ANSES Opinion
Request No 2022-SA-0198
Related Request No 2015-SA-0128

The Director General

Maisons-Alfort, 11 June 2025

Supplemented OPINION[1] of the French Agency for Food, Environmental and Occupational Health & Safety

on the development of long-term TRVs for several perfluorinated compounds

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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, the evaluation of the nutritional characteristics of food and the protection of the environment by assessing the impact of regulated products.
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 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 11 June 2025 shall prevail.

On 8 November 2022, ANSES received a formal request from the Directorate General for Competition, Consumer Affairs and Fraud Control (DGCCRF), the Directorate General for Labour (DGT), the Directorate General for Food (DGAL), the Directorate General for Risk Prevention (DGPR) and the Directorate General for Health (DGS) to undertake the following expert appraisal: request for an opinion on the assessment of health risks of and exposure to PFAS and the prioritisation of substances with a view to taking risk management measures.

This work was carried out as part of the memorandum of understanding between ANSES, the DGS and the DGPR for the implementation of the scientific expert appraisal work programme on toxicity reference values (TRVs), established in December 2022.

1. BACKGROUND AND PURPOSE OF THE REQUEST

Per- and polyfluoroalkyl substances (PFAS) make up a large class of over 4000 chemical compounds. Manufactured since the 1950s and used in many industrial applications and everyday consumer goods, particularly because of their water- and oil-repellent properties,

[1] This version cancels and replaces the initial version of 16 May 2025. The changes made are listed in Annex 1.

these compounds are found in a wide variety of products (textiles, food packaging, fire-fighting foams, cosmetics, medical devices, plant protection products, etc.). The presence of PFAS in the environment is therefore exclusively the result of human activities. These chemical compounds are highly persistent in the environment, contaminating various environmental media such as water, air, soil, sediment and biota. They are therefore found in the food chain. Some PFAS accumulate in living organisms, in particular in humans, and have health effects.

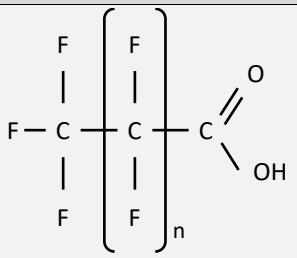
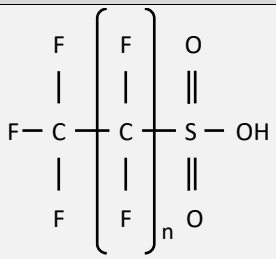
Due to growing concern about PFAS, numerous measures aimed at limiting their use and emissions have been implemented or are currently being assessed in France, Europe (via the Regulation on Registration, Evaluation and Authorisation of Chemicals (REACH)), the United States, Canada, etc. In Europe, certain PFAS (PFOA and PFOS) are already prohibited and a comprehensive restriction proposal to ban the manufacture and marketing of substances, mixtures and articles containing PFAS in Europe is currently being reviewed by the European Chemicals Agency (ECHA). In anticipation of this, on 27 February 2025[2], France adopted an Act prohibiting the manufacture, import, export and marketing of cosmetics, wax products (for skis) and clothing, shoes and their waterproofing agents containing PFAS, with the exception of protective clothing for professionals. This Act will take effect on 1 January 2026. In addition, an interministerial plan on PFAS was launched on 4 April 2024[3] to organise actions in response to growing concerns, taking health and environmental issues into account.

On 8 November 2022, ANSES received a formal request from the DGCCRF, DGT, DGAL, DGPR and DGS to undertake an expert appraisal on the assessment of health risks of and exposure to PFAS and the prioritisation of substances with a view to taking risk management measures. The request covered a particularly broad scope, in terms of both the number of substances in the PFAS class and the extent of the work, which led to several of the questions in the formal request being rephrased, including the one concerning the development of long-term oral TRVs for certain PFAS, alone and/or in mixtures, among the 20 analysed in water intended for human consumption (WIHC) (Table 1). In addition, as part of the establishment of ANSES's annual work programme on TRVs, the DGPR and DGS asked the Agency to include a fluorotelomer, 6:2 FTSA, in its expert appraisal. This substance is currently used in the automotive industry, particularly in the manufacture of batteries. In view of the European decision to ban the sale of combustion engine cars in 2035, leading to an increase in the production of batteries for electric cars, and the proposal to restrict PFAS via the REACH Regulation, with no alternative solution for this PFAS, it appeared necessary to have long-term oral and respiratory TRVs to be able to quantify the health risks associated with emissions of this substance.

This Opinion will only address the development of long-term oral and respiratory TRVs for certain PFAS considered individually and, where appropriate, the development of TRVs for a mixture of several PFAS among the 21 covered in this formal request, i.e. the 20 PFAS listed in Directive (EU) 2020/2184 (Table 1) and 6:2 FTSA. Only the linear forms of the 21 PFAS for which ANSES was consulted will be addressed; branched forms have not been included.

[2] French Act No 2025-188 of 27 February 2025 to protect the population from risks associated with perfluoroalkylated and polyfluoroalkylated substances (https://www.legifrance.gouv.fr/jorf/id/JORFTEXT000051260902, consulted on 6/03/2025)
[3] Interministerial plan on PFAS: the French government takes action to address health and environmental issues | Ministries for Land Planning and Ecological Transition, consulted on 25/04/2025

Table 1: List of the 20 PFAS to be taken into account in WIHC for the "Sum of PFAS" parameter (Directive (EU) 2020/2184 and Ministerial Order of 11 January 2007 as amended)

Perfluoroalkyl carboxylic acids[a]			Perfluoroalkyl sulfonic acids[a]		
					
n	**PFAS name**	**Acronym**	**n**	**PFAS name**	**Acronym**
2	Perfluorobutanoic acid	PFBA	3	Perfluorobutanesulfonic acid	PFBS
3	Perfluoropentanoic acid	PFPeA	4	Perfluoropentanesulfonic acid	PFPeS
4	Perfluorohexanoic acid	PFHxA	5	Perfluorohexanesulfonic acid	PFHxS
5	Perfluoroheptanoic acid	PFHpA	6	Perfluoroheptanesulfonic acid	PFHpS
6	Perfluorooctanoic acid	PFOA	7	Perfluorooctanesulfonic acid	PFOS
7	Perfluorononanoic acid	PFNA	8	Perfluorononanesulfonic acid	PFNS
8	Perfluorodecanoic acid	PFDA	9	Perfluorodecanesulfonic acid	PFDS
9	Perfluoroundecanoic acid	PFUnDA	10	Perfluoroundecanesulfonic acid	PFUnDS
10	Perfluorododecanoic acid	PFDoDA	11	Perfluorododecanesulfonic acid	PFDoDS
11	Perfluorotridecanoic acid	PFTrDA	12	Perfluorotridecanesulfonic acid	PFTrDS

[a] In view of their pKa[4] values, at the pH of WIHC, these substances are all present in anionic form.

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 (January 2024)".

The expert appraisal falls within the sphere of competence of the Expert Committee on Health reference values (HRV Committee). ANSES entrusted the expert appraisal to the Working Group (WG) on PFAS TRVs. The methodological and scientific aspects of the work were presented to the HRV Committee between 7 November 2024 and 23 May 2025. The work was adopted by the HRV Committee at its meeting on 10 April 2025. The work supplemented by the recommended TRVs for PFHxA was then adopted by the HRV Committee at its meeting on 23 May 2025 (see Annex 1).

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 following website: https://dpi.sante.gouv.fr/.

[4] Ka is the acidity constant, usually expressed as the logarithmic constant pKa = $-\log_{10}$ Ka

3. ANALYSIS AND CONCLUSIONS OF THE HRV COMMITTEE

3.1. Definitions and working method

ANSES defines a TRV as a generic term encompassing all types of toxicological indicators that can be used to establish a relationship between a quantity or concentration of a chemical agent and an adverse effect (threshold effect) or between a quantity or concentration of a chemical agent and a probability of effect (no-threshold effect) across an entire population. By definition, TRVs are established to protect the population as a whole, including sensitive sub-populations (e.g. children, the elderly, etc.), from the adverse effects caused by the chemical agent (ANSES, pending publication). TRVs are specific to a chemical agent and to a route (oral, respiratory, dermal) and duration (short, medium or long term) of exposure. There are therefore short-term TRVs for exposure lasting from one day to two weeks, medium-term TRVs for exposure lasting more than two weeks and less than one year, and long-term TRVs for exposure lasting more than one year.

TRVs can be used as part of quantitative health risk assessments carried out at population level, exclusively in a given exposure context, and thus help in the choice of risk management measures. They can also be used to establish guidance values (e.g. health-based guidance values for WIHC) or maximum regulatory levels in food. Lastly, they can serve to prioritise chemical agents, in which case they often enable the toxicity of such agents to be assessed (ANSES, pending publication).

Depending on the body of data and knowledge available on the biological mechanism(s) of action of the chemical agent of interest, two main types of long-term TRVs can be developed: “threshold” TRVs and “no-threshold” TRVs. In practice, proposing a TRV involves the steps set out in Figure 1.

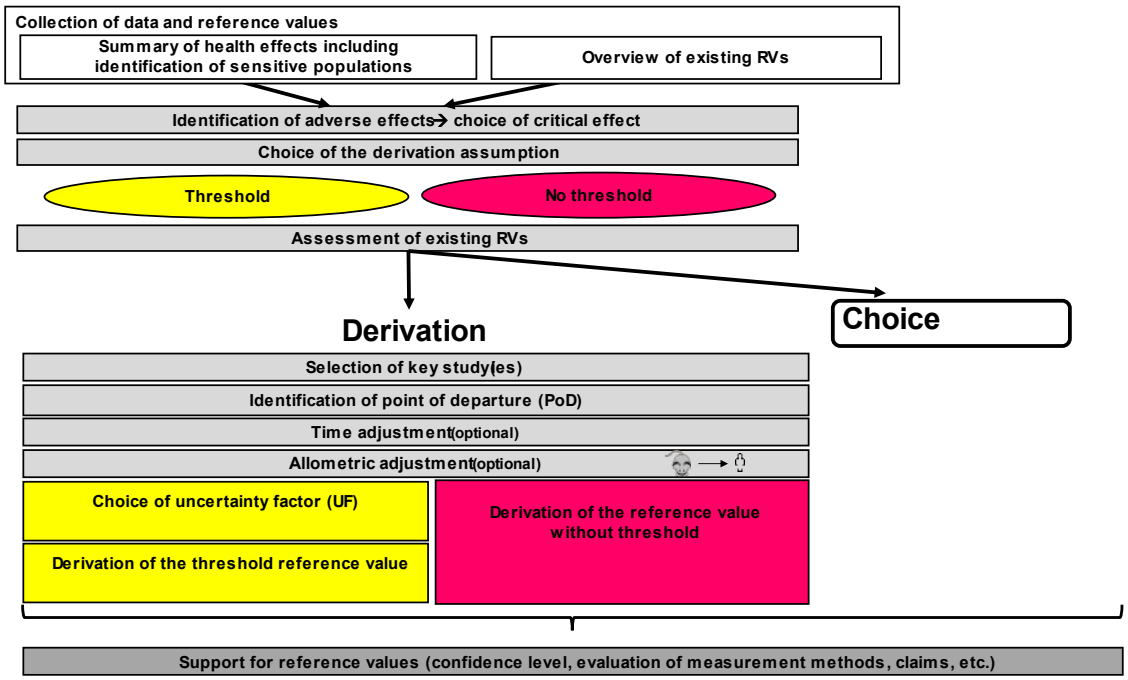


Figure 1: The various steps in proposing a TRV

- **The WG's working method**

In parallel, the WG decided to adopt an approach considering the substances individually and compile a list of approaches for dealing with PFAS mixtures.

- **TRVs for individual substances**

To propose TRVs for each of the substances considered, the WG referred to ANSES's guide for developing and choosing reference values (ANSES, pending publication).

Long-term oral TRVs were developed based on data specific to the different compounds, where available. The WG also developed long-term respiratory values based on long-term oral TRVs by route-to-route extrapolation when no respiratory data were available.

Given the very high volume of new data, particularly epidemiological data, and the large number of existing TRVs for certain substances, the WG gave priority to substances for which there were few (or no) TRVs and only a moderate volume of data available for proposing TRVs within the time available, i.e. PFBA, PFPeA, PFHxA, PFPeS, PFHpS, PFNS, PFDS, PFUnDS, PFDoDS, PFTrDS and 6:2 FTSA. For substances for which a large number of TRVs (≥ 6) and/or recent data were available (> 80 publications selected based on their title and abstract) – i.e. PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFBS, PFHxS and PFOS – it was not possible to produce a toxicological profile or propose TRVs within the time available.

For each of the 11 PFAS selected, a toxicological profile was drawn up taking into account summary reports from internationally recognised bodies in order to determine the effects observed in humans and animals. This work was supplemented by a scientific literature review performed by querying the PubMed and Scopus databases on 23 November 2023[5]. These toxicological profiles describe the health effects of each of the 11 PFAS in humans and animals based on *in vivo* and *in vitro* epidemiological, toxicological and genotoxicity studies.

The available oral and respiratory TRVs, published up to January 2025 by the main health and safety organisations recognised at supranational, European or national/regional level, were identified. This work was based on ANSES's scientific and technical support note on perfluorinated compounds in WIHC (ANSES 2023).

- **Available approaches for determining TRVs for PFAS when no specific data are available**

For PFAS for which few or no data were available, it was not possible to develop TRVs based on specific data for these compounds. The WG's experts identified the approaches available for determining TRVs without specific data for these PFAS, considering reports published in the scientific literature and by the main health and safety organisations recognised at international, European or national level.

