The Director General

Maisons-Alfort, 10 November 2011

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

on "the development of oral toxicity reference values based on the reprotoxic effects for dichloroacetic acid, trichloroacetic acid and dibromoacetic acid"

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 24 July 2009 in order to prepare oral toxicity reference values for dichloroacetic acid, trichloroacetic acid and dibromoacetic acid.

1. BACKGROUND AND PURPOSE OF THE REQUEST

On 22 December 2006 the French Director General for Health (DGS) of the Ministry of Health and Sports, the Director General for Pollution and Risk Prevention (DGPR) and the Director for Water and Biodiversity of the Ministry of Ecology, Energy, Sustainable Development and Land Planning made a formal request to the Agency for assessment of the health risks associated with public bathing areas not governed by current regulations. While addressing the formal request on **"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",** the expert working group on "Assessment of the health risks associated with water quality and swimming pool water treatment products and processes" considered the establishment of specific toxicity reference values (TRVs) to protect from the proven reprotoxic effects of haloacetic acids (HAAs).

Together with chloral hydrate, haloacetic acids are the main chlorinated by-products of disinfection found in drinking water and swimming pool water, where they are generally found at the highest concentrations. They are produced when chlorine reacts with natural organic matter.

Dichloroacetic acid (DCA) is the HAA most frequently found in French and international swimming pool water (around 600 µg/L, WHO, 2006). Trichloroacetic acid (TCA) is the second most common HAA in French and international swimming pool water (around

150 µg/L, WHO, 2006), while dibromoacetic acid (DBA) has been found in French and international swimming pool water at concentrations of about 10 µg/L (WHO, 2006).

In July 2009 the Agency entrusted the preparation of toxicity reference values (TRVs) for these substances to the "Toxicity reference values" working group.

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 (US EPA, 1998). 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 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.

This expert appraisal falls within the field of competence of the Expert Committee (CES) on Assessment of the risks related to chemical substances. ANSES entrusted the appraisal to the "Toxicity reference values" working group. The methodological and scientific aspects of the work were regularly submitted to the CES between 22 October 2009 and 18 February 2010. The work was adopted by the CES on Assessment of the risks related to chemical substances at its meeting of 7 January 2010 for dichloroacetic acid and trichloroacetic acid, and of 18 February 2010 pour dibromoacetic acid.

The scientific aspects of this Opinion are based on the final report from this collective expert appraisal, entitled "Preparation of oral TRVs based on the reprotoxic effects for dichloroacetic acid, trichloroacetic acid and dibromoacetic acid" dated April 2010.

3. ANALYSIS AND CONCLUSIONS OF THE CES

Dichloroacetic acid (DCA), trichloroacetic acid (TCA) and dibromoacetic acid (DBA) are non-volatile, highly polar and highly soluble chemicals. The primary route of exposure is ingestion and TRVs have therefore been established for the oral route.

■ TRV for dichloroacetic acid (CAS 79-43-6)

1. *TRV for developmental effects*

- *Choice of the critical effect*

Cardiovascular malformations were observed in three studies in rats. Other malformations (urogenital and ocular) have also been observed, in the presence of maternal toxicity. However, the moderate nature of the maternal toxicity, the type of malformations (cardiovascular damage), and their occurrence during *in vitro* study do not support the view that maternal toxicity plays a decisive role. Moreover, the choice of dose range used in these studies makes it impossible to consider these maternal effects as occurring before the cardiac effects.

The experts selected the increased incidence of cardiac malformations as the critical effect for establishing an oral TRV.

- *Choice of the study*

Among the studies, the one by Smith *et al.* (1992)[1] was considered to be of good quality (rated Klimisch 1). It comprised four tested doses and shows a dose-effect relationship (Table 1).

[1] Smith MK *et al.,* 1992. Developmental toxicity of dichloroacetate in the rat. Teratology 46(3), 217-223.

