The Director General

Maisons-Alfort, 23 June 2017

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

on the "development of chronic reference values by the oral route for four perfluorinated compounds: perfluorohexanoic acid (PFHxA), perfluorohexane sulfonic acid (PFHxS), perfluorobutanoic acid (PFBA), and perfluorobutane sulfonic acid (PFBS)"

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 published on its website. This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 23 June 2017 shall prevail.

On 9 June 2015, ANSES issued an internal request to carry out the following expert assessments: development of chronic reference values by the oral route for the following perfluorinated compounds: perfluorohexanoic acid (PFHxA, CAS No. 307-24-4), perfluorohexane sulfonic acid (PFHxS, CAS No. 355-46-4), perfluorobutanoic acid (PFBA, CAS No. 375-22-4) and perfluorobutane sulfonic acid (PFBS, CAS No. 375-73-5).

1. BACKGROUND AND PURPOSE OF THE REQUEST

This expert opinion follows the Agency's work on perfluorinated compounds published in a 2015 report (ANSES, 2015b), in response to a formal request from the Directorate General for Health in June 2009 on reprotoxic and/or endocrine-disrupting (ED) substances (Request No. 2009-SA-0331).

This report identified four priority perfluorinated compounds: perfluorobutanoic acid (PFBA), perfluorobutane sulfonic acid (PFBS), perfluorohexanoic acid (PFHxA) and perfluorohexane sulfonic acid (PFHxS). This selection was made according to several criteria including the regulatory status (REACh and sectoral regulations), the body of data available for each of the compounds, their uses and changes in use, the reference values already available, etc. These four perfluorinated compounds were therefore examined separately in order to assign toxicity reference values (TRVs) to them.

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, 2015a).

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;
- application of uncertainty factors to the critical dose to account for uncertainties.

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

An indicative toxicity value (iTV) is a toxicological benchmark that can be used for assessing a risk. It is an indicative value that is less robust than the TRV and therefore has a low confidence level.

An iTV may be proposed when the necessary conditions for establishing a TRV are not met and a quantitative health risk assessment (QHRA) is required in a given exposure context:

1. if there are **insufficient data** available on the substance to characterise the hazard it presents or if there is **doubt as to the harmful nature of the effect**. In this case, ANSES will conduct literature monitoring for these substances with a view to replacing the iTVs by TRVs if new data allow it;
2. in the event of **time and/or resource constraints**. In this case, the iTV will be developed as far as possible within the time available, to meet the decision-makers' policy imperatives, and then additional work will be carried out subsequently, if appropriate, to propose a TRV.

Based on the WHO/IPCS method proposing a step-by-step approach to health risk assessment, whose first step consists of a preliminary assessment (screening), the iTV can be used to rule out a risk in a conservative, first-level risk assessment approach (WHO/IPCS, 2010).

Unlike a TRV, an iTV should only be used to respond to the specific situation and context that justified its establishment. The conditions of application must therefore be clearly explained for each iTV proposed. As with TRVs, the use and interpretation of iTVs must take into account the route, duration and period of exposure, the type of effect with which it is associated, and the target population for which it is intended. The way in which iTVs are established depends on the available data on the substances' biological mechanisms of action and on commonly accepted assumptions. A distinction is therefore made between an iTV with a dose threshold and an iTV without a dose threshold. An iTV is developed by following the same establishment steps as a TRV.

iTVs are not published on the ANSES website separately from the simplified risk assessments that justified their development.

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 2015 and February 2017. It was adopted by the CES "Substances" at its meeting on 23 February 2017.

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

■ Perfluorobutanoic acid (PFBA) - CAS No. 375-22-4

- Toxicokinetics

With regard to the T_{max}[1] values and bioavailability calculated in oral experiments in rats and mice, absorption of PFBA appears to be both rapid and relatively complete.
Following oral exposure, the low volume of distribution values indicate that PFBA is very poorly distributed in tissues. All the data converge towards a rapid elimination of PFBA. In all the species studied (rats, mice, monkeys), urine is the main route of excretion. Disposal *via* faeces is negligible.

- Toxicity

Three repeated exposure toxicity studies in animals (28 days, 90 days and developmental toxicity) are currently available for assessing this compound. They mainly showed effects on the thyroid, on development, and on the liver.

Regarding the effects on the thyroid, in the 28-day and 90-day studies by Butenhoff *et al.* (2012), in addition to an increase in absolute thyroid weight, a decrease in serum total T4 and free T4 levels (with no change in TSH[2] levels) was observed.