- **Approach for taking PFAS mixtures into account in risk assessments**

The available methods used to propose TRVs for the class of perfluorinated compounds or for a mixture of several perfluorinated compounds were identified, considering reports published in the scientific literature and by the main health and safety organisations recognised at international, European or national level.

[5] Studies published after November 2023 were therefore not taken into account.

3.2. PFBA and its salts

3.2.1.Toxicological profile of PFBA and its salts

- **Toxicokinetics**

In rats, the oral absorption of PFBA is rapid and relatively complete.

In humans, PFBA is detected in the brain, liver, lungs and kidneys with respective median concentrations of 1.4 $ng \cdot g^{-1}$, 3 $ng \cdot g^{-1}$, 807 $ng \cdot g^{-1}$ and 263 $ng \cdot g^{-1}$. In animals, the low volume of distribution values indicate that PFBA is poorly distributed in tissues in mice (between 110 and 290 $mL \cdot kg\ bw^{-1}$ regardless of sex at the doses studied) and monkeys (526 ± 68 and 443 ± 59 $mL \cdot kg\ bw^{-1}$ in males and females) following oral exposure.

No human or animal studies on the metabolism of PFBA were identified.

All the data converge towards a rapid elimination of PFBA. In all the animal species studied (mice, rats and monkeys), urine is the main route of excretion. Elimination in faeces is negligible (Chang et al. 2008). Given the PFBA plasma half-lives in rats and mice, there may be a mechanism by which renal tubular reabsorption of this substance becomes saturated in these species.

- **Acute/short-term toxicity**

No short-term toxicity studies in humans were identified. In Sprague-Dawley (SD) rats, no hepatic effects were observed following exposure by gavage to up to 184 $mg \cdot kg\ bw^{-1} \cdot day^{-1}$ for five days; the same was true after they were exposed via feed to 20 $mg \cdot kg\ bw^{-1} \cdot day^{-1}$ for two weeks (3M 2007; Ikeda et al. 1985 cited in ATSDR[6] 2021 and US EPA[7] 2022). No other effects were identified. Only an increase in absolute and relative liver weights was observed following dietary administration of 78 $mg \cdot kg\ bw^{-1} \cdot day^{-1}$ to male C57Bl/6 mice for 10 days (Permadi et al. 1992, 1993 cited in ATSDR 2021 and US EPA 2022).

- **Subacute, subchronic and chronic toxicity**
    - Hepatic effects

No subacute, subchronic or chronic toxicity studies in humans were identified for hepatic effects.

Based on experimental subacute, subchronic and chronic toxicity studies, an isolated increase in absolute liver weight (indicative of liver hypertrophy) was described in male rats (Butenhoff et al. 2012) and male mice (Foreman et al. 2009). This increase was accompanied by hepatocellular hypertrophy, inflammation and focal necrosis attributed to PPAR[8]α activation in mice (Foreman et al. 2009). An increase in liver weight was also induced in murine PPARα-null mice that nonetheless expressed human PPARα (hPPARα). However, according to the authors, the incidence and severity of the focal necrosis induced by PFBA were similar in the PPARα-mutant mice expressing hPPARα, showing that human and mouse PPARα are not associated with the same hepatotoxic response induced by PFBA (Foreman et al. 2009). Moreover, no significant change in the marker liver enzymes for toxicity (AST, ALT[9]) was observed in the exposed male mice (Foreman et al. 2009; Crebelli et al. 2019).

[6] Agency for Toxic Substances and Disease Registry
[7] United States Environmental Protection Agency
[8] Peroxisome proliferator-activated receptor
[9] AST: aspartate aminotransferase; ALT: alanine aminotransferase

In addition, liver hypertrophy has been observed following exposure to PFBA, in several studies conducted in rats and/or mice. Isolated liver hypertrophy is not necessarily an adverse effect as it can be a sign of adaptation to exposure. However, one study conducted in mice showed that at the highest doses (175 and 350 mg·kg bw^{-1}·day^{-1}), the increase in absolute liver weight was associated with hepatocyte hypertrophy and then necrosis with inflammatory hepatic infiltrates, providing evidence that PFBA is hepatotoxic in mice. Further studies established that this hepatotoxicity was attributable to peroxisomal proliferation, which means that it could not be directly extrapolated to humans (Foreman et al. 2021; Mukherjee et al. 1994; IARC 1995).

- Thyroid effects

In humans, of four available cross-sectional studies, two showed a negative association between PFBA and either thyroid-stimulating hormone (TSH) or free thyroxine (fT4) (Kim et al. 2016; Ji et al. 2017a; Li et al. 2023a; Tan et al. 2024). As the epidemiological studies undertaken were cross-sectional, they were unable to make a causal inference between exposure to PFBA and thyroid hormone levels. In animals, only one study showed thyroid effects (Butenhoff et al. 2012). In this study, quantitative parameters of follicular hyperplasia/hypertrophy did not show PFBA to have any effects regardless of the dose. Only the microscopic observation results, described as subjective by the authors themselves, seemed to indicate a non-dose-dependent increase in the incidence of follicular hypertrophy/hyperplasia (no statistical results were reported in the study) after 28 and 90 days (Butenhoff et al. 2012). Due to analytical interference, the variations in circulating T4 concentrations reported in this study could not be interpreted. No change in circulating TSH concentrations was found.

Therefore, based on the available data, it was not possible to characterise any adverse effects of PFBA on the thyroid or the hypothalamic-pituitary-thyroid axis.

- Other effects (cardiovascular, immunological and renal)

The epidemiological studies identified were all cross-sectional studies that did not enable any causal interference to be drawn between PFBA and effects on kidney function (Xi et al. 2022; Wang et al. 2019; Yang et al. 2025), the immune system (Grandjean 2020; Zeng et al. 2020) or the cardiovascular system (Fu et al. 2014; Bao et al. 2017).

In animals, the haematological effects observed in the study by Butenhoff et al. (2012) were considered by the authors to be minor and not as being adverse effects on red blood cell turnover based on the absence of bone marrow and spleen damage. No macroscopic or microscopic changes were observed in the spleen, thymus or mesenteric lymph nodes of the rats.

- **Reprotoxicity and developmental toxicity**

No data on fertility in humans were identified. A few epidemiological studies reported an increased risk of prematurity, but the statistical analyses were not corrected for multiple comparisons (Yu et al. 2022; Qin et al. 2023).

In animals, the study by Das et al. (2008) identified some effects on fertility (increased foetal resorptions at 350 mg·kg bw^{-1}·day^{-1}) and development (delayed eye opening, delayed vaginal

opening and delayed balano-preputial separation from the lowest dose tested, i.e. 35 mg·kg bw^{-1}·day^{-1}). The experts consider these to be adverse effects.

- **Genotoxicity**

Markers of cellular toxicity, oxidative stress and DNA strand breaks were measured in the liver following administration of 5 mg·kg bw^{-1}·day^{-1} of PFBA to male C57Bl/6 mice for five weeks via drinking water (Crebelli et al. 2019). In this study, no genotoxic effects were determined by the analysis of micronuclei in blood reticulocytes and spleen lymphocytes or by the Comet assay on testis cells for effects on germ cells.

- **Carcinogenicity**

In humans, no association was observed between serum PFBA concentrations and numerous biological parameters in a cross-sectional study of 282 patients with lung cancer (Huang et al. 2024). No specific studies on the carcinogenicity of PFBA in animals were identified.

- **Sensitive population groups**

No studies in humans are available to determine whether exposure to PFBA may more specifically affect sensitive sub-populations or individuals at certain stages of life.

3.2.2. Proposed long-term oral TRV for PFBA and its salts

3.2.2.1. Choice of the critical effect

Only effects on fertility and development were identified by the experts as being adverse effects. They were observed in a study in mice (Das et al. 2008).

Concerning effects on fertility, Das et al. reported an increase in complete foetal resorptions[10] at the dose of 350 mg·kg bw^{-1}·day^{-1}. However, there is ambiguity in the description of the number of pregnant females (particularly among those sacrificed on gestation day (GD) 18), leading to inconsistencies with the number reported in the table of the publication presenting the results for complete foetal resorptions. **Therefore, the experts did not select this effect on fertility as the critical effect.**

PFBA induced developmental effects in mice exposed during gestation (Das et al. 2008). Delayed eye opening was observed in pups (with an average delay of 1.1, 1.2 and 1.5 days at 35, 175 and 350 mg·kg bw^{-1}·day^{-1} respectively). The harmful nature of delayed eye opening is clearly recognised by the US EPA (2022). It is also a marker of developmental effects to be taken into account under the REACH Regulation.

In 2017, ANSES did not select delayed eye opening as the critical effect, considering that the dose-response relationship was inconsistent, that the methodology for characterising this parameter was not described in the publication and that this effect was not corroborated by other criteria for developmental delays, such as delayed incisor emergence. However, the experts now consider that delayed eye opening should be selected as the critical effect given that:

[10] Number of pregnant females with full-litter loss

1) not only were the average times to eye opening significantly longer, regardless of the exposure dose (compared with the control group), but these times also increased monotonically with exposure,
2) the assessment of eye opening was based on visual inspection at regular intervals (i.e. daily) by the researchers, starting before the day when signs were expected to appear (as recommended for vaginal opening and balano-preputial separation in sub-paragraph 46 of Regulation (EU) No 900/2014),
3) delayed eye opening does not need to be corroborated by the occurrence of other developmental delays (sub-paragraph 31 of Regulation (EU) No 900/2014 and OECD Guidelines 426 and 416).

At the highest doses, other developmental effects have been reported: delayed vaginal opening by 2.3 and 3.2 days in female offspring at 175 and 350 mg·kg bw^{-1}·day^{-1} and delayed balano-preputial separation by 2.2 days in male offspring at 350 mg·kg bw^{-1}·day^{-1}. The harmful nature of these effects is unquestionable and clearly recognised by the US EPA and the guidelines of the Organisation for Economic Co-operation and Development (OECD).

Therefore, the experts considered that effects on development (delayed eye opening, delayed vaginal opening, delayed balano-preputial separation) were adverse effects that should be taken into account. Delayed eye opening was selected as the critical effect, as this effect occurred at the lowest dose tested.

3.2.2.2. Choice of the assumption for establishing the TRV

For most non-carcinogenic effects, it is considered, on the basis of current knowledge and by default, that toxicity is only expressed above a threshold dose. Therefore, the **experts considered that the effects on development resulted from a threshold dose mechanism.**

3.2.2.3. Analysis of the existing TRVs

In 2017, ANSES had proposed an indicative long-term oral toxicity value (iTV) based on hepatic effects. As this value was based on a critical effect different from the one selected by the experts in the current expert appraisal, it was not retained.

In 2022, medium- and long-term threshold TRVs were developed by the US EPA. The long-term TRV was adopted in 2023 by the Texas Commission on Environmental Quality (TCEQ). The US EPA developed organ-specific values for hepatic, thyroid and developmental effects based on the same studies considered by ANSES in 2017. Concerning the candidate values established for developmental effects, which were selected by the experts as the critical effect, the US EPA performed benchmark dose (BMD) modelling based on individual data from the study by Das et al. (2008) and calculated a BMDL[11] using the Hill model with parameter restrictions. Although it is not recommended as a first approach, this model was considered valid by the US EPA. However, no other models fit the data correctly. As a result, the experts did not retain the US EPA's TRVs based on developmental effects.

Not retaining the existing TRVs, the experts proposed developing a long-term oral TRV for PFBA and its salts.

[11] Lower limit of the confidence interval of the benchmark dose

3.2.2.4. Establishing the TRV

3.2.2.4.1. Choice of the key study

Only the study by Das et al. (2008), conducted in mice following exposure to PFBA from GD1 to GD17, showed effects on development and, in particular, delayed eye opening. This study was considered to be of good quality (rated Klimisch 1). **The study by Das et al. (2008) was therefore selected as the key study.**

3.2.2.4.2. Choice of the point of departure

In the study by Das et al. (2008), delayed eye opening was observed from the lowest dose tested of 35 mg·kg bw^{-1}·day^{-1}; this could therefore be considered a LOAEL[12]. Based on the individual data of Das et al. (2008) from the US EPA report, it was not possible to perform BMD modelling using model averaging and Bayesian inference as recommended in the ANSES guide (ANSES, pending publication).

Therefore, a LOAEL of 35 mg·kg bw^{-1}·day^{-1} was selected as the PoD.

3.2.2.4.3. Allometric adjustment

An allometric adjustment was performed to reduce the uncertainty regarding inter-species variability. According to ANSES's methodological guide, allometric adjustment should be performed using validated physiologically based pharmacokinetic (PBPK) models or, when these are not available, by applying the US EPA's default formulas or using kinetic data (ANSES, pending publication). For the oral route, the default formula for calculating a human equivalent dose (HED) is as follows:

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

For PFBA, there are no validated PBPK models. Using the default formula based on body weight ratio, with an average body weight of 0.045 kg for a pregnant mouse, considering the average body weight of a female mouse and weight gain of 22 g in pregnant control mice as reported in the study by Das et al., and a body weight of 70 kg for humans, the $LOAEL_{HED}$ is 5.6 mg·kg bw^{-1}·day^{-1} (35 x $(0.045 / 70)^{1/4}$).

The $LOAEL_{HED}$ of 5.6 mg·kg bw^{-1}·day^{-1} was selected.

3.2.2.4.4. Choice of uncertainty factors

The TRV was calculated from the selected $LOAEL_{HED}$, applying an **overall uncertainty factor (UF) of 250**, corresponding to:

- inter-species variability ($UF_{A\text{-}TD}$): 2.5, to account for toxicodynamic variability and residual toxicokinetic uncertainties;
- inter-individual variability (UF_H): 10 by default;
- variability depending on the type of PoD used ($UF_{L/B}$): √10, due to the use of a LOAEL;
- variability depending on the type of study (UF_S): 1, as the key study was a developmental study considering that pregnancy is a more relevant window of exposure for the induction of developmental effects than a lifelong study;

[12] LOAEL/C: Lowest Observed Adverse Effect Level/Concentration

- variability depending on data completeness (UF_D): √10, to account for the low volume of data, in particular the absence of studies on neurological toxicity and neurodevelopment.

