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Colophon

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Synopsis

Mixture exposure to PFAS: A Relative Potency Factor approach

PFAS is a large group of poly- and perfluoroalkyl compounds. They have a dirt-repellent effect and are therefore used, for example, in finishing clothing. For the best known PFAS, PFOA and PFOS, much information is available about the properties have been researched, as have the quantity people may be exposed to without causing negative effects on health.

In 2016, RIVM derived such a quantity for PFOA. However, much less is known about most of the other compounds in this group of substances. PFASs often occur together as contamination in soil, groundwater or drinking water. To be able to better assess the risks of this type of contamination, the RIVM has investigated the extent to which it is possible to express the harmfulness of a number of PFASs in relation to PFOA. It was concluded that this can be done by using so-called Relative Potency Factors (RPFs). Here the exposure to a PFAS mixture is expressed as a comparable amount of PFOA. This method can be used for dealing with pollution with PFASs in the environment, e.g. in cases involving contamination in soil, groundwater or drinking water. Measured PFAS quantities are simply expressed in PFOA units, so that they can be compared with PFOA standards for soil or (drinking) water.

The use of the RPF method does, however, have an important condition, namely that a (limited) set of comparable toxicity data for individual PFAS compounds is available. For the relevant health effect (on the liver of test animals), such information was available for 11 PFAS compounds. This effect has been investigated, because the liver reacts most sensitively to PFOA in humans and laboratory animals. The effect is an enlargement of the liver (hypertrophy). This is an unwanted effect.

Keywords: perfluor compounds, mixture exposure, Relative Potency Factors

Publiekssamenvatting

Gecombineerde blootstelling aan PFAS: een benadering met factoren voor relatieve potentie

PFAS is een grote stofgroep van poly- en perfluoralkylverbindingen. Ze hebben onder andere een vuilafstotende werking en worden daarom bijvoorbeeld in kleding verwerkt. Van de bekendste, zoals PFOA en PFOS, is onderzocht welke eigenschappen ze hebben en welke hoeveelheid mensen ervan binnen mogen krijgen zonder negatieve effecten op de gezondheid te veroorzaken.

In 2016 heeft het RIVM voor PFOA zo'n hoeveelheid afgeleid. Van de meeste andere verbindingen in deze stofgroep is echter veel minder bekend. PFAS stoffen komen vaak gezamenlijk als verontreiniging voor in grond, grondwater of drinkwater. Om de ernst van dergelijke verontreiniging beter te kunnen inschatten, heeft het RIVM onderzocht in hoeverre het mogelijk is om de schadelijkheid van een aantal PFAS ten opzichte van PFOA uit te drukken. Dat kan door gebruik te maken van zogeheten Relative Potency Factors (RPF). Hierbij wordt de blootstelling aan een PFAS-mengsel uitgedrukt in een vergelijkbare hoeveelheid PFOA. Deze methode kan worden gebruikt bij het omgaan met verontreiniging met PFAS in het milieu, zoals bij een verontreiniging in grond, grondwater of drinkwater. Gemeten PFAS-hoeveelheden worden eenvoudig in PFOA-eenheden uitgedrukt, zodat ze vergeleken kunnen worden met voor bodem of (drink)water geldende PFOA-normen.

Aan het gebruik van de RPF-methode kleeft wel een belangrijke voorwaarde, namelijk dat een (beperkte) set aan vergelijkbare toxiciteitsgegevens voor individuele PFAS-verbindingen beschikbaar is. Voor het relevante gezondheidseffect (op de lever van proefdieren) bleek dergelijke informatie voor elf PFAS verbindingen beschikbaar. Dit effect is onderzocht omdat de lever bij mens en proefdieren het gevoeligst op PFOA reageert. Het ongewenste effect is een vergroting van de lever (hypertrofie).

Kernwoorden: perfluorverbindingen, mengselblootstelling, toxiciteitsfactoren

Contents

Summary

The exposure to Per- and PolyFluoroAlkyl Substances (PFASs) via drinking water and soil can be assessed by a mixture of 19 different congeners, with congeners basically differing in carbon chain length (C2 C18).

Evaluating the toxic risk associated with such exposure warrants the exposure to be expressed as one single aggregated exposure metric. Relative Potency Factors (RPF) enable the calculation of such an aggregated exposure metric.

By definition, RPFs express the toxic potency of individual mixture components relative to the so-called Index Compound (IC), the latter being one of the mixture components with well-known occurrence and toxicity and, hence, the availability of a (life-long) human exposure level which is without toxic risk (Health-Based Guidance Value, HBGV). For this reason, the PFAS PerFluoroOctanoic Acid (PFOA) was chosen in this report as the IC.