Table 1: Incidence of foetal malformations as a result of maternal exposure to DCA on gestation days 6 to 15 (Long-Evans rats)

Dose mg/kg bw/d	Number of litters affected	Number of litters
0	0	39
14	1	18
140*	2	19
400*	6	19

** significant: p< 0.05*

- *Choice of the critical dose*

In accordance with the Agency's recommendations concerning the TRV establishment method based on effects on reproduction and development[2], the dose can be used to calculate an acute TRV for developmental effects. Establishment of a Benchmark Dose (BMD) was proposed, based on the results presented in Table 1.

The available data were modelled with the Dutch RIVM's Proast 18.2 software in order to prepare a Benchmark Dose. A significant dose-response relationship between increased cardiac malformations and the daily dose of exposure to DCA appears in the key study (Table 1) (Kruskal-Wallis test).
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) level. This is predominantly the BMDL, i.e. the benchmark dose lower confidence limit. The experimental data were fitted by the models developed by the RIVM for dichotomous data (gamma, logistic, multistage, probit, Weibull models, etc.).

The model chosen was the one that best fitted to the experimental data using the maximum likelihood method (log likelihood): the log-logistic model was selected for estimating the lower limit of the 90% confidence interval[3] of a dose corresponding to a 5% increased incidence of foetal malformations compared to the unexposed group. This model is the one whose maximum likelihood was closest to the "full" model. The $BMD_{5\%}$ and $BMDL_{5\%}$ were calculated, as the 5% threshold is generally used in reprotoxicity studies. The **$BMD_{5\%}$** is equal to **45.2 mg/kg bw/d** and the **$BMD_{5\%}L_{90\%}$ to 24.6 mg/kg bw/d.**

- *Choice of uncertainty factors and allometric adjustment*

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 following equation (US EPA, 2006):

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

The average rat weight was determined from the data described in the study to be 339 g and was measured on day 20, while the human weight is estimated at 70 kg. Doses are expressed in mg/kg/d.

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

[3] The lower limit of the 95% confidence interval (one-sided) used by the US EPA is equivalent to the lower limit of the 90% confidence interval (two-sided) used by the RIVM.

Therefore, the equivalent dose in humans = the animal dose * 0.26 (which amounts to applying a factor of 3.8 that takes toxicokinetic variability into account).

To take toxicodynamic variability and residual uncertainties into account, an 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). The value of the uncertainty factor for interspecies variability is therefore 2.5.
A final default value of 2.5 was chosen for intraspecies variability, UF_H = 10 (intraspecies or interindividual variability).

2. *TRV for effects on fertility*

- *Choice of the critical effect*

The experts selected the effects observed on the male reproductive organs, particularly the decrease in sperm count, to establish an oral TRV.

- *Choice of the study*

The study by Toth *et al.*[4] in rats can be rated Klimisch 2. Damage was observed in the testes at all doses (Table 2). The study can therefore be used to calculate a chronic TRV for developmental effects.

Table 2: Reduction in sperm count (10^6/g) in the epididymis as a result of exposure to DCA in male Long-Evans rats

Dose (mg/kg bw/d)	Mean (sperm count (10^6)/g) in the epididymis	Standard deviation	Number of animals
0	630.0	204.8	19
31.25	582.5	137.0	18
62.5*	502.6	163.5	18
125*	367.8	91.6	19

** significant: p<0.05*

- *Choice of the critical dose*

In accordance with the Agency's recommendations concerning the TRV establishment method based on effects on reproduction and development, the CES proposed establishing a benchmark dose.

The available data were modelled with the RIVM's Proast software in order to prepare a Benchmark Dose (BMD). A significant dose-response relationship between the decrease in spermatozoa and the daily dose of exposure to DCA appears in the key study (Table 2).
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) level. This is predominantly the BMDL, i.e. the benchmark dose lower confidence limit.

[4] Toth GP *et al.,* 1992. Adverse male reproductive effects following subchronic exposure of rats to sodium dichloroacetate. Fundamental and Applied Toxicology: Official Journal of the Society of Toxicology 19(1), 57-63.