3.2.2.5. Proposed long-term oral TRV and confidence level

A long-term TRV of **0.02 mg·kg bw^{-1}·day^{-1}** was proposed based on the ratio of the $LOAEL_{HED}$ to the total UF. This TRV applies to the ammonium salt of PFBA (the substance used in the key study) as well as to PFBA and its sodium and potassium salts. The overall confidence level for this TRV was estimated at 2.3/5; this is a **moderate-low** confidence level.

3.2.3. Proposed long-term respiratory iTV for PFBA and its salts

Given the variability of the data on saturated vapour pressure, the volatility of the substance is uncertain.

In the absence of respiratory toxicity data, a route-to-route extrapolation was proposed to derive a respiratory reference value from the PoD for the long-term oral TRV described above. Such a route-to-route extrapolation is possible when the critical effect is a systemic effect. **Due to the moderate-low confidence level assigned to the oral TRV, the experts proposed establishing an indicative toxicity value (iTV) for the respiratory route. This indicative value is less robust than a TRV and therefore has a low confidence level.** The iTV can be used to rule out a risk, in a conservative approach.

Since no PBPK models were available, route-to-route extrapolation was performed based on the equation below, using default absorption data in the absence of PFBA-specific absorption data.

$$LOAEC_{HEC}[13] = (LOAEL_{HED} \times Absorption_{oral} \times BW)/(Respiratory\ volume \times Absorption_{resp.}) = 9.8\ mg \cdot m^{-3}$$

Where $LOAEL_{HED}$ = 5.6 mg·kg bw^{-1}·day^{-1}, body weight (BW) = 70 kg, respiratory volume = 20 m^3·day^{-1}, oral absorption = 50% and respiratory absorption = 100%.

The experts selected a $LOAEC_{HEC}$ of 9.8 mg·m^{-3} as the PoD after oral-to-respiratory extrapolation. The iTV was calculated from the selected $LOAEC_{HEC}$ using an overall UF of 790, corresponding to the same UFs as for the oral TRV ($UF_{A\text{-}TD}$ = 2.5; UF_H = 10; UF_S = 1; $UF_{L/B}$ = √10) except for UF_D, which was set at 10 to account for the absence of data for the respiratory route. **The proposed long-term respiratory iTV is 12.4 µg·m^{-3}.** To be able to propose a more robust respiratory value, the experts recommend undertaking respiratory toxicity studies.

3.3. PFPeA and its salts

3.3.1. Toxicological profile of PFPeA and its salts

- **Toxicokinetics**

Few toxicokinetic data are available for PFPeA, but this substance is found in cord blood at a median concentration of 0.2 ng/mL^{-1} (Shah-Kulkarni et al. 2016) and in seminal fluid at a median concentration of 1.7 ng/mL^{-1} (Song et al. 2018).

[13] HEC: human equivalent dose

Subacute, subchronic and chronic toxicity

Around 15 epidemiological studies have looked at the toxicity of PFPeA. Ten of these were cross-sectional studies, which did not allow causal inferences to be made; moreover, their analyses were not corrected for multiple comparisons. Of the five case-control studies identified, one found an association between serum PFPeA concentrations and non-alcoholic fatty liver disease (Wu et al. 2024). However, as this article requires further analysis and questions for the authors, it could not be taken into account within the time available.

3.3.2.Proposed long-term oral TRV for PFPeA and its salts

Due to the methodological limitations of the epidemiological studies undertaken, no long-term oral TRV could be derived from them. Moreover, in the absence of animal data, it was not possible to derive a TRV from specific data for PFPeA and its salts. At this stage, the experts have not taken a position on a TRV based on a read-across approach with other PFAS.

3.4. PFHxA and its salts

3.4.1.Toxicological profile of PFHxA and its salts

- **Toxicokinetics**

Absorption is rapid in rodents (Chengelis, Kirkpatrick, Myers, et al. 2009; Iwabuchi et al. 2017; Gannon et al. 2011). PFHxA is extensively absorbed with an average time to reach maximum concentration (Tmax) of one hour in rats.

In humans, PFHxA is detected in the lungs, brain, kidneys, liver and bones with respective median concentrations of 207 $ng \cdot g^{-1}$, 141 $ng \cdot g^{-1}$, 2.7 $ng \cdot g^{-1}$, 68.3 $ng \cdot g^{-1}$ and 1.5 $ng \cdot g^{-1}$ of fresh tissue (Pérez et al. 2013). In plasma, PFHxA is mainly bound to proteins (> 99% bound to albumin) (Bischel et al. 2011). PFHxA crosses the placental barrier (Zhang et al. 2013). In animals, detectable but non-quantifiable concentrations of PFHxA were found in the heart, kidneys, liver and lungs after 24 hours in rats given a single dose of 100 $mg \cdot kg\ bw^{-1}$ by gavage (Gannon et al. 2011). In all tissues, measured concentrations peaked within a few hours (Gannon et al. 2011; Iwabuchi et al. 2017). Similarly, the highest concentrations were observed in the liver and femur in male CD-1 mice (Burkemper et al. 2017 cited in US EPA 2023).

No metabolites were detected in the liver or urine after oral administration to mice and rats.

No pharmacokinetic studies on the elimination of PFHxA in humans were identified. Using pharmacokinetic models, the serum half-life in humans was estimated to be between 13.7 and 327 days (Russell, Nilsson, and Buck 2013; Wallis et al. 2023). In monkeys, clearance values and serum values were similar between males and females after the first four hours, suggesting that there are no marked gender differences (Chengelis, Kirkpatrick, Myers, et al. 2009). In rats and mice, urine is the main route of elimination (77.8% to 83.4%), followed by faeces (9.6% to 12.9% of the administered dose) (Iwai 2011). In male rats, PFHxA is eliminated relatively quickly and has the lowest bioaccumulation among six perfluorinated compounds (PFBA, PFHxA, GenX, PFOA, PFBS and PFOS) (Toutain et al. 2004; Gomis et al. 2018).

- **Acute/short-term toxicity**

In rats, increased mortality (animals found dead or euthanised *in extremis*) and morbidity (renal papillary necrosis and/or stomach erosion/ulceration) were observed in males and females given 450 $mg \cdot kg\ bw^{-1} \cdot day^{-1}$ of PFHxA by gavage for four days (Kirkpatrick 2005). In a 14-day

study in which the sodium salt of PFHxA was administered in single doses of 0, 175, 550, 1750 or 5000 mg·kg bw^{-1}·day^{-1} by gavage, one out of four female rats treated at the dose of 1750 mg·kg bw^{-1}·day^{-1} and three out of three female rats treated at the dose of 5000 mg·kg bw^{-1}·day^{-1} died during treatment (Loveless et al. 2009).

Two subacute studies conducted in rats by gavage are available: a 28-day study (NTP 2022) and a 32- to 44-day study (Kirkpatrick 2005). In the first study, rats were exposed by gavage to repeated doses of 0, 62.5, 125, 250, 500 and 1000 mg·kg bw^{-1}·day^{-1}. The following were observed:

- a decrease in body weight in males and females at 1000 mg·kg bw^{-1}·day^{-1};
- hepatic effects with increases in mean absolute liver weight from 500 mg·kg bw^{-1}·day^{-1} in both sexes, and in mean relative liver weight from 500 mg·kg bw^{-1}·day^{-1} in females and from 250 mg·kg bw^{-1}·day^{-1} in males, as well as biochemical changes[14];
- renal effects with increases in mean relative kidney weight from 500 mg·kg bw^{-1}·day^{-1} in males and at 1000 mg·kg bw^{-1}·day^{-1} in females, and in absolute kidney weight at 1000 mg·kg bw^{-1}·day^{-1} in females;
- dose-dependent effects on haematological parameters:
    - decreases in red blood cell counts, haematocrit, mean corpuscular haemoglobin concentration (MCHC) and haemoglobin concentration from 62.5 mg·kg bw^{-1}·day^{-1} in males and from 250 mg·kg bw^{-1}·day^{-1} in females;
    - an increase in mean corpuscular volume (MCV) from 250 mg·kg bw^{-1}·day^{-1} in males and from 500 mg·kg bw^{-1}·day^{-1} in females;
    - an increase in reticulocyte counts from 500 mg·kg bw^{-1}·day^{-1} (males and females) correlated with a decrease in red blood cell counts;
    - an increase in platelet counts from 500 mg·kg bw^{-1}·day^{-1} in males and at 1000 mg·kg bw^{-1}·day^{-1} in females.

  Histological examinations showed an increase in splenic extramedullary haematopoiesis and the incidence of erythroid hyperplasia of the bone marrow in males and females at 500 mg·kg bw^{-1}·day^{-1}.
- effects on thyroid function (decreased plasma concentrations of triiodothyronine (T3), free thyroxine (fT4) and total thyroxine (total T4) at all the doses tested in males only, with no change in TSH);
- effects on the nasal cavity with an increased incidence of degeneration and hyperplasia of the olfactory epithelium of minimal to moderate severity in male and female rats from 250 mg·kg bw^{-1}·day^{-1}, as well as an increased incidence of suppurative inflammation of the olfactory epithelium in males at 1000 mg·kg bw^{-1}·day^{-1} and in females from 500 mg·kg bw^{-1}·day^{-1}. It should be noted that the NTP (National Toxicological Program) report does not mention any analysis of the respiratory epithelium;
- a decrease in absolute thymus weight in males exposed to 1000 mg·kg bw^{-1}·day^{-1}.

[14] Dose-dependent decreases in total protein and globulin concentrations in males from 125 mg·kg bw^{-1}·day^{-1} and at 1000 mg·kg bw^{-1}·day^{-1} in females, irregular decrease in albumin concentrations in males, increase in the albumin/globulin ratio in males from 250 mg·kg bw^{-1}·day^{-1} and in females at 1000 mg·kg bw^{-1}·day^{-1}, decrease in cholesterol concentrations at all doses in males, increases in ALT and AST enzyme activity from 500 mg·kg bw^{-1}·day^{-1} in males and females, increase in alkaline phosphatase (ALP) from 500 mg·kg bw^{-1}·day^{-1} in males and at 1000 mg·kg bw^{-1}·day^{-1} in females, increase in sorbitol dehydrogenase activity at 1000 mg·kg bw^{-1}·day^{-1} in males and increase in total bile acid concentrations at 1000 mg·kg bw^{-1}·day^{-1} in males and females.

One study also reported immunological effects with thymic atrophy in female rats given a dose of 300 to 450 mg·kg bw^{-1}·day^{-1} of PFHxA by gavage for 32 to 44 days, as well as thymic atrophy and necrosis in most of the male and female rats receiving 450 mg·kg bw^{-1}·day^{-1} for 44 days (Kirkpatrick 2005).

- **Subchronic and chronic toxicity**
  - Hepatic effects

Five cross-sectional studies investigated the relationship between exposure to PFHxA on the one hand and blood biochemical parameters and circulating concentrations of certain liver enzymes on the over (Hall et al. 2023; Fan et al. 2014 cited in ANSES 2017; Jiang et al. 2014; Liu et al. 2022; Nian et al. 2019 cited in US EPA). These epidemiological studies were all cross-sectional, which means they did not allow causal inferences to be made; moreover, they were conducted without correction for multiple comparisons.

Relative and absolute liver weights increased in a dose-dependent manner in three subacute and subchronic studies. Increases in relative liver weight in male rats were observed at doses ≥ 200–250 mg·kg bw^{-1}·day^{-1} while increases in female rats were only observed at doses ≥ 500 mg·kg bw^{-1}·day^{-1}. More specifically, in the 28-day study, relative liver weight increased (14%) in male rats at 250 mg·kg bw^{-1}·day^{-1}, where a similar increase (15%) was observed in female rats at 500 mg·kg bw^{-1}·day^{-1} (NTP 2022). In two subchronic toxicity studies, relative liver weight increased by 22% at 200 mg·kg bw^{-1}·day^{-1} in males (no change was observed in females) (Chengelis, Kirkpatrick, Radovsky et al. 2009), and by 63% and 37% at 500 mg·kg bw^{-1}·day^{-1} in males and females (Loveless et al. 2009), respectively. The effects of PFHxA on relative liver weight disappeared after 28 days in the recovery group (Chengelis, Kirkpatrick, Radovsky et al. 2009). Increases in absolute liver weight were observed from 500 mg·kg bw^{-1}·day^{-1} in male and female rats in the subacute and subchronic studies. Liver weight was not assessed in the chronic toxicity study (Klaunig et al. 2015).

Four studies assessed liver histopathology in rats. One of the observed effects of exposure to PFHxA was hepatocellular hypertrophy, noted in the subacute and subchronic studies, with a dose-dependent relationship and greater susceptibility in males (LOAEL = 100 mg·kg bw^{-1}·day^{-1}).

Other indicators of liver dysfunction or damage were also mentioned, such as increases in ALT, AST and ALP from 100 mg·kg bw^{-1}·day^{-1} in male and female rats for subacute and subchronic exposure (NTP 2022; Chengelis, Kirkpatrick, Radovsky, et al. 2009; Loveless et al. 2009).