Based on the critical PFAS/PFOA toxicity in experimental animals, i.e. liver toxicity, RPFs were derived for all 20 relevant PFASs.
The derived RPFs were used to express the occurrence of PFASs in drinking water and soil in terms of PFOA equivalents.

1 Introduction

Per- and PolyFluoroAlkyl Substances (PFASs) are a class of man-made chemicals with a wide range of industrial and commercial applications, which has resulted in their ubiquitous presence in the environment (ATSDR, 2015; CONCAWE, 2016; Bull et al., 2014).
Due to emissions to air, water and soil, PFASs are present in soil, groundwater, surface water and sediments. PFASs have been measured in the blood serum of workers, inhabitants near to plants producing PFASs, as well as in the general population, the latter arising from exposure through contaminated food and drinking water (see e.g. Noorlander et al. 2011, Eschauzier, 2013; Zafeiraki et al. 2015 and Gebbink et al. 2017 for the occurrence of PFASs in Dutch food and drinking water).

To evaluate the toxic risk of chemical exposure, a so-called Health-Based Guidance Value (HBGV) should be available. A HBGV refers to the life-long human daily exposure which is without toxic effect. A HBGV may be derived for any of the relevant human routes of exposure, i.e. inhalation, dermal exposure or oral exposure. As in the case of PFASs, the current exposure of the general population occurs mainly via food and drinking water, the interest lies with an oral HBGV.

Suitable human epidemiological data or toxicological data obtained from experimental animals may be used to derive a HBGV. In practice, the latter implies the availability of a complete toxicity dossier, i.e. acute toxicity (single dose), sub-acute toxicity (28 day exposure), semi-chronic toxicity (90-day exposure), chronic toxicity (two-year exposure), reproductive toxicity, as well as genotoxicity studies. In the case of PFASs, such a complete dossier is only available for PFOA and PFOS, thereby in fact precluding the derivation of a HBGV for any of the other PFASs. HBGVs, based on the extrapolation of animal toxicity to man, are currently in place for perfluorooctane sulfonic acid (PFOS) and PFOA (Table 1.1). As shown, various institutions have derived different values for the HBGV. This variability stems from differences in the particular animal toxicity study used as the Point of Departure (PoD) to derive the HBGV and the way the uncertainty in the extrapolation was addressed.

Hitherto, no HBGVs have been derived for PFASs other than PFOA and PFOS. Exception to the rule here is FSANZ who considered PFHxS equipotent to PFOS. Furthermore, in the risk assessment, PFAS mixture components are evaluated independently from each other. Here, too, an exception to the rule exists in that, in drinking water, US EPA and the state of Vermont consider the occurrence of PFOA and PFOS to act additively (US EPA, 2016a, b; Vermont, 2016).

PFAS compounds often occur together as contamination in soil, groundwater or drinking water. Because HBGVs are absent for most compounds, the level of contamination can only be assessed based on the content of PFOA and PFOS. To be able to better assess the risk of this type of contamination, the study investigates if it is possible to express the risk of the measured PFAS substances relative to PFOA.

With this knowledge, and additional information on the occurrence of PFAS in the environment, it is possible to give guidance to authorities and companies on how to deal with the presence of PFAS in soil, groundwater, surface water and sediments. The outcome of this study will be used to develop a risk assessment framework for PFAS contamination in the Netherlands (Slenders et al., 2018).

Table 1.1 Health-Based Guidance Values (ng/kg bw/day) for PFASs.

Substance	**HBGV**
PFHxS	FSANZ (2017): 20
PFOS	EFSA (2008): 150 [1]
	ATSDR (2015): 30 [2]
	US EPA (2016b): 20 [2]
	FSANZ (2017): 20 [2]
PFOA	EFSA (2008):1500 [1]
	US EPA (2016b): 20
	ATSDR (2015): 20
	RIVM (Zeilmaker, 2016): 12.5
	NJWQI (2016): 2
	FSANZ (2017): 160

1currently under revision;
2critical toxicity: PFOS: liver hypertrophy (ATSDR); foetal toxicity (US EPA, FSANZ); PFOA: liver hypertrophy (NJWQI, RIVM, ATSDR); foetal toxicity (US EPA, FSANZ)

2 Scope

RIVM was asked to screen the toxicity assessment of PFASs and to develop, if possible, a toxicological framework for the mixture exposure to these compounds. The substances of interest are presented in Table 2.1 (Arcadis, personal communication).

For the purpose of this work, a literature screening was performed with regard to basic information necessary to evaluate PFASs toxicity, i.e. the availability of animal toxicity data. Study information for PFOS and PFOA was not recorded, since they are the most studied members of the group and HBGVs are already in place for these compounds.