The experimental data were fitted by the models developed by the RIVM for continuous data.
The model chosen was the one that best fitted to the experimental data using the maximum likelihood method (log likelihood) and corresponds to the exponential model. This model is the one whose maximum likelihood was closest to the "full" model. The model was selected for estimating the lower limit of the 90% confidence interval of a dose corresponding to a 32.5% decrease in sperm count (or one times the standard deviation, SD[5]). The BMD_{1xSD}[6] and $BMD_{1xSD}L_{90\%}$ were calculated and are equal to **91.1** mg/kg bw/d and **72.1** mg/kg bw/d respectively.

- *Choice of uncertainty factors and allometric adjustment*

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 following equation[7]:

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

The average rat weight was determined from the data described in the study to be 484 g, before sacrifice, while the human weight is estimated at 70 kg. Doses are expressed in mg/kg/d.

Therefore, the equivalent dose in humans = the animal dose * 0.29 (which amounts to applying a factor of 3.5 that takes toxicokinetic variability into account).
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).
A final default value of 10 was chosen for intraspecies variability, UF_H = 10 (intraspecies or interindividual variability).

[5] This approach has been proposed by the US EPA as the default approach when no information is available on the choice of level of effect observed during biochemical and biological analysis in the control group.
[6] This amounts to considering as the threshold value the mean (sperm count in the untreated group) minus one times the standard deviation of the untreated group, and corresponds to a 32.5% decrease in spermatozoa.
[7] This equation comes from the recommendations of the US EPA (US EPA, 2006).

Conclusions and recommendation of the collective expert appraisal relating to the TRVs for DCA

- **TRV for developmental effects**

The CES proposes an oral TRV specifically for the effects of DCA on foetal development (teratogenic effects) applicable **for acute exposure**.

Critical effect	Critical dose*	Uncertainty factor	TRV
Increased cardiac malformations in Long-Evans rats Smith *et al.,* 1992	$BMD_{5\%}L_{90\%}$=24.6 mg/kg bw/d Allometric adjustment $BMD_{5\%}L_{90\%\ HED}$ = 6.5 mg/kg bw/d	**UF 25** UF_A 2.5 UF_H 10	**TRV = 260 µg/kg bw/d**

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

- **TRV for effects on fertility**

The CES proposes an oral TRV specifically for the effects of DCA on fertility applicable **for chronic exposure**.

Critical effect	Critical dose*	Uncertainty factor	TRV
Decrease in spermatozoa in Long-Evans rats Toth *et al.,* 1992	$BMD_{1xSD}L_{90\%}$ = 72 mg/kg bw/d Allometric adjustment $BMD_{1xSD}L_{90\%HED}$ = 21 mg/kg bw/d	**UF 25** UF_A 2.5 UF_H 10	**TRV = 840 µg/kg bw/d**

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

The CES draws the Agency's attention to the fact that the proposed TRVs for the reprotoxic effects (developmental toxicity and effects on fertility) would not provide protection from DCA's other toxic effects, cancer in particular. The CES therefore recommends a critical analysis of existing TRVs, TRVs based on the carcinogenic effects of DCA, and if necessary, developing a specific TRV for carcinogenic effects.

TRV for trichloroacetic acid (CAS 76-03-9)

- *Choice of the critical effect*

After summarising the previously detailed studies, the critical effect selected is the cardiac malformations resulting from maternal exposure to TCA during organogenesis. The studies by Singh (2006)[8] also showed neurological and testicular effects in the absence of significant maternal toxicity, but the choice of dose range used in these studies makes it impossible to consider these effects as occurring before the cardiac effects.

The experts therefore selected the increased incidence of cardiac malformations as the critical effect.