Based on the subacute and subchronic toxicity data, exposure to PFHxA has induced hepatic effects such as hepatocellular hypertrophy (NOAEL = 50 mg·kg bw^{-1}·day^{-1}, LOAEL = 200 mg·kg bw^{-1}·day^{-1}) and increases in relative liver weight (for subacute exposure: NOAEL = 125 mg·kg bw^{-1}.day^{-1}, LOAEL = 250 mg·kg bw^{-1}·day^{-1}; and for subchronic exposure: NOAEL = 50 mg·kg bw^{-1}·day^{-1}, LOAEL = 200 mg·kg bw^{-1}·day^{-1}) and absolute liver weight (for subacute exposure: LOAEL = 500 mg·kg bw^{-1}·day^{-1}, NOAEL = 250 mg·kg bw^{-1}·day^{-1}; and for subchronic exposure: NOAEL = 100 mg·kg bw^{-1}·day^{-1}, LOAEL = 500 mg·kg bw^{-1}·day^{-1}). Other indicators of liver dysfunction or damage were also mentioned, such as increases in ALT, AST and ALP from 100 mg·kg bw^{-1}·day^{-1} in male and female rats for subacute and subchronic exposure.

- Haematological effects

Haematological effects were investigated in a single cross-sectional epidemiological study that did not allow for causal inference and was conducted without correction for multiple comparisons (Jiang et al. 2014 cited in US EPA 2023).

In the 90-day studies, decreases in MCHC, red blood cell counts, haemoglobin concentration, haematocrit and platelets were observed at doses > 200 mg·kg bw^{-1}·day^{-1} in rats (both sexes), while an increase in reticulocyte counts was noted in males only at 200 mg·kg bw^{-1}·day^{-1}. An increase in neutrophil counts was also observed from 20 mg·kg bw^{-1}·day^{-1} along with an increase in platelet counts at 500 mg·kg bw^{-1}·day^{-1}, while eosinophil and basophil counts decreased at 500 mg·kg bw^{-1}·day^{-1} in males (Loveless et al. 2009; Chengelis, Kirkpatrick, Radovsky, et al. 2009). In a 32- to 44-day study, a decrease in haemoglobin concentration was observed in rats from 50 mg·kg bw^{-1}·day^{-1}, while a decrease in MCHC and an increase in reticulocyte counts were observed only in rats that received 450 mg·kg bw^{-1}·day^{-1} (Kirkpatrick 2005).

The results of the chronic toxicity study showed no haematological effects at doses of up to 100 mg·kg $bw^{-1}day^{-1}$ in males and 30 mg·kg bw^{-1}·day^{-1} in females. At 200 mg·kg bw^{-1}·day^{-1}, a decrease in erythrocyte counts and haemoglobin concentration and an increase in reticulocyte counts were observed at 25 and/or 51 weeks, but not at 104 weeks, in females only (Klaunig et al. 2015).

Based on animal toxicity data, damage to the haematopoietic system (decrease in red blood cell counts, haemoglobin concentration and haematocrit and increase in reticulocyte counts) was observed at 450 mg·kg bw^{-1}·day^{-1} for an exposure period of 32 to 44 days (NOAEL = 150 mg·kg bw^{-1}·day^{-1}) (Kirkpatrick 2005), 200 mg·kg bw^{-1}·day^{-1} for an exposure period of 90 days (NOAEL = 50 mg·kg bw^{-1}·day^{-1}) (Chengelis, Kirkpatrick, Radovsky, et al. 2009)~~)~~, 500 mg·kg bw^{-1}·day^{-1} for an exposure period of 90 days (NOAEL = 100 mg·kg bw^{-1}·day^{-1}) (Loveless et al. 2009) and 200 mg·kg bw^{-1}·day^{-1} for an exposure period of 104 weeks (NOAEL = 100 mg·kg bw^{-1}·day^{-1}) (Klaunig et al. 2015).

- Respiratory effects

No long-term exposure studies investigating effects on the respiratory system were identified in humans.

A subchronic toxicity study in rats showed atrophy and degeneration of the olfactory epithelium at 100 and 500 mg·kg bw^{-1}·day^{-1} in both sexes, although these were not found 30 and 90 days after the end of exposure, as well as metaplasia of the nasal respiratory epithelium at 500 mg·kg bw^{-1}·day^{-1}, which was still present 30 or 90 days after the end of exposure (NOAEL = 20 mg·kg bw^{-1}·day^{-1}) (Loveless et al. 2009). Loveless et al. hypothesised that nasal tissue may be sensitive to the irritant properties of surfactants such as PFHxA. However, no studies targeting irritation were identified for PFHxA. It is also possible that reflux associated with gavage with the test compound contributed to the nasal lesions observed (Damsch et al. 2011). Loveless et al. considered that, given the NOAEL of 20 mg·kg bw^{-1}·day^{-1} and the very low levels of potential human exposure, this effect is unlikely to occur in humans.

These nasal cavity lesions were also observed in the short-term (28-day) NTP study.

In the study by Chengelis et al., conducted using the same design as the one by Loveless et al. over an exposure period of 90 days, the authors did not mention any changes in the nasal cavity in rats. In a chronic study conducted in rats exposed for two years by gavage (Klaunig

et al. 2015), the authors did not describe any changes in the nasal cavity but noted localised inflammation and/or epithelial necrosis in the larynx or pulmonary airways (congestion and/or haemorrhage, increase in alveolar macrophages) (in males: from 2.5 mg·kg bw^{-1}·day^{-1}, in females: from 5 mg·kg bw^{-1}·day^{-1}); these were thought to be secondary to accidental aspiration of the substance.

The authors of the NTP study considered that the pattern of nasal pathology observed in the study by Loveless et al. (2009) was not consistent with the lesions observed due to a reflux effect induced by gavage (NTP 2022). No lesions were observed in the oesophagus or in the posterior regions of the nasal cavity, where reflux effects related to gavage are generally observed. The NTP concluded that the nasal lesions observed with PFHxA were not attributable to gastric reflux but were likely to be systemic effects of this substance. Nevertheless, the experts note that, in the studies by the NTP and Loveless et al., damage to two different and contiguous types of epithelia (olfactory and respiratory) and the absence of reported lesions in the bronchial respiratory epithelium could suggest a local (related to gavage and/or reflux or an irritant effect) rather than a systemic effect (NTP 2022; Loveless et al. 2009). This is supported by the increased incidence of suppurative inflammation of the olfactory epithelium observed in the NTP's 28-day study. Therefore, considering all the studies, the experts cannot rule out adverse effects on the olfactory and nasal epithelia induced by PFHxA.

Based on subchronic toxicity data, changes in the olfactory epithelium (reversible upon cessation of exposure) have been observed after administration of PFHxA by gavage in animals. At the highest dose tested, metaplasia of the nasal respiratory epithelium (non-reversible upon cessation of exposure) was associated with these lesions of the olfactory epithelium. This enabled the experts to identify a NOAEL of 20 mg·kg bw^{-1}·day^{-1} and a LOAEL of 100 mg·kg bw^{-1}·day^{-1} based on the effects of degeneration and atrophy of the olfactory epithelium observed in male and female rats. Considering all the studies, the experts cannot rule out adverse effects on the olfactory and nasal epithelia induced by PFHxA.

- Renal effects

The three available epidemiological studies were cross-sectional and therefore did not allow a causal inference to be made concerning exposure to PFHxA and effects on the kidneys (Wang et al. 2019; Zhang et al. 2019; Seo et al. 2018 cited in US EPA 2023).

An increase in relative kidney weight (but not absolute weight) was observed in two subchronic studies in rats: at 500 mg·kg bw^{-1}·day^{-1} in both sexes (Loveless et al. 2009), at 10, 50 and 200 mg·kg bw^{-1}·day^{-1} in males and only at 50 mg·kg bw^{-1}·day^{-1} in females (Chengelis, Kirkpatrick, Radovsky, et al. 2009). No histopathological abnormalities in kidney tissue were reported after subchronic exposure.

Following chronic exposure, papillary renal necrosis of minimal to marked severity and tubular degeneration of minimal to moderate severity were observed in female rats at 200 mg·kg bw^{-1}·day^{-1} (Klaunig et al. 2015). Renal function parameters (urine density, specific gravity, urine pH, total urine volume) varied non-monotonically with the dose and duration of exposure in males and females, with variations observed after 26 weeks of treatment but not after 52 weeks.

Based on animal toxicity data, the effects of PFHxA on the renal system remain uncertain due to conflicting results for similar exposure durations and doses.

- Effects on the endocrine system

The three epidemiological studies that explored effects on the endocrine system were cross-sectional studies and therefore did not allow causal inferences to be drawn (Seo et al. 2018 cited in US EPA 2023).

In rats, no effects on the weight of the thyroid and other endocrine glands (adrenal glands, epididymis, testes, uterus, ovaries, thymus) were observed following subchronic exposure to PFHxA (Chengelis, Kirkpatrick, Radovsky et al. 2009; Loveless et al. 2009).

Three publications studied histopathological changes in endocrine tissues, including the thyroid, pituitary gland and pancreas, in rodents exposed to PFHxA (Chengelis, Kirkpatrick, Radovsky, et al. 2009; Klaunig et al. 2015; Loveless et al. 2009). Only the subchronic study by Loveless et al. (2009) reported an increase in the incidence of thyroid follicular cell hypertrophy in female rats given 500 mg·kg bw^{-1}·day^{-1} (n = 4/10) and in male rats given 100 (n = 1 /10) and 500 mg·kg bw^{-1}·day^{-1} (n = 2/10) of PFHxA sodium salt by gavage for 90 days. The other two studies did not report any histopathological effects on the thyroid at doses of up to 200 mg·kg bw^{-1}·day^{-1} after subchronic (90 days) or chronic (two years) exposure to PFHxA.

In animals, histopathological changes in the thyroid have been observed at high doses (LOAEL = 500 mg·kg bw^{-1}·day^{-1}, NOAEL = 200 mg·kg bw^{-1}·day^{-1}), with persistent follicular cell hypertrophy (Loveless et al. 2009). Note that the 28-day study of the NTP (NTP 2022) also reported effects on the thyroid with a decrease in T3 and T4 (TT4 and fT4) in male rats from 62.5 mg·kg bw^{-1}·day^{-1}, with no change in TSH (no NOAEL).

- Other effects

Cardiovascular effects were only investigated in two cross-sectional epidemiological studies, which means that no causal inference could be made (Wittkopp et al. 2022; Lin et al. 2023).

Four epidemiological studies on immunological effects showed no association or were cross-sectional and therefore did not allow for causal inference (Dong et al. 2013; Qin et al. 2017 cited in ATSDR 2021; Ji et al. 2021; Kaur et al. 2023).

Two case-control studies did not show an association between exposure to PFHxA and type 2 or gestational diabetes (Duan et al. 2021; Zhang et al. 2023).

- **Reproductive and developmental toxicity**
  - <u>Fertility</u>

Four cross-sectional studies examined the potential link between exposure to several PFAS, including PFHxA, and fertility parameters such as certain sperm characteristics (motility and concentration), reproductive hormones and ovarian reserve. Two case-control studies also investigated the potential connection between exposure to several PFAS, including PFHxA, and the risk of developing polycystic ovary syndrome (PCOS) (Zhou et al. 2016 cited in US EPA 2023; Song et al. 2018; Rodríguez-Carrillo et al. 2023; Zhan et al. 2023; Björvang et al. 2021; Tian et al. 2023). They did not allow any conclusions to be drawn about a link between effects on fertility and exposure to PFHxA, because they were cross-sectional and therefore did not allow causal inference to be made, because they were not corrected for multiple comparisons, or because they did not show any association.

The data from the 28-day NTP study showed no changes in absolute epididymis tail, epididymis or testes weight (NTP 2022). A decrease in the number of sperm cells in the epididymis tail was observed at 1000 mg·kg bw^{-1}·day^{-1}.

Loveless et al. conducted a one-generation study (OECD 415) in female rats exposed by gavage to 0, 20, 100 and 500 mg·kg bw^{-1}·day^{-1} of PFHxA sodium salt for around 70 days prior to cohabitation, through gestation and lactation, for a total exposure duration of around 126 days. Males of the parental generation were exposed by gavage for 110 days. No treatment-related mortality was observed in males or females, regardless of the dose tested. Clinical signs of toxicity included stained skin/fur in males and females at 500 mg·kg bw^{-1}·day^{-1}. Effects were observed in the parents, including:

- a decrease in male body weight at 100 and 500 mg·kg bw^{-1}·day^{-1} associated with a decrease in overall body weight gain, of 12% and 29% respectively, compared to the control group;
- a decrease in average maternal body weight gain at 500 mg·kg bw^{-1}·day^{-1} during the first week of gestation, but not in subsequent weeks;
- an increase in body weight gain during lactation at 500 mg·kg bw^{-1}·day^{-1} (weight gain of 25 g vs 5 g in the control group), whereas a reduction in body weight gain was expected according to the authors.

PFHxA treatment was not found to have any effects on mating, fertility, gestation length, the number of implantation sites, oestral cyclicity, sperm parameters, litter size, sex ratio, clinical observations of the pups, survival of the pups or the developmental milestones of the F1 adults, at any of the doses tested. Overall body weight gain from day 0 to day 39 in F1 adults (after weaning) was comparable between the two sexes regardless of the dose. No changes in organ weights were observed in adult F1 males and females, nor were any macroscopic or pathological findings observed in animals designated for reproductive evaluation.

Three studies in rats exposed by gavage (exposure periods of 28 or 90 days) assessed the effects of PFHxA or its sodium salt on testis weight (Loveless et al. 2009; NTP 2022; Chengelis, Kirkpatrick, Radovsky, et al. 2009). Two studies reported an increase in relative (but not absolute) testis weight in rats exposed to 1000 mg·kg bw^{-1}·day^{-1} for 28 days (NTP 2022) or to 500 mg·kg bw^{-1}·day^{-1} for 90 days (Loveless et al. 2009). No treatment-related effects on male reproductive organ weights were reported (Chengelis, Kirkpatrick, Radovsky, et al. 2009). No changes in the female reproductive system were reported in the 28- and 90-day studies (Loveless et al. 2009; Klaunig et al. 2015; Iwai and Hoberman 2014; Chengelis, Kirkpatrick, Radovsky, et al. 2009).