With regard to the developmental effects, a statistically significant but moderate delay in eye opening was observed, with a weak dose-response relationship.

Regarding the liver effects, an increase in absolute (+45% in the 28-day study and +23% in the 90-day study, at the highest doses) and relative liver weight was observed in both studies by Butenhoff *et al.* (2012), associated with hepatocellular hypertrophy (only minimal in the 28-day study, minimal to mild in the 90-day study). In the 28-day study and the developmental study, a statistically significant decrease in cholesterol was also observed. With the exception of this reduction in cholesterol, all the biochemical parameter values measured were in the normal range, i.e. usually observed in Sprague-Dawley rats. They are not therefore toxicologically relevant. In both of the repeated toxicity studies, the authors also measured hepatic levels of mRNA transcripts of interest. A number of these transcripts (Acox, CYP4A1, etc.), which are markers of PPARα[3] activation, increased. There was also an increase in levels of CYP2B2, a marker of CAR[4] activation. Lastly, a decrease in the hepatic level of CYP1A1 was also observed, suggesting a decrease in AhR[5] activity.

- Establishment

[1] Tmax: time to reach the maximum concentration
[2] TSH: Thyroid-stimulating hormone
[3] PPARα: Peroxisome proliferator-activated receptor alpha
[4] CAR: Constitutive androstane receptor
[5] AhR: Aryl hydrocarbon receptor

- Choice of the critical effect

  - **Thyroid effects**

The CES did not consider it appropriate to establish a TRV based on the changes in hormone concentrations mentioned above, as the authors themselves doubted the reliability of the results obtained (problem carrying out the measurements).

  - **Effects on development**

The authors did not detail their methodology for assessing the delay in eye opening. Moreover, this was not corroborated by any other criteria for developmental delays (such as delayed incisor emergence). The CES did not consider it appropriate to establish a TRV based on these changes.

  - **Liver effects**

Given the differences in mechanism of action and in the consequences of PPARα activation between humans and rodents, established on the basis of humanised PPARα mice, this effect was not considered to be transposable to humans (Hall *et al.*, 2012). Transcript changes relating to PPARα activation cannot therefore support the development of a reference value based on liver effects (Hall *et al.*, 2012). As with PPARα, there seem to be major differences between murine and human CAR, both in its ligands and in the responses mediated by this receptor, with the European Society of Toxicologic Pathology (ESTP) even considering their activation to be a non-harmful effect (Hall *et al.*, 2012). In particular, the response concerning lipogenesis seems to be different between humans and rodents. Extrapolating these observations made on a rodent model to humans therefore seems questionable for selecting them as the critical effect (Lynch *et al.*, 2014; Yang *et al.*, 2013; Hall *et al.*, 2012).

According to documents from the US Environmental Protection Agency (US EPA) (2002) and the ESTP (Hall *et al.*, 2012), with hepatocyte hypertrophy, in the absence of histological changes, evidence of hepatocyte damage characterised by a dose-dependent, biologically significant and consistent change in at least two liver parameters is required to characterise an adverse effect on the liver. These conditions are not met in this case. According to these criteria, therefore, the liver effects should not be regarded as harmful.

Nevertheless, although when taken independently of each other none are sufficient for establishing a TRV (magnitude of the response) or can be totally transposed to humans, many effects (hypertrophy and functional signs) related to liver and lipid metabolism are observed following exposure to PFBA. In addition, even though this was a mechanistic study, minimal to mild hepatic necrosis was observed in addition to hypertrophy in male wild-type mice exposed to PFBA for 28 days in the study by Foreman *et al.* (2009). Lastly, it also seems necessary in this analysis to take into account existing knowledge on the entire class of perfluorinated compounds, and in particular the two most studied compounds in this class, namely PFOS and PFOA. Clearly established hepatic toxicity for these two compounds, extending to the onset of hepatocytic adenomas in animals, cannot be ruled out in humans (EFSA, 2008; US EPA, 2016a, 2016b).

In view of all this information, the CES decided to select the liver effects as the critical effect. Given the doubts existing about the choice of critical effect and its harmful nature, the decision was made to establish an iTV for PFBA.

- Choice of the key study

The subchronic study (90 days), which had the longest exposure conditions among the available studies on this compound, was chosen for the selection of the starting point (Butenhoff *et al*. 2012).