- *Choice of the study*

The most appropriate study for calculating a toxicity reference value, highlighting the critical effect, is the study by Smith *et al.* (1989)[9], for the following reasons:

- general methodological quality, in particular the number of animals per group, the dose regime and the study of the foetuses,
- transparency of the results enabling a full statistical analysis.

This study was considered to be of good quality (rated Klimisch 1).

- *Choice of the critical dose*

Establishment of a Benchmark Dose was proposed, based on the results presented in Table 3.

Table 3: Incidence of foetal malformations resulting from maternal exposure to TCA at gestation days 6 to 15 (Long-Evans rats)

Dose (mg/kg bw/d**)**	**Number of litters affected**	**Number of litters**
0	1	26
330*	6	19
800*	12	17
1200*	11	14
1800*	8	8

** significant p< 0.05*

The available data were modelled with the RIVM's Proast 18.2 software in order to prepare a Benchmark Dose (BMD). A significant dose-response relationship between increased cardiac malformations and the daily dose of exposure to TCA appears in the key study (Table 3) (Kruskal-Wallis test).
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) level. This is predominantly the

[8] Singh R. 2006 Neuroembryopathic effect of trichloroacetic acid in rats exposed during organogenesis.
[9] Smith MK *et al.*, 1989. Teratogenic activity of trichloroacetic acid in the rat. Teratology 40(5), 445-451.

BMDL, i.e. the benchmark dose lower confidence limit. The experimental data were fitted by the models developed by the RIVM for dichotomous data (gamma, logistic, multistage, probit, Weibull models, etc.).
The model chosen was the one that best fitted to the experimental data using the maximum likelihood method (log likelihood): the gamma model was selected for estimating the lower limit of the 90% confidence interval[10] of a dose corresponding to a 5% increased response compared to the unexposed group. This model is the one whose maximum likelihood was closest to the "full" model. The **$BMD_{5\%}$** is equal to **80.2** mg/kg bw/d and the **$BMD_{5\%}L_{90\%}$ to 28.6** mg/kg bw/d.

- *Choice of uncertainty factors and allometric adjustment*

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 following equation (US EPA, 2006):

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

The average rat weight was determined from the data described in the study to be 320 g and was measured on day 20, while the human weight is estimated at 70 kg. Doses are expressed in mg/kg/d.

Therefore, the equivalent dose in humans = the animal dose * 0.26 (which amounts to applying a factor of 3.8 that takes toxicokinetic variability into account).

To take toxicodynamic variability and residual uncertainties into account, an 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). The value of the uncertainty factor for interspecies variability is therefore 2.5.

A final default value of 10 was chosen for intraspecies variability (AFSSET 2007), UF_H = 10 (intraspecies or interindividual variability).

[10] The lower limit of the 95% confidence interval (one-sided) used by the US EPA is equivalent to the lower limit of the 90% confidence interval (two-sided) used by the RIVM

Conclusions and recommendation of the collective expert appraisal

The CES proposes an oral TRV specifically for the developmental effects of TCA applicable for acute exposure:

Type of TRV	Critical effect	Critical dose	UF**	TRV
Acute threshold TRV for developmental effects, oral route	Increased cardiac malformations in Long-Evans rats Study by Smith *et al.,* 1989	$BMD_{5\%}L_{90\%}$= 28.6 mg/kg bw/d* <u>Allometric adjustment</u> $BMD_{5\%}L_{90\%HED}$ = 7.4 mg/kg bw/d	UF 25 UF_A 2.5 UF_H 10	**TRV = 300 µg/kg bw/d**

* $BMD_{5\%}L_{90\%}$: lower limit of the 90% confidence interval of the benchmark dose corresponding to a 5% increase in response compared to the unexposed group.
**UF: overall uncertainty factor (applied), UF_A: interspecies variability; UF_H: interindividual variability
*** $BMD_{5\%}L_{90\%}$ HED: adjusted $BMD_{5\%}L_{90\%}$ (allometric adjustment), HED: Human Equivalent Dose; in order to take toxicodynamic variability into account, an uncertainty factor, UF_A, was set at 2.5.