Based on toxicity studies in rats, no effects on fertility were identified.

- Developmental toxicity

The epidemiological studies identified did not allow any conclusions to be drawn about a link between the risk of premature birth or pregnancy outcomes and exposure to PFHxA, because they were cross-sectional and therefore did not allow causal inferences to be made, because they were not corrected for multiple comparisons, or because they did not show any association (Jin et al., 2020 cited in US EPA 2023; Zheng et al. 2024; Yu et al. 2022; Li et al. 2023).

Loveless et al. conducted several experimental protocols, including a one-generation reproductive study and a developmental study (Loveless et al. 2009). In the one-generation reproductive study (OECD 415), female rats were exposed by gavage to 0, 20, 100 and 500 mg·kg bw^{-1}·day^{-1} of PFHxA sodium salt for around 70 days prior to cohabitation, through

gestation and lactation, for a total duration of around 126 days. Males of the parental generation were exposed by gavage for 110 days. No treatment-related effects were observed on the pups, survival of the pups or the developmental milestones of the F1 adults, regardless of the dose tested. A decrease in average pup weight at PND[15] 0, 7, 14 and 21 was observed at 500 mg·kg bw^{-1}·day^{-1}.

In a study on development (OECD 41) described in the same publication, 22 female rats per group received a daily dose of 0, 20, 100 or 500 mg·kg bw^{-1}·day^{-1} by gavage, from GD6 to GD20. The authors did not observe any treatment-related deaths or post-mortem macroscopic abnormalities in the mothers regardless of the dose. Maternal toxicity occurred at 500 mg·kg bw^{-1}·day^{-1} (reductions in total weight gain from GD6 to GD21 and in overall net gain). Developmental toxicity was limited to a decrease in foetal weight at 500 mg·kg bw^{-1}·day^{-1}.

In a combined reproductive and developmental toxicity study (Charles River 2011, cited in ANSES 2017), PFHxA was administered by gavage to pregnant Crl:CD1 mice (n = 20/dose) at doses of 0, 100, 350 and 500 mg·kg bw^{-1}·day^{-1} from GD6 to GD18. In the mothers, a slight increase in salivation was observed at 350 mg·kg bw^{-1}·day^{-1}; this increase was slight to moderate at 500 mg·kg bw^{-1}·day^{-1}. A decrease in weight gain during lactation was also observed at 500 mg·kg bw^{-1}·day^{-1}. At 500 mg·kg bw^{-1}·day^{-1}, an increase in the number of stillbirths was reported. Viability indices were reduced on day 7 at 350 and 500 mg·kg bw^{-1}·day^{-1} (and also on day 4 for this group). Developmental delays were observed from 350 mg·kg bw^{-1}·day^{-1} (delayed eye opening at 350 mg·kg bw^{-1}·day^{-1}; decrease in the percentage of pups per litter with eyes open at PND14 from 350 mg·kg bw^{-1}·day^{-1}). Lastly, terminal body weights in the females of the F1 generation were reduced at 350 mg·kg bw^{-1}·day^{-1} and relative liver weight was reduced in males at 500 mg·kg bw^{-1}·day^{-1}.

In another 28-day exposure study, no changes in gestation length were observed in rats given 315 mg·kg bw^{-1}·day^{-1} of PFHxA (Kirkpatrick 2005). This study also showed no clinical signs or changes in the survival or developmental milestones of the pups between PND1 and PND4. No changes in litter size or the survival or body weight of the pups and no occurrence of internal malformations were observed in the offspring of rats given 315 mg·kg bw^{-1}·day^{-1} before mating up to lactation day 4.

Based on animal toxicity studies on development in rats and mice, the experts identified the decrease in pup weight at PND0 as an adverse effect, with a LOAEL of 500 mg·kg bw^{-1}·day^{-1} and a NOAEL of 100 mg·kg bw^{-1}·day^{-1} according to the study by Loveless et al. (2009).

- **Genotoxicity**

The genotoxic effects of PFHxA have been assessed in several mammalian cell systems and in prokaryotes *in vitro*. These tests with PFHxA sodium salt were negative for mutagenicity in several strains of *Escherichia coli* and *Salmonella typhimurium* in the presence and absence of metabolic activation, in mammalian cells *in vitro* and in human peripheral blood lymphocytes.

In vivo, the micronucleus test in circulating erythrocytes in male and female rats following twice-daily gavage at doses of 31.3 to 500 mg·kg bw^{-1}·day^{-1} was negative for PFHxA.

Based on the results of these available *in vitro* and *in vivo* studies, the experts concluded that PFHxA has no genotoxic potential.

[15] Post-natal day

- **Carcinogenicity**

In a case-control study with a small population size, no association was found between PFHxA exposure and the risk of breast cancer.

No increase in the incidence of tumours, regardless of the type and all types combined, was observed in males or females at all doses in the two-year chronic exposure study by gavage in rats (Klaunig et al. 2015).

The only chronic toxicity study undertaken in rats did not show an increase in the incidence of tumours, regardless of the type and all types combined.

- **Sensitive population groups**

No data are currently available to identify any population groups that may be sensitive to PFHxA.

3.4.2. Proposed long-term oral TRV for PFHxA and its salts

3.4.2.1. Choice of the critical effect

The available studies on PFHxA highlighted effects on the liver, kidneys, development, respiratory system, thyroid, haematological system and body weight (in adults and offspring). Among these, the three effects that occurred at the lowest doses were effects on the upper respiratory tract, the liver (hepatic hypertrophy) and body weight. Questions arose for each of these effects.

The effect on the nasal cavity was observed in two studies involving gavage in rats: 1) a subchronic study (90 days) with atrophy and degeneration of the olfactory epithelium and metaplasia of the respiratory epithelium in males and females (Loveless et al. 2009) and 2) an acute study (28 days) with hyperplasia and degeneration of the olfactory epithelium, associated with inflammatory lesions in males and females (NTP 2022). The dose of 20 mg·kg bw^{-1}·day^{-1} was identified as the NOAEL based on the study by Loveless et al. (2009). These studies also highlighted damage to two types of epithelia (olfactory and respiratory), which may suggest a local effect. Although there are arguments suggesting that the nasal effects were local irritant effects, which is supported by the increased incidence of suppurative inflammation of the olfactory epithelium observed in the NTP's 28-day study, the experts consider that a systemic effect cannot be ruled out.

Hepatic effects were observed in rats in two 90-day studies and one two-year study (Klaunig et al. 2015; Loveless et al. 2009; Chengelis, Kirkpatrick, Radovsky, et al. 2009). Loveless et al. demonstrated an increase in absolute and relative liver weights at 500 mg·kg bw^{-1}·day^{-1} in both sexes, dose-dependent hepatocellular hypertrophy at 500 mg·kg bw^{-1}·day^{-1} in females and males, and an increase in AST (from 100 mg·kg bw^{-1}·day^{-1}) and ALT (from 20 mg·kg bw^{-1}·day^{-1}) in males only. These increases in AST and ALT were reversible three months after the end of exposure. The same effects were observed in the study by Chengelis et al., only in male rats at 200 mg·kg bw^{-1}·day^{-1} (increased relative liver weight and ALT and hepatocellular hypertrophy), as were an increase in ALP and a decrease in cholesterol and total protein (Chengelis, Kirkpatrick, Radovsky, et al. 2009). The increases in ALT and ALP were reversible 28 days after the end of exposure. The authors suggested that the male rats were more sensitive than the females, with a NOAEL of 20 mg·kg bw^{-1}·day^{-1} and a LOAEL of 100 mg·kg bw^{-1}·day^{-1}. This may have been due to faster elimination in females than in males, reducing the residence time in the target organ. These two studies also showed an increase in peroxisomal β-oxidation activity: from 100 mg·kg bw^{-1}·day^{-1} in males and at

500 mg·kg bw^{-1}·day^{-1} in females in the studies by Loveless et al. (2009) and at 200 mg·kg bw^{-1}·day^{-1} in males in the one by Chengelis, Kirkpatrick, Radovsky, et al. (2009). Although some of the hepatic effects were mild and reversible and did not lead to cytolysis, steatosis or necrosis during the exposure period in question, the experts considered that their association with other systemic effects at the same doses would justify them being classified as adverse effects. The NOAEL would be 20 mg·kg bw^{-1}·day^{-1} in males and the LOAEL 100 mg·kg bw^{-1}·day^{-1}.

Lastly, a decrease in body weights was observed in a one-generation reproductive study in parents from 100 mg·kg bw^{-1}·day^{-1} in males and at 500 mg·kg bw^{-1}·day^{-1} in females (Loveless et al. 2009). Therefore, the NOAEL for this effect is 20 mg·kg bw^{-1}·day^{-1} and the LOAEL is 100 mg·kg bw^{-1}·day^{-1}.

Each of the three effects (hepatic, respiratory and on body weight) in isolation could not be considered a critical adverse effect. However, considering that these three types of effects occur at the same dose level, the experts selected their combination as the critical effect.

3.4.2.2. Choice of the assumption for establishing the TRV

For most non-carcinogenic effects, it is considered, on the basis of current knowledge and by default, that toxicity is only expressed above a threshold dose. **Therefore, the experts considered that the effects on the nasal cavity, liver and body weight in adult rats resulted from a threshold dose mechanism.**

3.4.2.3. Analysis of the existing TRVs

Six medium- and long-term threshold TRVs have been established for the oral route: ANSES (2017), Michigan (2019), MDH (2021), US EPA (2023), TCEQ (2023) and OEHHA (2024).

The TRV established by ANSES in 2017 was based on renal effects observed in the chronic study by Klaunig et al. (2015). Several methodological biases were identified in this study, including increased mortality unrelated to treatment; this was observed at all doses, including in male and female controls (lesions due to gavage, mechanical injury or reflux).

The US EPA and TCEQ TRVs were based on a decrease in pup weights observed at PND0 in the one-generation reproductive study of Loveless et al. (2009). Based on the description of the statistical analysis of the data in the key study, it is not clear which effect was taken into account (decrease in individual weights or in average litter weight).

The Minnesota Department of Health (MDH) and the Office of Environmental Health Hazard Assessment (OEHHA) established their TRVs based on respiratory effects from the same study (Loveless et al. 2009). Although there are arguments suggesting that the nasal effects were local irritant effects, which is supported by the increased incidence of suppurative inflammation of the olfactory epithelium observed in the NTP's 28-day study, the experts consider that a systemic effect cannot be ruled out.

Therefore, not retaining the existing long-term TRVs, the experts proposed establishing a long-term oral TRV for PFHxA and its salts.

3.4.2.4. Establishing the TRV

3.4.2.4.1. Choice of the key study and PoD

According to the ANSES method for establishing TRVs (ANSES, pending publication), good-quality data in humans should be preferred to data obtained in animals when they are available, which was not the case here.

Among the available experimental data, the study by Loveless et al. was considered to be of good quality (rated Klimisch 1) (Loveless et al. 2009). It highlighted:

- effects on the olfactory epithelium of the nasal cavity in male and female rats, with an increase in the incidence of lesions (atrophy and degeneration) at 100 and 500 mg·kg bw^{-1}·day^{-1} in both sexes,
- an increased incidence of metaplasia of the nasal respiratory epithelium at 500 mg·kg bw^{-1}·day^{-1}, present 30 or 90 days after the end of exposure,
- hepatotoxic effects in male rats only, with an increase in ALT from 20 mg·kg bw^{-1}·day^{-1} and an increase in AST from 100 mg·kg bw^{-1}·day^{-1},
- effects on the body weights of male parents (P1) at 100 and 500 mg·kg bw^{-1}·day^{-1}.

The experts chose the study by Loveless et al. (2009) as the key study. A NOAEL of 20 mg·kg bw^{-1}·day^{-1} was selected as the PoD based on all the respiratory, hepatic and body weight effects observed in adult rats.

3.4.2.4.2. Allometric adjustment

An allometric adjustment was performed to reduce the uncertainty regarding inter-species variability. According to ANSES's methodological guide, allometric adjustment should be performed using validated PBPK models or, when these are not available, by applying the US EPA's default formula based on the body weight ratio or by using kinetic data (ANSES, pending publication).

For PFHxA, there were no sufficiently robust or predictive PBPK models, but unlike for the other PFAS studied in this report, kinetic data, particularly clearance data, were available, allowing a second approach to be used to perform this allometric adjustment. Depending on the type of critical effect, the US EPA selected clearance data from either rats or mice, according to sex ($Cl_{female\ rats}$ = 0.383 and $Cl_{male\ rats}$ = 0.163). To estimate clearance in humans, the US EPA used two approaches:

- clearance calculated from the average elimination constant in humans, taken from the study by Nilsson et al. (2013), and the average volume of distribution (Vd) in monkeys, i.e. $1.84 \cdot 10^{-3}$ L·hr^{-1}·kg bw^{-1},
- clearance estimated by allometry from the set of $t_{1/2}$ values in different species (allometric scaling approach).

The US EPA selected the clearance calculated from the human study by Nilsson et al. (2013).

However, the elimination kinetics characterised by Nilsson et al. could not be used to perform an allometric adjustment because they were based on apparent clearance taken from a study in which exposure in humans was not controlled. Therefore, the experts did not adopt the approach based on kinetic data and used the default formula recommended in the methodological guide (ANSES, pending publication), with an average weight of 0.250 kg for rats and 70 kg for humans, leading to a $NOAEL_{HED}$ of 4.8 mg·kg bw^{-1}·day^{-1}.

The $NOAEL_{HED}$ of 4.8 mg·kg bw^{-1}·day^{-1} was selected.