- Choice of the critical dose

Considering all the effects occurring at 30 mg/kg/day in this study (statistically significant increase in absolute and relative liver weight, hepatocellular hypertrophy, decrease in the mRNA transcript marker of AhR, etc.), this dose was considered to be the LOAEL[6]. The NOAEL[7], the level directly below in the 6 mg/kg/day study, was selected as the critical dose.

- Adjustments

In order to reduce the degree of uncertainty due to inter-species variability when determining a human equivalent dose (HED), an allometric adjustment was performed using the following equation:

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

$$NOAEL_{HED}\ = 6\ \mathrm{mg/kg/d}\ \times \left(\frac{0.523\ \mathrm{kg}}{70\ \mathrm{kg}}\right)^{1/4}\ =\ 1.764\ mg/kg/d$$

- Choice of uncertainty factors

The iTV was calculated from the $NOAEL_{HED}$ using the following uncertainty factors (ANSES, 2015a):

- Inter-species variability (UF_A): 2.5

The allometric adjustment performed enabled a human equivalent dose 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): 3

This was a subchronic study, with animals exposed for 90 days. To take account of possible effects occurring at lower doses after longer exposure, it was considered appropriate to apply a UF_S of 3.

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

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

- Inadequacy of the data (UF_D): 1

The three repeated exposure toxicity studies in animals (28 days, 90 days and developmental toxicity) available on PFBA were sufficient for assessing the toxicity of this compound.

[6] LOAEL: Lowest Observed Adverse Effect Level
[7] NOAEL: No Observed Adverse Effect Level

An overall uncertainty factor of 75 was thus used to determine the iTV for PFBA.

- Proposed chronic iTV by ingestion

$$iTV = \frac{1.764\ mg/kg/d}{75} = 0.0235 \cong 0.024\ mg/kg/d$$

- **Perfluorohexane sulfonic acid (PFHxS) - CAS No. 355-46-4**

  - Toxicokinetics

No data on the toxicokinetics of PFHxS are available.

  - Toxicity

Many studies in humans are available for assessing the toxicity of PFHxS, but cannot be used to derive TRVs. In addition, to date, only one repeated-dose toxicity study combined with a reproductive and developmental toxicity test (OECD 422) has been used to assess this compound (Butenhoff *et al*., 2009). It mainly showed effects on the thyroid and liver.
Concerning the effects on the thyroid, an increase in thyroid hypertrophy/hyperplasia was observed.
Concerning the liver effects, an increase in absolute and relative liver weight at the two highest doses (+20% and +56% for absolute weight, no data for relative weights) combined with hepatocellular hypertrophy (9/10 rats, 8 minimal, 1 slight at 3 mg/kg/day, and 10/10 rats, 4 minimal, 5 mild and 1 moderate at 10 mg/kg/day) was observed. A statistically significant increase in alkaline phosphatase (ALP) (+37%) was observed at 10 mg/kg/day. A statistically significant increase in albumin (5%) and in the albumin/globulin ratio (19%), as well as a decrease in cholesterol (-42%) and triglycerides (-68%) were also observed at the highest dose, although the toxicological significance of these changes is questionable.

  - Establishment

    - Choice of the critical effect

      - **Thyroid effects**

According to the authors, the increase in hypertrophy/hyperplasia may be the consequence of hepatic hypertrophy. The absence of statistical testing and hormone assays means that this mechanistic argument cannot be substantiated. Without confirmation of the mechanism of action, the thyroid hypertrophy appears too weak to establish a reference value.

      - **Liver effects**

According to documents from the US EPA (2002) and ESTP (Hall *et al.*, 2012), with hepatocyte hypertrophy, in the absence of histological changes, evidence of hepatocyte damage characterised by a dose-dependent, biologically significant and consistent change in at least two liver parameters is required to characterise an adverse effect on the liver. These conditions are not met in this case. According to these criteria, therefore, the liver effects should not be regarded as adverse.
Nevertheless, although when taken independently of each other none are sufficient for establishing a TRV (magnitude of the response) or can be totally transposed to humans, many effects

(hypertrophy and functional signs) related to liver and lipid metabolism are observed following exposure to PFHxS. It also seems necessary in this analysis to take into account existing knowledge on the entire class of perfluorinated compounds, and in particular the two most studied compounds in this class, namely PFOS and PFOA. Clearly established hepatic toxicity for these two compounds, extending to the onset of hepatocytic adenomas in animals, cannot be ruled out in humans (EFSA, 2008; US EPA, 2016a, 2016b).