The CES draws the Agency's attention to the fact that none of the data in humans or animals show any effects of TCA on fertility.

TRV for dibromoacetic acid (CAS 631-64-1)

- *Choice of the critical effect*

The effects of DBA observed in animal experiments mainly concern male fertility:

- Effects on spermiation and spermatid development,
- Decreased sperm count and motility,
- Abnormal sperm morphology,
- Epididymal and testicular abnormalities.

Impaired spermatogenesis (retention of step 19 spermatozoa in the tubules beyond stage VIII) is an effect found in several studies, and at doses of the same order of magnitude.

The experts selected impaired spermatogenesis as the critical effect of DBA.

- *Choice of the study*

The most appropriate study for calculating a toxicity reference value, highlighting the critical effect, is the study by Christian *et al.* (2002)[11], for the following reasons:

- general methodological quality, in particular the number of animals per group, the dose regime and the two-generation study,
- transparency of the results enabling a full statistical analysis.

This study was considered to be of good quality (rated Klimisch 1). Christian *et al.* (2002) conducted a two-generation study in which groups of Sprague-Dawley rats (30 per gender and per dose) were administered DBA continuously in their drinking water at concentrations of 0, 50, 250 or 650 mg/L. The dose range had been calculated from a previous study by the same authors (Christian *et al.*, 2001).

In the 2002 study, the authors found a reduction in water consumption at all doses and for each F1 generation. In subjects from the group exposed to the highest dose, from the P and F1 generations, clinical signs associated with the reduced water consumption were described (decreased body weight, reduced food consumption). All the doses administered to the F1 generation during lactation resulted in a decrease in body weight, which required weaning to be delayed to day 29.

This study describes impaired spermatogenesis, with step 19 spermatids retained in the seminiferous tubules at stages IX and X, together with changes in the epididymal tubules (increase in the amount of exfoliated cells and residual bodies in the epididymal tubules, atrophy and hypospermia) in rats from both P and F1 generations exposed to 250 and 650 ppm.
Table 4 summarises the results of the two-generation study by Christian *et al.* and the incidence of impaired spermatogenesis (namely step 19 spermatids retained in the seminiferous tubules) in the parental generation. According to Fisher's test, the dose of

[11] Christian MS *et al.,* 2002. Oral (drinking water) two-generation reproductive toxicity study of dibromoacetic acid (DBA) in rats. International Journal of Toxicology 21(4), 237-276

22.4 mg/kg bw/day is statistically significant (with p=0.01) and is therefore the LOAEL. Consequently, the NOAEL is 4.5 mg/kg bw/d.[12]

Table 4: Incidence of impaired spermatogenesis (step 19 spermatids retained in the seminiferous tubules) - parental generation

Dose (mg/kg bw/d**)**	**Number of testes examined**	**Number of testes affected**
0	30	4
4.5	30	3
22.4*	30	13
52.4	30	23

**statistically significant, Fisher's test (one-sided), with p=0.01*

Several studies have shown toxicity on the male reproductive system (impaired spermatogenesis, decreased sperm motility, abnormal sperm morphology). These effects are found at doses of the same order of magnitude as the NOAEL identified in the study by Christian *et al.* (2002).

- *Choice of the critical dose*

The available data were modelled with the RIVM's Proast software in order to prepare a Benchmark Dose (BMD). A significant dose-response relationship between retention of step 19 spermatids and the daily dose of exposure to DBA appears in the key study (Table 4).

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) level. This is predominantly the BMDL, i.e. the benchmark dose lower confidence limit.
The experimental data were fitted by the models developed by the RIVM for continuous data.