3.4.2.4.3. Choice of uncertainty factors

The TRV was calculated from a $NOAEL_{HED}$ using the following UFs (ANSES, pending publication):

- 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 toxicokinetic 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): $\sqrt{10}$, as the key study was a subchronic study (90 days);
- use of a point of departure ($UF_{L/B}$): 1 due to the use of a NOAEL;
- inadequacy of the data (UF_D): $\sqrt{10}$, in light of the low volume of data available (no functional immunotoxicity studies or two-generation studies).

An overall uncertainty factor of 250 was therefore used to establish the TRV.

3.4.2.4.4. Proposed long-term oral TRV and confidence level

A long-term TRV of **20 µg·kg bw^{-1}·day^{-1}** was calculated based on the ratio of the $NOAEL_{HED}$ to the overall uncertainty factor. This TRV applies to the sodium salt of PFHxA (the substance used in the key study) as well as to the acid and its potassium and ammonium salts. An overall confidence level of 2.4/5 was estimated for this TRV; this is a moderate-low confidence level.

3.4.3. Proposed long-term respiratory iTV for PFHxA and its salts

Given the variability of the data on saturated vapour pressure, the volatility of the substance is uncertain. No toxicokinetic or toxicity studies were identified for the respiratory route.

In the absence of respiratory toxicity data, a route-to-route extrapolation was proposed to derive a long-term respiratory reference value from the PoD for the long-term oral TRV described above. Such a route-to-route extrapolation is possible when the critical effect is a systemic effect. **The experts proposed establishing an iTV for the respiratory route. This indicative value is less robust than a TRV and therefore has a low confidence level.** The iTV can be used to rule out a risk, in a conservative approach.

In the absence of kinetic models, route-to-route extrapolation was performed based on the adjusted PoD from the study by Loveless et al. (2009), i.e. the $NOAEL_{HED}$ of 4.8 mg·kg bw^{-1}·day^{-1}. In the absence of data on the respiratory absorption of PFHxA in humans and animals, as well as on its digestive absorption in humans, and although studies conducted in rodents indicated digestive absorption close to 100%, default values were selected in accordance with the ANSES methodological guide (ANSES, pending publication), i.e. 50% for the oral route and 100% for the respiratory route:

$$NOAEC_{HEC} = (NOAEL_{HED} \times Absorption_{oral} \times BW)/(Respiratory\ volume \times Absorption_{resp.})$$

Where $NOAEL_{HED}$ = 4.9 mg·kg bw^{-1}·day^{-1}, body weight (BW) = 70 kg and respiratory volume = 20 m^3/day

The experts selected a $NOAEC_{HEC}$ of 8.6 mg·m^{-3} as the PoD after oral-to-respiratory extrapolation. The TRV was calculated from a $NOAEC_{HEC}$ using an overall UF of 790, corresponding to the same UFs (UF_A = 2.5; UF_H = 10; UF_S = $\sqrt{10}$; $UF_{L/B}$ = 1), except for UF_D,

which was set at 10 to take account of the absence of data for the respiratory route. **The proposed long-term respiratory iTV is 11 µg·m^{-3}.** To be able to propose a more robust respiratory value, the experts recommend undertaking respiratory toxicity studies.

3.5. PFPeS and its salts

3.5.1. Toxicological profile of PFPeS and its salts

A significant positive association between plasma PFPeS concentrations and the risk of infertility associated with PCOS was found in a case-control study conducted in women aged 20 to 40 (Zhan et al. 2023). No other studies in humans or animals have specifically investigated the toxicity of PFPeS.

3.5.2. Proposed long-term oral TRV for PFPeS and its salts

Due to the methodological limitations of the epidemiological studies undertaken, no long-term oral TRV could be derived from them. Moreover, in the absence of animal data, it was not possible to derive a TRV from specific data for PFPeS and its salts. At this stage, the experts have not taken a position on a TRV based on a read-across approach with other PFAS.

3.6. PFHpS and its salts

3.6.1. Toxicological profile of PFHpS and its salts

Only data in humans were identified.

- **Toxicokinetic data**

No studies on absorption or metabolism were identified in humans.

Mean serum half-lives for PFHpS were estimated at between 1.46 and 4.55 years in two observational studies in a population exposed to PFAS via contaminated drinking water (Xu et al. 2020; Li et al. 2022) and at 7.4 years in a study of Australian fire-fighters heavily exposed to PFAS (Nilsson et al. 2022a).

Three studies reported transplacental transfer of PFHpS in humans (Kim et al. 2011; Eryasa et al. 2019 cited by UBA[16] 2023; Bangma et al. 2020).

- **Acute and subacute toxicity**

No acute or subacute toxicity data were identified.

- **Subchronic and chronic toxicity**

Most of the epidemiological studies were cross-sectional studies examining the relationship between PFHpS exposure and thyroid hormones (markers: circulating concentrations of fT4/TT4, fT3 (free T3)/TT3 (total T3) and TSH) (Xing et al. 2024; Inoue 2019; Nilsson et al., 2022b), markers of liver function (ALT, apolipoprotein B, α-fœtoprotein) (Nilsson et al. 2022b; Chen et al. 2024; Dai et al. 2024), renal function impairment (marker: glomerular filtration rate) (Su et al. 2022), the occurrence of cardiovascular disease (Nilsson et al. 2022b; Goodrich et al. 2023), the risk of dyslipidaemia using serum cholesterol and lipid levels as markers (Nilsson et al. 2022b; Averina et al. 2021; Liu et al. 2024), the risk of diabetes (marker: blood glucose)

[16] Umweltbundesamt (the German Environment Agency)

(Qu et al. 2024) and the prevalence of osteoporosis (marker: bone density) (Fan et al. 2023). These cross-sectional studies did not allow for causal inference and were not corrected for multiple comparisons.

The only significant findings from longitudinal data concerned symptoms of bronchitis/lung disease, respiratory health and allergies during childhood as reported by parents (Impinen et al. 2019; Kvalem et al. 2020). The associations were corrected to account for multiple comparisons. The risk of classification bias was too high to use these studies for identifying an adverse effect. An American cross-sectional pilot study found an association with a decrease in SARS-CoV-2 anti-spike antibody (IgG) levels (Kaur et al. 2023).

In a cross-sectional study (Averina et al. 2021), the fourth quartile was positively associated with hypertension with an OR of 1.74 ($CI_{95\%}$: 0.97-3.11, p = 0.063) in comparison with the first quartile. This association with hypertension was at the limit of statistical significance.

Of the epidemiological studies investigating the relationship between PFHpS exposure and height & weight development (a BMI[17] marker), only the study by Dai et al. (2023) had a longitudinal design. However, the results differed depending on the categorisation of the dependent variable (discrete or continuous) and no correction for multiple comparisons was performed.

- **Reproductive and developmental toxicity**
  - Fertility

A single cross-sectional study nested within a Chinese prospective cohort of couples consulting at the preconception stage found a positive association between PFHpS concentrations, sperm counts and concentrations without changes in semen volume, and progressive and total sperm motility using an adjusted model after correcting for multiple comparisons (Luo et al. 2022).

In a Norwegian cohort study, in a model adjusted for potential confounding factors, an association was found between reduced PFHpS concentrations and women with short cycles[18], only for women in the study who had previously had at least one pregnancy. However, no correction for multiple comparisons was performed (Singer et al. 2018).

Of the epidemiological studies that investigated the relationship between PFHpS exposure and puberty, only the study by Ernst et al. had a longitudinal design (Ernst et al. 2019). After adjusting for potential confounding factors, no association was found between prenatal exposure to PFHpS and age at puberty in boys or girls, using an overall indicator of pubertal development. When pubertal signs were studied individually, the results showed that on average, only stages B2 and B3 of breast development occurred earlier in girls. In boys, no association was found in the tertile analyses. In the continuous statistical analyses, associations were found in boys between increasing PFHpS concentrations and the early onset of stage G3, stage P4 and voice break. However, the events were self-reported and the associations observed were isolated and inconsistent. Furthermore, no correction for multiple comparisons was undertaken.

A cross-sectional study found an association in boys between plasma PFHpS concentrations (median = 0.16 $ng \cdot mL^{-1}$) and serum 11-deoxycorticosterone concentrations.

[17] Body mass index
[18] 17–24 days (vs normal cycles (25–31 days) and long cycles (>32 days))

- Developmental toxicity

The studies that examined the links between exposure to PFHpS and pregnancy outcomes were either cross-sectional studies or studies without correction for multiple comparisons (Liew et al. 2020; Shen et al. 2022; Ding et al. 2023). The study by Meng et al. showed that the association between maternal exposure to PFHpS and shorter length of gestation was still present after adjusting for the plasma concentrations of the five other PFAS measured, with no increased risk of preterm birth (Meng et al. 2018). Therefore, these effects could not be considered adverse effects.

A significant association between prenatal exposure to PFHpS and reduced working memory performance was not observed in the general population but was noted in children with symptoms of attention-deficit/hyperactivity disorder (ADHD) with a small sample size in a cohort study (Skogheim 2020).

- **Genotoxicity**

No genotoxicity studies were identified.

- **Carcinogenicity**

Itoh et al. conducted a hospital-based case-control study on exposure to 20 PFAS, including PFHpS, and the risk of breast cancer (401 cases and 401 controls). After adjusting for confounding factors (no correction for multiple comparisons), an inverse association was found for the third tertile of exposure compared to the first tertile. In a continuous analysis, plasma concentrations of PFHpS were associated with a decreased risk of breast cancer in a model adjusted for confounding factors (Itoh et al. 2021).

- **Sensitive population groups**

No data were found enabling sensitive population groups to be identified.

3.6.2. Proposed long-term oral TRV for PFHpS and its salts

Due to the methodological limitations of the epidemiological studies undertaken, no long-term oral TRV could be derived from them. Moreover, in the absence of animal data, it was not possible to derive a TRV from specific data for PFPeS and its salts. At this stage, the experts have not taken a position on a TRV based on a read-across approach with other PFAS.

3.7. PFNS and its salts

3.7.1. Toxicological profile of PFNS and its salts

No data on kinetics, (sub)acute toxicity, (sub)chronic toxicity, fertility, genotoxicity or carcinogenicity were identified in humans or animals. Only one cross-sectional study investigated the impact of maternal exposure to 14 PFAS, including PFNS, on the risk of prematurity and various pregnancy outcomes in 506 mother-child pairs (Shen et al. 2022). This cross-sectional study did not allow for causal inference and was conducted without correction for multiple comparisons.

3.7.2. Proposed long-term oral TRV for PFNS and its salts

Due to the methodological limitations of the epidemiological studies undertaken, no long-term oral TRV could be derived from them. Moreover, in the absence of animal data, it was not possible to derive a TRV from specific data for PFNS and its salts. At this stage, the experts have not taken a position on a TRV based on a read-across approach with other PFAS.

3.8. PFDS and its salts

3.8.1. Toxicological profile of PFDS and its salts

One cross-sectional study showed a link between serum PFDS concentrations and increased systolic blood pressure in women (Bao et al. 2017). Another cross-sectional study found an association between PFDS concentrations and decreased triglycerides in cord blood in pregnant women exposed to PFAS during the attacks of 11 September 2001 (Spratlen et al. 2019). These cross-sectional studies did not allow for causal inference and were not corrected for multiple comparisons.

3.8.2. Proposed long-term oral TRV for PFDS and its salts

Due to the methodological limitations of the epidemiological studies undertaken, no long-term oral TRV could be derived from them. Moreover, in the absence of animal data, it was not possible to derive a TRV from specific data for PFDS and its salts. At this stage, the experts have not taken a position on a TRV based on a read-across approach with other PFAS.

3.9. PFUnDS and its salts

No studies were identified in the literature search for PFUnDS and its salts. Therefore, in the absence of data, it was not possible to derive a TRV from specific data for PFUnDS and its salts. At this stage, the experts have not taken a position on a TRV based on a read-across approach with other PFAS.

3.10. PFDoDS and its salts

No studies were identified in the literature search for PFDoDS and its salts. Therefore, in the absence of data, it was not possible to derive a TRV from specific data for PFDoDS and its salts. At this stage, the experts have not taken a position on a TRV based on a read-across approach with other PFAS.

3.11. PFTrDS and its salts

No studies were identified in the literature search for PFTrDS and its salts. Therefore, in the absence of data, it was not possible to derive a TRV from specific data for PFTrDS and its salts. At this stage, the experts have not taken a position on a TRV based on a read-across approach with other PFAS.

3.12. 6:2 FTSA and its salts

3.12.1. Toxicological profile of 6:2 FTSA and its salts

- **Toxicokinetic data**

Four toxicokinetic studies are available: three studies (one *in vitro* study and two *in vivo* studies in rats), not described in detail in the REACH registration dossier for 6:2 FTSA, and one study in mice summarising the results of a report by the US Army Public Health Center (Narizzano et al. 2021; Narizzano, Bohannon, and Quinn 2021).

No information on the absorption of 6:2 FTSA was identified. 6:2 FTSA has been found in the blood of both humans and rodents (Yeung et al. 2008; Lee and Mabury 2011; Loi et al. 2013; Eriksson et al. 2017; Narizzano, Bohannon, and Quinn 2021; study report, 2007 cited in the 2019 REACH registration dossier). In humans, it has also been detected in cerebrospinal fluid (Hong et al. 2024). 6:2 FTSA can cross the placental barrier (Yang et al. 2016). No metabolites of 6:2 FTSA were identified in an *in vitro* study in rat livers (study report, unnamed, 2008, cited in the REACH registration dossier) or in a study by gavage in *Peromyscus leucopus* 'white-footed' mice (Narizzano, Bohannon, and Quinn 2021). 6:2 FTSA was primarily excreted in urine (65-68%) after rats were exposed by gavage. The calculated urinary excretion half-lives ranged from 20.9 to 23.75 hours.