In view of all this information, the CES decided to select the liver effects as the critical effect. Given the doubts existing about the choice of critical effect and its adverse nature, the decision was made to establish an iTV for PFHxS.

- Choice of the key study

Only one experimental study is available for establishing an iTV (Butenhoff *et al.*, 2009). It follows the OECD 422 guideline (Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test), and was therefore selected as the key study.

- Choice of the critical dose

Considering all the effects occurring at 3 mg/kg/day in this study (statistically significant increase in absolute and relative liver weight, hepatocellular hypertrophy in 9 out of 10 rats), this dose was considered to be the LOAEL.
The NOAEL, the level directly below in the study of 1 mg/kg/day, was therefore selected as the critical dose.

- Adjustments

In order to reduce the value of uncertainty due to inter-species variability when determining a human equivalent dose, an allometric adjustment was performed using the following equation:

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

$$NOAEL_{HED}\ = 1\ \mathrm{mg/kg/d}\ \times \left(\frac{0.490\ \mathrm{kg}}{70\ \mathrm{kg}}\right)^{1/4}\ =\ 0.289\ mg/kg/d$$

- Choice of uncertainty factors

The iTV was calculated from the $NOAEL_{HED}$ using the following uncertainty factors (ANSES, 2015a):

- Inter-species variability (UF_A): 2.5

The allometric adjustment performed enabled a human equivalent dose 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): 3

In the study by Butenhoff *et al.* (2009), male rats were exposed for a minimum of 42 days. It was therefore considered relevant to apply a UF_S of 3.

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

Because establishment of the iTV was based here on a NOAEL, a specific $UF_{B/L}$ was not necessary.

- Inadequacy of the data (UF_D): 1

Only one experimental study was available for assessing the toxicity of PFHxS, but it combines repeated-dose toxicity and reproduction/developmental toxicity. In addition, many studies in humans are available on this compound. Consequently, the CES considered that a UF_D was not necessary.

An overall uncertainty factor of 75 was thus used to determine the iTV for PFHxS.

- Proposed chronic iTV by ingestion

$$iTV = \frac{0.289\ mg/kg/d}{75} = 0.0038 \cong \mathbf{0.004\ mg/kg/d}$$

- **Perfluorobutane sulfonic acid (PFBS) - CAS No. 375-73-5**

- Toxicokinetics

Orally, T_{max} values suggest rapid absorption of PFBS.
However, no conclusion can be reached as to the extent of oral absorption of PFBS in rats based on the calculation of bioavailability in males and females from the available data.
In both monkeys and rats, the serum elimination half-lives reported in the available studies indicate that PFBS metabolism in males seems to be lower than in females.
Urine is the major route of elimination of the compound, whether from the oral or intravenous route. Moreover, this elimination is rapid.

- Toxicity

To date, two repeated exposure toxicity studies in animals (90 days and two-generations) can be used for assessing this compound. They mainly showed effects on the kidney and liver. The kidney effects were the most reproducible in the available studies: tubular hyperplasia was observed in the 90-day study available and in the two-generation study, in both parents and F1 generation offspring (Lieder *et al.*, 2009a, 2009b).

- Establishment

- Choice of the critical effect

The CES therefore selected the effects on the kidney as the critical effect. The CES stresses that this choice will protect against potential liver effects.

- Choice of the key study

Although tubular hyperplasia was also observed in the subchronic exposure study (Lieder *et al*., 2009a), the key study selected was the two-generation study (for effects observed in the F0 generation) (Lieder *et al*., 2009b). The kidney effects are described by the authors in more detail in the latter.

- Choice of the critical dose

The experimental data established on tubular hyperplasia were modelled using PROAST software to establish a Benchmark Dose (BMD). It should be noted that only the results at the two highest doses were provided by the authors of the publication and could therefore be used for modelling.

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, known as the Benchmark Response (BMR). This BMR corresponds to an excess risk of 10%, as recommended by ANSES and the European Food Safety Authority (EFSA) for quantal data (EFSA, 2017).