The model chosen was the one that best fitted to the experimental data using the maximum likelihood method (log likelihood) and corresponds to the logProbit model. This model is the one whose maximum likelihood was closest to the "full" model. The model was selected for estimating the lower limit of the 90% confidence interval of a dose corresponding to a 5% increased response compared to the unexposed group. The $BMD_{5\%}$ and $BMDL_{5\%}$ were calculated, as the 5% threshold is generally used in reprotoxicity studies. The **$BMD_{5\%}$** is equal to **8.0 mg/kg/d** and the **$BMD_{5\%}L_{90\%}$** to **2.7 mg/kg/d.**

[12] Effects on general toxicity (e.g. on the significant increase in the number of macrophages in the spleen) were observed from 68-73 mg/kg bw and per day, or at higher doses

- *Choice of uncertainty factors and allometric adjustment*

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 following equation (US EPA, 2006):

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

The average terminal rat weight was determined from the data described in the study to be 549 g and was measured at the end of the study, while the human weight is estimated at 70 kg. Doses are expressed in mg/kg bw/d.

Therefore, the equivalent dose in humans = the animal dose * 0.30 (which amounts to applying a factor of 3.3 that takes toxicokinetic variability into account).

To take toxicodynamic variability and residual uncertainties into account, an 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). The value of the uncertainty factor for interspecies variability is therefore 2.5.

A final default value of 10 was chosen for intraspecies variability, UF_H = 10 (intraspecies or interindividual variability).

Conclusions and recommendation of the collective expert appraisal

The experts propose an oral TRV specifically for the effects of DBA on fertility[13] applicable for chronic exposure.

Type of TRV	Critical effect	Critical dose	UF*	TRV
Chronic threshold TRV for effects on fertility, oral route	Increase in impaired spermatogenesis in Sprague-Dawley rats Study by Christian *et al.* 2002	$BMD_{5\%}$ $L_{90\%}$ = 2.7 mg/kg bw/d Allometric adjustment $BMD_{5\%}$ $L_{90\%\ HED}$ = 0.8 mg/kg bw/d	UF 25 UF_A 2.5 UF_H 10	**TRV = 30 µg/kg bw/d**

* $BMD_{5\%}L_{90\%}$: lower limit of the 90% confidence interval of the benchmark dose corresponding to a 5% increase in response compared to the unexposed group.
**UF: overall uncertainty factor (applied), UF_A: interspecies variability; UF_H: interindividual variability
*** $BMD_{5\%}L_{90\%}$ HED: adjusted $BMD_{5\%}L_{90\%}$ (allometric adjustment), HED: Human Equivalent Dose; in order to take toxicodynamic variability into account, an uncertainty factor, UF_A, was set at 2.5.

[13] In the study by Christian *et al.* (2002), the chosen effects on spermatogenesis are not associated with decreased fertility. However, the CES believes that these effects may be precursors to the effect on fertility

DBA is a chemical that can affect foetal development in animals. Effects on other organs have also been observed, such as weight changes in some organs (liver, kidneys, pituitary and adrenal glands, thymus), (Christian *et al.* 2002). The CES therefore recommends a critical analysis of the effects on foetal development, and where appropriate, the preparation of such a TRV.

The CES draws the Agency's attention to the fact that the proposed TRVs for the reprotoxic effects would not provide protection from other toxic effects, cancer in particular. The CES therefore recommends a critical analysis of existing TRVs, especially those based on DBA's carcinogenic effects, and if necessary, developing a specific TRV for these effects.

4. THE AGENCY'S CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions and recommendations of the Expert Committee (CES) on Assessment of the risks related to chemical substances, relating to the preparation of oral toxicity reference values for dichloroacetic acid, trichloroacetic acid and dibromoacetic acid.