- **Acute and subacute toxicity**

No studies in humans were identified.

An LD_{50}[19], by the oral route (gavage) in rats, of 300 to 2000 mg·kg bw^{-1} was reported in the REACH registration dossier for 6:2 FTSA (2017 study report cited in the 2019 REACH registration dossier).

In a study of 20 adult male CD-1 mice exposed by gavage for 28 days to a single dose of 0 or 5 mg·kg bw^{-1}·day^{-1} of 6:2 FTSA ammonium salt, an increase in absolute and relative liver weights was observed, as were increases in serum AST and albumin concentrations (Sheng et al. 2017). Histopathological examination identified hepatocyte hypertrophy and necrosis. Sheng et al. also observed an increase in hepatic inflammatory markers[20]. According to the authors, the overall liver findings suggested signs of hepatic lesions following exposure to 6:2 FTSA at a dose of 5 mg·kg bw^{-1}·day^{-1} (LOAEL).

In a repeated-dose toxicity study in male Crl:CD-1 mice exposed to 0, 3, 30, 300 and 3000 ppm via feed for 14 days, the following effects were observed: decreases in average body weight and weight gain at 3000 ppm, increases in relative and absolute average liver weights from 300 ppm, and liver discolouration (1/5 of mice at 300 ppm and 4/5 at 3000 ppm). Based on these results, the registrant identified a NOAEL of 30 ppm (study report, 1995, cited in the REACH registration dossier).

A dose-determination study was conducted in Wistar rats exposed by gavage for 14 days. The authors noted a decrease in weight gain and food consumption, changes in creatinine and

[19] Median lethal dose

[20] Tumour necrosis factor alpha (TNFα) cytokines in serum and liver, interleukin (IL)-10 and IL-1β in serum and IL-6 in liver

urea, and an increase in kidney weight in males at 50 and 100 mg·kg bw^{-1}·day^{-1} and in females at 100 mg·kg bw^{-1}·day^{-1} (study report, 2018, cited in the REACH registration dossier).

- **Subchronic and chronic toxicity**

Only one cross-sectional study was identified (Carlsson et al. 2023). Because of its design, this cross-sectional study was of limited relevance for making a causal inference and was not corrected for multiple comparisons.

In a combined repeated-dose toxicity and reproductive and developmental toxicity screening study (OECD 422), the summary of which was taken from the REACH registration dossier, Wistar rats received 0, 5, 15 or 45 mg·kg bw^{-1}·day^{-1} of 6:2 FTSA potassium salt for 90 days by gavage (study report, 2018, cited in the REACH registration dossier). Males were treated for 10 weeks before mating, during mating and until sacrifice after a total of 90 days of exposure, while females were treated for 10 weeks before mating and during mating, gestation and lactation (until around lactation day 14). In males, an increase in MCHC was observed at 15 mg·kg bw^{-1}·day^{-1} along with a decrease in the average percentage of monocytes at the lowest dose (5 mg·kg bw^{-1}·day^{-1}). At 45 mg·kg bw^{-1}·day^{-1}, a decrease in body weight was observed in males[21] and females[22]. Mean plasma concentrations of total protein and albumin were decreased in males at 5 and 45 mg·kg bw^{-1}·day^{-1} (in the absence of a dose-response relationship and given the limited effect, this effect was not considered harmful by the authors, although it was related to treatment). The mean serum urea concentration was higher in males at the highest dose. In males only, an increase in relative kidney weight was observed at 5 and 45 mg·kg bw^{-1}·day^{-1} while an increase in absolute kidney weight was only noted at the lowest dose; there was mild to moderate (multi)focal tubular dilatation only at the highest dose. In females, the mean relative heart weight was reduced from 15 mg·kg bw^{-1}·day^{-1}, with no change in absolute heart weight and with no histopathological changes reported, making it difficult to assess the adverse nature of these effects. Considering the renal effects observed at 45 mg·kg bw^{-1}·day^{-1}, the authors identified a NOAEL of 15 mg·kg bw^{-1}·day^{-1} for the 90-day study.

In a repeated toxicity study combined with a reproductive and developmental study, Bohannon et al. exposed white-footed mice to 0, 0.2, 1, 5 or 25 mg·kg bw^{-1}·day^{-1} for 112 days by gavage (Bohannon et al. 2023). The F1 generation was only exposed *in utero* and via lactation and was then euthanised on PND10. Only an increase in spleen weights was observed in males of the parental generation exposed to 5 mg·kg bw^{-1}·day^{-1}. Assessment of immune function via the plaque-forming cell (PFC) assay showed a reduction in the number of PFCs in males and females of the parental generation after exposure to 5 and 25 mg·kg bw^{-1}·day^{-1}, with the effect being more pronounced in males than in females.

- **Reproductive and developmental toxicity**
    - Fertility

In the combined repeated-dose toxicity and reproductive and developmental toxicity screening study described above (study report, 2018), only postnatal survival was marginally reduced (one pup missing or dead at 5 and 15 mg·kg bw^{-1}·day^{-1}, two pups missing or dead at

[21] Between day 49 and day 70 of treatment
[22] Between day 0 and day 7, between day 21 and day 28, and between day 56 and day 70

45 mg·kg bw^{-1}·day^{-1}). The registrant concluded that 6:2 FTSA had no effect on male or female fertility or reproductive performance (NOAEL > 45 mg·kg bw^{-1}·day^{-1}).

In the study by Narizzano et al., total sperm counts were not affected in white-footed mice exposed by gavage for 28 days to 0, 2.5, 6 and 12.5 mg·kg bw^{-1}·day^{-1} of 6:2 FTSA (Narizzano et al. 2021). In the study by Bohannon et al. in which white-footed mice were exposed to 6:2 FTSA for 112 days, no effects on reproduction were observed in either the parental generation or the F1 generation (Bohannon et al. 2023).

- Effects on development

In the study by Bohannon et al. in which white-footed mice were exposed to 6:2 FTSA for 112 days, no effects on development were observed in either the parental generation or the F1 generation (Bohannon et al. 2023).

In the combined repeated-dose toxicity and reproductive and developmental toxicity screening study described above, only the following effects were observed: increased mean anogenital distance in F1 females at 15 mg·kg bw^{-1}·day^{-1} and increased T4 levels at the two lowest doses only in males on PND13. Although the registrant considered these results to be unrelated to the treatment[23] (NOAEL > 45 mg·kg bw^{-1}·day^{-1}), the increase in T4 levels could suggest an adverse effect at 5 mg·kg bw^{-1}·day^{-1} according to the UBA and the Michigan Department of Environment, Great Lakes, and Energy (Michigan Department of Environment, Great Lakes, and Energy 2020; UBA 2023).

- **Genotoxicity**

The REACH registration dossier describes several tests carried out with 6:2 FTSA, most of which had negative results with or without metabolic activation (Ames test, induction of chromosome number changes in Chinese hamster ovary (CHO) cells, *in vivo* studies by gavage: chromosomal aberrations in bone marrow in mice, induction of micronuclei in bone marrow in ICR mice, unscheduled DNA synthesis test in the liver in male SD rats, comet assay in the liver and stomach in male SD rats). The only positive result was related to the induction of structural chromosomal aberrations in CHO cells treated for four hours (± S9), although the result was negative after 20 hours of treatment in the absence of metabolic activation.

- **Carcinogenicity**

No human or animal studies were identified.

- **Sensitive population groups**

No data are currently available to identify any population groups that may be sensitive to 6:2 FTSA.

[23] Due to large variation between values within the groups (high standard deviation compared to historical control data) and lack of statistical significance at the highest dose

3.12.2. Proposed long-term oral TRV for 6:2 FTSA and its salts

3.12.2.1. Choice of the critical effect

Only a few experimental studies in laboratory animals are available for 6:2 FTSA. A combined repeated-dose toxicity and reproductive and developmental toxicity screening study showed a decrease in mean relative heart weight without any change in absolute heart weight. This effect was only observed in females and was not confirmed histopathologically. Therefore, the experts did not select this effect as the critical effect, given the difficulty of assessing its adverse nature in the absence of histopathological follow-up and information concerning the extent of the decrease.

A recent study observed immunological effects in mice with an increase in spleen weights in males at a single dose (5 mg·kg bw^{-1}·day^{-1}) as well as a decrease in the number of PFCs in males and females at 5 and 25 mg·kg bw^{-1}·day^{-1}. The PFC (haemolytic plaque) assay is a simple and highly sensitive *ex vivo* test of immune function. It measures the ability of an organism to trigger an immune response with antibody production following the administration of an antigen. Variations in haemolysis plaques reflect damage to immune cells in lymphoid organs, most commonly the spleen, impairing the production of specific antibodies. In mice receiving an injection of sheep red blood cells, this test was found to be sensitive, reproducible and highly predictive for measuring immunotoxic effects (Luster 1988 and 1992). It should be noted that this test is recommended by the NTP for assessing immunotoxicity. Therefore, this test showed a decrease in the number of antibody-producing cells, suggesting an immunosuppressive effect. However, the mechanism of action remained unclear.

The experts therefore selected immunological effects (decrease in PFCs) as the critical effects, as these were considered the most robust.

3.12.2.2. Choice of the assumption for establishing the TRV

For most non-carcinogenic effects, it is considered, on the basis of current knowledge and by default, that toxicity is only expressed above a threshold dose. Therefore, **the experts considered that the immunological effects resulted from a threshold dose mechanism.**

3.12.2.3. Analysis of the existing TRVs

Without going as far as proposing a TRV, the UBA selected two PoDs (associated with UFs) related on the one hand to hepatic effects in mice, observed in a 28-day study (Sheng et al. 2017), and on the other to a decrease in heart weights in rats, observed in a combined repeated-dose toxicity and reproductive and developmental toxicity screening study.

In 2020, Michigan proposed a long-term oral TRV based on the decrease in heart weights, applying a UF of 3000, including a UF_D of 10 to account, among other things, for the lack of data on immunotoxic effects and for the deficiencies in the results relating to the heart.

The experts did not select the TRV of Michigan, nor the PoDs used by the UBA, as these were not based on the critical effect selected; they also considered that a 28-day single-dose study was not relevant for deriving a long-term TRV; moreover, they found the effect on heart weight to be irrelevant.

As a consequence, given these limitations, the experts did not retain the existing TRVs and proposed developing a long-term oral TRV.

3.12.2.4. Establishing the TRV

3.12.2.4.1. Choice of the key study

According to the ANSES method for developing TRVs (ANSES, pending publication), good-quality data in humans should be preferred to data obtained in animals when they are available, which was not the case here. Among the available experimental data, only the study by Bohannon et al. (2023) was considered to be of good quality (rated Klimisch 1). It found immunotoxic effects in mice exposed for 112 days. An increase in spleen weights was observed at 5 mg·kg bw^{-1}·day^{-1} in males only. The authors also found a decrease in the number of PFCs at the two highest doses (5 and 25 mg·kg bw^{-1}·day^{-1}) in both males and females, with a more pronounced effect in males.

The experts chose the study by Bohannon et al. (2023) as the key study.

3.12.2.4.2. Choice of the point of departure

The data from the study by Bohannon et al. (2023) highlighted a dose-response relationship between the decrease in PFCs and exposure to 6:2 FTSA. This was modelled using the EFSA web application for Bayesian Benchmark Dose Modelling as described in ANSES's methodological guide (ANSES, pending publication). As it was not possible to quantitatively define a biologically relevant response level to inform the selection of a benchmark response (BMR) for the effect considered, BMDLs were modelled with various BMRs (5%, 8% and 10% corresponding to 1SD) for males, females and both sexes. The BMDLs modelled from pooled male and female data were not retained due to a sex covariate effect. The experts chose a default BMR value of 10%, considering that the PFC assay is a sensitive test for identifying an immunosuppressive effect. Of the $BMDL_{10}$ values, for protective purposes, the experts chose the lowest value, i.e. 2.05 mg·kg bw^{-1}·day^{-1}.

The experts selected the $BMDL_{10}$ of 2.05 mg·kg bw^{-1}·day^{-1} as the point of departure, as it corresponds to the lower bound of the credible interval for the BMD for a BMR of 10%.

3.12.2.4.3. Allometric adjustment

An allometric adjustment was performed to reduce the uncertainty regarding inter-species variability. According to the ANSES guide, allometric adjustment should be performed using predictive PBPK models or, if these are not available, using kinetic data or by applying the US EPA's default formulas (ANSES, pending publication). For 6:2 FTSA, there are no validated PBPK models or kinetic data. Therefore, an HED was calculated using the equation given in Section 3.2.2.4.3, using an average body weight of 19 g for female control mice at the end of the study and a body weight of 70 kg for humans, i.e. a $BMDL_{HED}$ of 0.26 mg·kg bw^{-1}·day^{-1}.

3.12.2.4.4. Choice of uncertainty factors

The TRV was calculated from a $BMDL_{HED}$, applying an **overall UF of 790**, corresponding to:

- inter-species variability (UF_A): 2.5, to account for toxicodynamic variability and residual toxicokinetic uncertainties;
- inter-individual variability (UF_H): 10 by default;
- subchronic to chronic transposition (UF_S): $\sqrt{10}$, as the key study was a long-term study (112 days);
- use of a point of departure ($UF_{L/B}$): 1 due to the use of a BMDL;

- inadequacy of the data (UF_D): 10, in light of the low volume of data available.

3.12.2.4.5. Proposed long-term oral TRV and confidence level

A **long-term TRV of 0.33 µg·kg bw^{-1}·day^{-1}** was calculated based on the ratio of the $BMDL_{HED}$ to the total UF. This TRV applies to 6:2 FTSA (the substance used in the key study), as well as its sodium, potassium and ammonium salts. The overall confidence level for this TRV was estimated at 2.1/5; this is a **moderate-low** confidence level.