In the case of PFBS, data in females were selected as they seemed to be more sensitive for this effect.
The values selected were as follows:

$BMC_{10\%}$: 40.6 mg/kg/d
$BMD_{10\%}L_{95\%}$: 24 mg/kg/d

- Adjustments

In order to reduce the value of uncertainty due to inter-species variability when determining a human equivalent dose, an allometric adjustment was performed using the following equation:

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

$$BMD_{10\%}L_{95\%\ HED}\ = 24\ \mathrm{mg/kg/d}\ \times \left(\frac{0.285\ \mathrm{kg}}{70\ \mathrm{kg}}\right)^{1/4}\ =\ 6.06\ mg/kg/d$$

- Choice of uncertainty factors

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

- Inter-species variability (UF_A): 2.5

The allometric adjustment performed enabled a human equivalent dose 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): 3

As the selected key study was a 2-generation study in which animals were exposed for 70 days, and no chronic exposure studies were available, it was decided to apply a UF_S of 3.

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

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

- Inadequacy of the data (UF_D): 1

The toxicological data available on PFBS were deemed sufficient for assessing the toxicity of the compound.

An overall uncertainty factor of 75 was thus used to determine the TRV for PFBS.

- Proposed chronic TRV by ingestion

$$TRV = \frac{6.06\ mg/kg/d}{75} = 0.081 \cong \mathbf{0.08\ mg/kg/d}$$

- Confidence level:

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

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

Moderate: the toxicological data are generally sufficient for assessing this compound. However, most of the available studies, although of good quality, were produced by the group of Butenhoff *et al.* (Bijland *et al*., 2011; Lieder *et al.,* 2009a & b; Olsen *et al*., 2009).

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

Moderate: tubular hyperplasia is an effect observed in all studies that assessed the overall toxicity of this compound. Nevertheless, this effect was discussed relatively little by the authors.

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

High: this is a well-detailed study that follows OECD guidelines and good laboratory practice.

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

Moderate: it was possible to establish a BMD, but only on two doses. The results at the two lowest doses were not presented by the authors.

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

■ Perfluorohexanoic acid (PFHxA) - CAS No. 307-24-4

- Toxicokinetics

Following oral exposure, PFHxA absorption appears to be rapid, with a T_{max} of about 1 hour.
Regarding the systemic distribution of PFHxA, an intravenous study appears to show differences between males and females, with a higher serum half-life in males. However, the oral study does not report any such difference between males and females.
Whether administered orally or intravenously, PFHxA is excreted rapidly, mainly in urine.

- Toxicity

The available studies on PFHxA have shown various effects, mainly on the liver and kidneys.
In the liver, an increase in absolute and relative liver weight associated with hepatocellular hypertrophy, and a statistically significant increase in aspartate aminotransferases (ASTs) and alanine aminotransferases (ALTs) were observed in the two available subchronic exposure studies. Regarding effects on the kidney, the chronic exposure study showed papillary necrosis and tubular degeneration in females at 200 mg/kg/day (Klaunig *et al.*, 2015). These lesions were associated with a statistically significant increase in urinary volume and a statistically significant decrease in specific severity at the same dose in females at 26 weeks of treatment.

- Establishment

    - Choice of the critical effect

        - **Liver effects**

According to the US EPA (2002), ALTs and ASTs have to be increased by at least a factor of 2 or 3 to be regarded as relevant, which was not the case in these two studies. In addition, the chronic exposure study did not show any increase in these two parameters.

        - **Kidney effects**

In view of the severity of the lesions observed in the kidney (papillary necrosis and tubular degeneration), the CES decided to select them as the critical effect. The CES stresses that this choice will protect against potential liver effects.

    - Choice of the key study

The key study selected was the study by Klaunig *et al.* (2015), the only study available for chronic exposure (2 years). This is a fairly good quality study, but it did not follow OECD guidelines. It is also important to note that a considerable number of animals died before the end of the study, independently of the toxic effects of the substance.

    - Choice of the critical dose

Due to the absence of a dose-response relationship (effect occurring at the highest dose), a BMD could not be established. As a result, the NOAEL is the dose directly below the identified LOAEL, namely 200 mg/kg/d. The NOAEL is therefore 30 mg/kg/d.

    - Adjustments

In order to reduce the value of uncertainty due to inter-species variability when determining a human equivalent dose, an allometric adjustment was performed using the following equation:

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

$$NOAEL_{HED}\ = 30\ \mathrm{mg/kg/d}\ \times \left(\frac{0.338\ \mathrm{kg}}{70\ \mathrm{kg}}\right)^{1/4} = 7.91\ mg/kg/d$$

- Choice of uncertainty factors

The TRV was calculated from the $NOAEL_{HED}$ using the following uncertainty factors (ANSES, 2015a):

- Inter-species variability (UF_A): 2.5

The allometric adjustment performed enabled a human equivalent dose 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

As the key study selected was a chronic study, a UF_S was not necessary.