In accordance with the findings of the collective expert report, the Agency proposes the following oral TRVs:

Dichloroacetic acid

Type of TRV	Critical effect	Critical dose	UF**	TRV
Acute threshold TRV for developmental effects, oral route	Increased cardiac malformations in Long-Evans rats Study by Smith *et al.,* 1992	$BMD_{5\%}L_{90\%*}$ = 24.6 mg/kg bw/d Allometric adjustment $BMD_{5\%}L_{90\%\ HED***}$ = 6.5 mg/kg bw/d	**UF 25** UF_A 2.5 UF_H 10	**TRV = 260 µg/kg bw/d**
Chronic threshold TRV for effects on fertility, oral route	Decrease in spermatozoa in Long-Evans rats Study by Toth *et al.,* 1992	$BMD_{1xSD}L_{90\%}^{***}$*= 72 mg/kg bw/d Allometric adjustment $BMD_{1xSD}L_{90\%HED}$ = 21 mg/kg bw/d	**UF 25** UF_A 2.5 UF_H 10	**TRV = 840 µg/kg bw/d**

* $BMD_{5\%}L_{90\%}$: lower limit of the 90% confidence interval of the benchmark dose corresponding to a 5% increase in response compared to the unexposed group.
**UF: overall uncertainty factor (applied), UF_A: interspecies variability; UF_H: interindividual variability
*** $BMD_{5\%}L_{90\%}$ HED: adjusted $BMD_{5\%}L_{90\%}$ (allometric adjustment), HED: Human Equivalent Dose; in order to take toxicodynamic variability into account, an uncertainty factor, UF_A, was set at 2.5.
****$BMD_{1xSD}L_{90\%}$, the chosen level of effect corresponds to one times the standard deviation: this amounts to considering as the threshold value the mean (sperm count in the untreated group) minus one times the standard deviation of the untreated group, and corresponds to a 32.5% decrease in spermatozoa.

Trichloroacetic acid

Type of TRV	Critical effect	Critical dose	UF**	TRV
Acute threshold TRV for develop-mental effects, oral route	Increased cardiac malformations in Long-Evans rats Study by Smith *et al.*, 1989	$BMD_{5\%}L_{90\%}$= 28.6 mg/kg bw/d* Allometric adjustment $BMD_{5\%}L_{90\%HED}$ = 7.4 mg/kg bw/d	**UF 25** UF_A 2.5 UF_H 10	**TRV = 300 µg/kg bw/d**

* $BMD_{5\%}L_{90\%}$: lower limit of the 90% confidence interval of the benchmark dose corresponding to a 5% increase in response compared to the unexposed group. **UF: overall uncertainty factor (applied), UF_A: interspecies variability; UF_H: interindividual variability. *** $BMD_{5\%}L_{90\%}$ HED: adjusted $BMD_{5\%}L_{90\%}$ (allometric adjustment), HED: Human Equivalent Dose; in order to take toxicodynamic variability into account, an uncertainty factor, UF_A, was set at 2.5.

Dibromoacetic acid

Type of TRV	Critical effect	Critical dose	UF*	TRV
Chronic threshold TRV for effects on fertility, oral route	Increase in impaired spermatogenesis in Sprague-Dawley rats Study by Christian *et al.* 2002	$BMD_{5\%}$ $L_{90\%}$ = 2.7 mg/kg bw/d Allometric adjustment $BMD_{5\%}$ $L_{90\%\ HED}$ = 0.8 mg/kg bw/d	**UF 25** UF_A 2.5 UF_H 10	**TRV = 30 µg/kg bw/d**

* $BMD_{5\%}L_{90\%}$: lower limit of the 90% confidence interval of the benchmark dose corresponding to a 5% increase in response compared to the unexposed group.
**UF: overall uncertainty factor (applied), UF_A: interspecies variability; UF_H: interindividual variability
*** $BMD_{5\%}L_{90\%}$ HED: adjusted $BMD_{5\%}L_{90\%}$ (allometric adjustment), HED: Human Equivalent Dose; in order to take toxicodynamic variability into account, an uncertainty factor, UF_A, was set at 2.5.

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The Director General

Marc Mortureux

KEY WORDS

Dichloroacetic acid, trichloroacetic acid, dibromoacetic acid, reprotoxicity, development, fertility, toxicity reference values, critical dose, uncertainty factors, health effects, general population