3.12.2.5. Proposed long-term respiratory TRV for 6:2 FTSA

In the absence of measured physical and chemical data, in particular data on saturated vapour pressure, the experts considered that it was not possible to determine whether the substance is volatile or not. No toxicokinetic or toxicity studies were identified for the respiratory route. In the absence of respiratory toxicity data, a route-to-route extrapolation was proposed to derive a long-term respiratory reference value from the PoD for the long-term oral TRV described above. Such a route-to-route extrapolation is possible when the critical effect is a systemic effect. **Due to the moderate-low confidence level assigned to the oral TRV, the experts proposed establishing an indicative toxicity value (iTV) for the respiratory route. This indicative value is less robust than a TRV and therefore has a low confidence level.** The iTV can be used to rule out a risk, in a conservative approach.

In the absence of kinetics models, route-to-route extrapolation was performed based on the equation below, using default absorption data in the absence of specific absorption data.

$$BMCL^{24}_{HEC} = (BMDL_{HED} \times Absorption_{oral} \times BW)/(Respiratory\ volume \times Absorption_{resp.}) \mathbf{= 0.46\ mg \cdot m^{-3}}$$

Where $BMDL_{HED}$ = 0.26 mg·kg bw^{-1}·day^{-1}, body weight (BW) = 70 kg, respiratory volume = 20 m^3/day, oral absorption = 100%, respiratory absorption = 50%.

The experts selected a $BMCL_{HEC}$ of 0.46 mg·m^{-3} as the PoD after oral-to-respiratory extrapolation. The TRV was calculated from a BMC_{HEC} using the same UFs that were used to establish the oral TRV. **The long-term respiratory iTV is 0.6 µg·m^{-3}.** To be able to propose a more robust respiratory value, the experts recommend undertaking toxicity studies by this route.

3.13. Review of existing approaches for determining TRVs when no specific data are available

For PFAS for which the data are not sufficient to establish a TRV, some organisations have adopted the TRV of another compound based on structural and/or effect similarities. Approaches such as read-across and grouping by chain length can be used to determine pragmatic TRVs (TCEQ 2023; US EPA 2024a; Patlewicz et al. 2024; Anderson et al. 2022). Moreover, some organisations have proposed using new approach methods (NAMs) to determine PoDs (RIVM 2018 and 2021; US EPA 2024b).

Only a review of approaches for determining TRVs in the absence of data was carried out. A critical analysis of these approaches will be performed when this expert appraisal is updated.

[24] BMCL: Lower limit of the confidence interval of the benchmark concentration.

3.14. Review of approaches for taking account of PFAS mixtures in risk assessments

The experts conducted a review of existing methods for taking account of PFAS mixtures in risk assessments.

In order to assess PFAS mixtures, it is ideal to have studies conducted with such mixtures. The experts found that epidemiological studies have investigated various mixtures of PFAS. However, the experts did not review either these studies or the methods for analysing mixture-specific data.

Some organisations have proposed using TRVs considering a sum of PFAS: EFSA proposed a TRV of 4.4 ng·kg bw^{-1}·day^{-1} for a mixture of four PFAS (PFOS, PFOA, PFNA, PFHxS) (EFSA 2020).

Approaches based on the assumption of dose additivity have also been used to assess cumulative risks for mixtures:

- the hazard index (HI) method, with three articles proposing HIs for PFAS (Borg et al. 2013; Bil et al. 2023; Mumtaz, Buser, and Pohl 2021);
- the point of departure index (PoDI) approach. No publications using the PoDI approach to PFAS were identified;
- relative potency factors (RPFs) or toxic equivalency factors (TEFs). RPFs have been proposed for 25 PFAS in various publications and by various organisations, as well as by the RIVM using PFOA as the reference compound (RIVM 2018 and 2021; Bil et al. 2021, 2022 and 2023; Gomis et al. 2018; Behnisch et al. 2021; Luz et al. 2019 cited in UBA 2023; MassDEP ORS 2019).

Only a review of the approaches to PFAS mixtures was carried out as part of this report. A critical analysis of these approaches will be performed when this expert appraisal is updated.

3.15. Conclusion and recommendations

Given the large volume of new data, particularly epidemiological data, and the large number of existing TRVs for certain PFAS, the experts prioritised substances for which there were no or few TRVs and for which it was possible to propose TRVs within the time available. As a result, this expert appraisal focused on the following 11 PFAS: PFBA, PFPeA, PFPeS, PFHpS, PFNS, PFDS, PFUnDS, PFDoDS, PFTrDS, PFHxA and 6:2 FTSA. For substances with a large number of TRVs (≥ 6) and/or a large volume of recent data (PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFBS, PFHxS and PFOS), the experts were unable to produce a toxicological profile or propose TRVs within the time available. Further work will be carried out on some of these compounds in order to propose TRVs in a future update to the report.

The experts developed long-term oral TRVs for PFBA, PFHxA and 6:2 FTSA and their salts. For these compounds, the experts also developed long-term respiratory iTV values established by route-to-route extrapolation.

For PFBA and its salts, the proposed long-term oral TRV is based on delayed eye opening. A moderate-low confidence level was assigned to this TRV.

For PFHxA and its salts, the proposed long-term oral TRV is based on all respiratory and hepatic effects and effects on body weight. A moderate-low confidence level was assigned to this TRV.

For 6:2 FTSA and its salts, the proposed long-term oral TRV is based on immune system effects demonstrated by a decrease in PFCs. A moderate-low confidence level was assigned to this TRV.

For the eight other PFAS and their salts, the experts were unable to propose TRVs in the absence of data (PFUnDS, PFTrDS, PFDoDS) or due to a lack of usable data for deriving TRVs based on specific data for these substances (PFPeA, PFPeS, PFHpS, PFNS, PFDS).

In addition, the experts reviewed approaches for determining TRVs in the absence of data, as well as approaches for PFAS mixtures, without taking a position on them. Their critical analysis will be carried out during an update to the report in order to assess whether it is possible to develop an approach for the PFAS class and/or propose TRVs for compounds for which no data are available among the 21 included in the formal request.

The experts recommend carrying out studies to obtain:

- information on the purity of the technical PFAS[25] used and the identity of their main impurities;
- measured (rather than modelled) experimental data for the physico-chemical properties of PFAS, including volatility and solubility;
- toxicity data, mainly from subchronic and chronic studies, as well as toxicokinetic data for PFAS for which few or no data are available;
- respiratory toxicity data, in particular for the compounds for which the experts developed iTVs (PFBA, PFHxA and 6:2 FTSA), in order to have more robust reference values, regardless of whether these compounds are volatile or not.

[25] The technical quality or grade of a product refers to one of the qualities under which products are marketed (to be distinguished from a pure product or a product used as an analytical reference, for example)

Table 2: Long-term oral TRVs and long-term threshold respiratory iTVs for various PFAS and their salts

Compounds		**PFBA and its salts**		**PFHxA and its salts**		**PFPeA**	**PFPeS**	**PFHpS**	**PFNS**	**PFDS**	**PFUnDS**	**PFDoDS**	**PFTrDS**	**6:2 FTSA and its salts**	
TRV	**Type**	**LT oral TRV**	**LT inhalation iTV**	**LT oral TRV**	**LT inhalation iTV**	**No TRV**								**LT oral TRV**	**LT inhalation iTV**
	Value	**20 µg·kg bw^{-1}·day^{-1}**	**12.4 µg·m^{-3}**	**20 µg·kg bw^{-1}·day^{-1}**	**11 µg·m^{-3}**									**0.33 µg·kg bw^{-1}·day^{-1}**	**0.6 µg·m^{-3}**
Critical effect		Effects on development (delayed eye opening)		All respiratory and hepatic effects and effects on body weight in adults		In the absence of data or due to a lack of usable data, it was not possible to derive a TRV based on specific data for these compounds and their salts								Effects on the immune system (↘ plaque-forming cells)	
Key study	**Reference**	Das et al. 2008		Loveless et al. 2009										Bohannon et al. 2023	
	Species	CD-1 mice		Crl:CD(SD) rats										White-footed mice	
	Exposure	Gavage, GD1-GD17		Gavage, 90 days										Gavage, 112 days	
Point of departure		LOAEL 35 mg·kg bw^{-1}·day^{-1}		NOAEL 20 mg·kg bw^{-1}·day^{-1} LOAEL 100 mg·kg bw^{-1}·day^{-1}										LOAEL 5 mg·kg bw^{-1}·day^{-1} NOAEL 1 mg·kg bw^{-1}·day^{-1} $BMDL_{10}$ 2.05 mg·kg bw^{-1}·day^{-1}	
Allometric adjustment		$LOAEL_{HED}$ 5.6 mg·kg bw^{-1}·day^{-1}		$NOAEL_{HED}$ 4.8 mg·kg bw^{-1}·day^{-1}										$BMDL_{HED}$ 0.26 mg·kg bw^{-1}·day^{-1}	
Route-to-route extrapolation		/	$LOAEC_{HEC}$ = $LOAEL_{HED}$ x BW/Respiratory vol x Abs_{oral}/Abs_{resp} = 9.8 mg·m^{-3}	/	$NOAEC_{HEC}$ = $NOAEL_{HED}$ x BW/Respiratory vol x Abs_{oral}/Abs_{resp} = 8.6 mg·m^{-3}									/	$BMCL_{HEC}$ = $BMDL_{HED}$ x BW/Respiratory vol x Abs_{oral}/Abs_{resp} $BMCL_{HEC}$ 0.46 mg·m^{-3}
Uncertainty factors (UFs)		250 ($UF_{A\text{-}TD}$ 2.5; UF_H 10; $UF_{L/B}$ √10; UF_S √10; UF_D √10)	790 ($UF_{A\text{-}TD}$ 2.5; UF_H 10; $UF_{L/B}$ √10; UF_S √10; UF_D 10)	250 ($UF_{A\text{-}TD}$ 2.5; UF_H 10; $UF_{L/B}$ 1; UF_S √10; UF_D √10)	790 ($UF_{A\text{-}TD}$ 2.5; UF_H 10; $UF_{L/B}$ 1; UF_S √10; UF_D 10)									790 ($UF_{A\text{-}TD}$ 2.5; UF_H 10; UF_L 1; UF_S √10; UF_D 10)	
Confidence level		Moderate-low	Low by definition	Moderate-low	Low by definition									Moderate-low	Low by definition

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions and recommendations of the CES on Health reference values (HRV Committee) on the proposed reference values for long-term exposure to several PFAS.

Given growing scientific and societal concerns about PFAS, the amount of data generated is increasing exponentially, particularly in epidemiology. There is also a large number of TRVs for certain PFAS. The experts therefore decided to prioritise substances for which recent data could be analysed, given the volume of data to be processed within the time available for the work. The expert appraisal therefore focused on 11 PFAS (among the 21 included in the formal request[26]), i.e. PFBA, PFPeA, PFPeS, PFHxA, PFHpS, PFNS, PFDS, PFUnDS, PFDoDS, PFTrDS and 6:2 FTSA, corresponding to those for which there were few (or no) TRVs and for which a moderate amount of data was available for proposing TRVs within the time available.

Long-term oral TRVs and long-term respiratory iTVs were developed for PFBA, PFHxA, 6:2 FTSA and their salts. These iTVs were derived from long-term oral TRVs by performing a route-to-route extrapolation in the absence of specific data for the respiratory route. For the eight other PFAS[27], it was not possible to derive TRVs based on specific effects due to the absence or a lack of usable data for determining the critical effect and deriving TRVs. For these PFAS, ANSES recommends conducting oral and respiratory (mainly subchronic and chronic) studies to obtain toxicity data, as well as toxicokinetic data so that specific TRVs can be developed.

Beyond the individual approach, an approach by class has been considered. However, the class of PFAS is vast and heterogeneous in terms of structure and health effects (increased cholesterol levels, cancer, effects on fertility and foetal development, effects on the liver, kidneys, etc.) These substances are also suspected of interfering with the endocrine (thyroid) and immune systems.

In light of this complexity and given the time available, at this stage it has only been possible to review the approaches used to determine TRVs in the absence of data for PFAS as well as the approaches used to take into account mixtures of PFAS in risk assessments.

ANSES is continuing its expert appraisal work on the development of TRVs for certain PFAS not examined among the 21 covered by the formal request, as well as for the eight for which sufficient data are not available to establish TRVs. The approaches for determining TRVs for PFAS for which no data are available and the approaches for taking account of PFAS mixtures in risk assessments will also be critically analysed. Depending on the nature of the results, these follow-up actions will be described in a supplemented version of this opinion or in separate opinions.

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[26] 20 PFAS analysed in WIHC + 6:2 FTSA
[27] i.e. PFPeA, PFPeS, PFHpS, PFNS, PFDS, PFUnDS, PFDoDS and PFTrDS

KEYWORDS

VTR, long terme, voie orale, voie respiratoire, inhalation, PFAS, perfluorés, PFBA, PFPeA, PFPeS, PFHxA, PFHpS, PFNS, PFDS, PFUnDS, PFDoDS, PFTrDS, 6:2 FTSA

TRV, long term, oral route, respiratory route, inhalation, PFAS, perfluorinated compounds, PFBA, PFPeA, PFPeS, PFHxA, PFHpS, PFNS, PFDS, PFUnDS, PFDoDS, PFTrDS, 6:2 FTSA

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ANNEX 1: TRACKING OF OPINION UPDATES

Date	Page	Description of the change
11 April 2025		Initial version
22 May 2025	10	Clarification added concerning the choice of body weight for mice used for the allometric adjustment
	13	Addition of Section 3.4 on PFHxA and its salts
	39	Addition of the proposed TRVs/iTVs for PFHxA in the conclusion