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

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

- Inadequacy of the data (UF_D): 1

The toxicological data available on PFHxA were sufficient for assessing the toxicity of the compound. A UF_D was therefore not necessary.

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

- Proposed chronic TRV by ingestion

$$TRV = \frac{7.91\ mg/kg/d}{25} = 0.316 \cong \mathbf{0.32\ mg/kg/d}$$

- Confidence level

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

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

High: the toxicological data are sufficient for assessing this compound. The studies available are of good quality.

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

Moderate: it is a sufficiently robust effect for establishing a TRV. However, it was only found in females at the highest dose, with no identifiable dose-response relationship.

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

Moderate: this is a well-detailed study. However, it did not follow the guidelines and did not state whether it complied with good laboratory practice. There was a large number of animals per dose. However, the differences between the doses in the trial were quite high, and a large number of animals died during the study.

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

Moderate: no dose-response relationship could be identified. A BMD could not be established, but a NOAEL was identified.

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

The reports were validated unanimously by the experts present (16 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 on "Characterisation of substance hazards and toxicity reference values" on the development of reference values by the oral route for the four perfluorinated compounds assessed.

Concerning PFBA and PFHxS, given the doubts existing about the choice of critical effect and its adverse nature, the decision was made to establish an Indicative Toxicity Value (iTV).
An iTV is established by ANSES when all the conditions required for establishing a TRV are not met (insufficient data, doubts about the adverse nature of the effect and/or time and/or resource constraints). This pragmatic approach aims to provide a temporary response to the health risk assessment objective pending sufficient qualitative and/or quantitative data, and can therefore help meet the expectations of risk managers in the presence of documented exposure situations. This iTV can only be used to respond to the specific situation and context that justified its establishment.

PFBA:

Critical effect (key study)	Critical concentration	UF	Reference value
Liver effects Butenhoff *et al.*, 2012	NOAEL = 6 mg/kg/d <u>Allometric adjustment</u> $NOAEL_{HED}$ = 1.764 mg/kg/d	75 UF_A: 2.5 UF_D: 1 UF_H: 10 UF_L: 1 UF_S: 3	**iTV = 0.024 mg/kg/d**

PFHxS:

Critical effect (key study)	Critical concentration	UF	Reference value
Liver effects Butenhoff *et al.*, 2009	NOAEL = 1 mg/kg/d <u>Allometric adjustment</u> $NOAEL_{HED}$ = 0.289 mg/kg/d	75 UF_A: 2.5 UF_D: 1 UF_H: 10 UF_L: 1 UF_S: 3	**iTV = 0.004 mg/kg/d**

For PFBS and PFHxA, TRVs were established:

PFBS:

Critical effect (key study)	Critical concentration	UF	Reference value
Kidney effects (tubular hyperplasia) Lieder *et al.,* 2009b	$BMD_{10\%}L_{95\%}$ = 24 mg/kg/d <u>Allometric adjustment:</u> $BMD_{10\%}L_{95\%\ HED}$ = 6.06 mg/kg/d	75 UF_A: 2.5 UF_D: 1 UF_H: 10 UF_L: 1 UF_S: 3	**TRV = 0.08 mg/kg/d** **Confidence level: Moderate**

PFHxA:

Critical effect (key study)	Critical concentration	UF	Reference value
Kidney effects (papillary necrosis and tubular degeneration) Klaunig *et al*., 2015	NOAEL = 30 mg/kg/d <u>Allometric adjustment:</u> $NOAEL_{HED}$ = 7.91 mg/kg/d	25 UF_A: 2.5 UF_D: 1 UF_H: 10 UF_L: 1 UF_S: 1	**TRV = 0.32 mg/kg/d** **Confidence level: Moderate**

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KEYWORDS

Valeur toxicologique de référence, VTR, Voie orale, chronique, Valeur Toxicologique Indicative, VTi, Acide perfluorobutanoïque, PFBA, Acide perfluorobutane sulfonique, PFBS, Acide perfluorohexanoïque, PFHxA, Acide perfluorohexane sulfonique, PFHxS
Toxicity reference value, TRV, perfluorobutyrate, perfluorobutane sulfonate, perfluorohexanoic acid, perfluorohexane sulfonate, oral route, chronic

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