.*, 2016). In this model, the default variation applied to each physiological parameter (tissue volumes, blood flows) was established at +/- 50%. Regarding body weight, data from the Second Individual and National Study on Food Consumption (INCA2) were used, resulting in an average of 60.5 kg +/- 20.62 for 3-79 year olds.

Estimates of venous concentrations for the median, 95th percentile and 5th percentile over 6 hours of exposure were obtained following 1000 iterations.

The estimate of this UF_{H-TK} was then obtained by calculating the ratio of the 95th percentile and the median venous concentration after 6 hours of exposure, i.e.: 0.84/0.63 = 1.33

$UF_H = UF_{H-TD} \times UF_{H-TK} = 3.16 \times 1.33 = 4.2$

- Subchronic to chronic transposition (UF_S): 1

A UF_S was considered, since the key study used for establishing the TRV was one in which the animals were exposed for 70 days. However, this exposure time covers a complete reproductive cycle, and no more sensitive effects have been observed in subchronic or chronic exposure studies. The use of a UF_S was therefore not regarded as relevant.

- 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 D4 were considered sufficient for establishing the TRV.

An overall uncertainty factor of **10.5** was thus used to establish the TRV for D4.

Calculation of the TRV

TRV = 15.2 ppm, or **183 mg·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:

Moderate: the effect is robust, found at similar levels in both studies assessing it. However, the mode of action is not specified.

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

High: this was a very detailed study that follows the OPPTS (US EPA) guidelines and was conducted according to GLP. This study is also supported by the one by Meeks *et al.* (2007).

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

High: the quality of the dose-response relationship is good, it was possible to establish a BMC. In addition, there is a good-quality PBPK model available that was used to derive the human equivalent dose.

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[7] WHO: World Health Organisation

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

The report was validated unanimously by the experts present (13 experts present).

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 chronic toxicity reference values for inhalation for D4.

Critical effect Key study	Critical concentration	UF	TRV
Decrease in the number of implantation sites Siddiqui *et al.*, 2007	$BMC_{5\%}L_{95\%} = 72.9$ ppm $BMC_{5\%}L_{95\%\ HEC} = 160$ ppm	10.5 UF_A: 2.5 UF_D: 1 UF_H: 4.2 UF_L: 1 UF_S: 1	**TRV = 183 mg·m^{-3}** or 15.2 ppm Confidence level **High**

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KEYWORDS

Octamethylcyclotetrasiloxane, D4, toxicity reference value, inhalation

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