Table 2.1 PFASs found in environmental matrices

Substance	**Abbre-viation**	**CAS No**	**Molecular formula**
Perfluoroalkane sulfonic acids			
Perfluorobutane sulfonic acid	PFBS	375-73-5	$C_4F_9SO_3H$
Perfluoropentane sulfonic acid	PFPeS	375-92-8	$C_5F_{11}SO_3H$
Perfluorohexane sulfonic acid	PFHxS	355-46-4	$C_6F_{13}SO_3H$
Perfluoroheptane sulfonic acid	PFHpS	355-46-4	$C_7F_{15}SO_3H$
Perfluorooctane sulfonic acid	PFOS	375-39-8	$C_8F_{17}SO_3H$
Perfluorodecane sulfonic acid	PFDS	335-77-3	$C_{10}F_{21}SO_3H$
Perfluoralkane carboxylic acids			
Perfluorobutanoic acid	PFBA	357-22-44	C_3F_7COOH
Perfluoropentanoic acid	PFPeA	2706-90-3	C_4F_9COOH
Perfluorohexanoic acid	PFHxA	307-24-4	$C_5F_{11}COOH$
Perfluoroheptanoic acid	PFHpA	375-85-9	$C_6F_{13}COOH$
Perfluorooctanoic acid	PFOA	335-67-1	$C_7F_{15}COOH$
Perfluorononanoic acid	PFNA	375-95-1	$C_8F_{17}COOH$
Perfluorodecanoic acid	PFDA	335-76-2	$C_9F_{19}COOH$
Perfluoroundecanoic acid	PFUnDA	2508-94-8	$C_{10}F_{21}COOH$
Perfluorododecanoic acid	PFDoDA	307-55-1	$C_{11}F_{23}COOH$
Perfluorotridecanoic acid	PFTrDA	72629-94-8	$C_{12}F_{25}COOH$
Perfluorotetradecanoic acid	PFTeDA	376-06-7	$C_{13}F_{27}COOH$
Perfluorohexadecanoic acid	PFHxDA	67905-19-5	$C_{15}F_{31}COOH$
Perfluorooctadecanoic acid	PFODA	16517-11-6	$C_{17}F_{35}COOH$
Precursors			
Fluorotelomer sulfonic acid	6: 2 FTS	27619-97-2	$C_8H_5F_{13}SO_3$
Fluorotelomer sulfonic acid	8: 2 FTS	39108-34-4	$C_{10}H_5F_{17}SO_3$

3 Methodology

3.1 Literature search

Human toxicity
The reference lists of several scientific reports (CONCAWE, 2016; ATSDR, 2015; DEPA, 2015; Bull et al., 2014; US EPA, 2016a; 2016b), related to the toxicity of PFASs were used for the identification of critical studies up to the year 2016, when the latest report was published. In order to examine whether any additional data were generated on the toxicity of PFAS from 2016 onwards, a literature search was performed in two search engines: SCOPUS and PubMed.

The studies identified were screened for relevance, based on their title and abstract, with the use of specific criteria (exclusion/inclusion) (Table 3.1). The European Chemical Agency's (ECHA) Database was also searched for relevant toxicity information using the CAS numbers of each individual substance (Annex I).

Table 3.1 Toxicity study selection criteria during literature search.

Inclusion Criteria	**Exclusion criteria**
General criteria	
Articles identified in one of the selected study reports: ATSDR (2015), CONCAWE (2016), DEPA (2015), EFSA (2014)	
Articles in search databases published between the years 2015 and 2017	Articles identified in search databases and published earlier than 2015
Articles in English language	Articles in languages other than English
Experimental studies	Reviews
Specific criteria	
articles involving *in vivo* experimental studies in mammals	*In vitro* assays (e.g. mouse embryonic stem cell), studies on alternative organisms (e.g. zebrafish)
Studies that fall within the concept of standard toxicity testing	Examples: Studies aimed at examining only the underlying mechanism of toxicity; studies without sufficient dose levels tested; studies using non-standard animal strains
Studies with oral exposure (gavage, feeding, or via drinking water)	Articles performed by other routes of exposure, e.g. inhalation, dermal, intraperitoneal
Repeated dose toxicity studies: sub-acute, sub-chronic and chronic: 28 days/ 42 days/90 days or more	Acute or short-term (14 days or less) toxicity studies
Reproductive/developmental toxicity studies; one-generation, multi-generations, developmental toxicity	

Kinetic data were collected and recorded as referenced in the following documents: FSANZ, 2017; NJDWQI, 2016; US EPA, 2016a and 2016b; Zeilmaker et al., 2016.

Secondary poisoning
Additional data specifically focused on secondary poisoning in birds and mammals were searched as well. This search was conducted on birds in general and in mammalian wildlife species, such as the mink. No useful data were found for such species, in accordance with a recent literature search for PFOA (Verbruggen et al., 2017). Only for PFOS were some useful chronic bird studies available (Moermond et al., 2010). However, a search on the Internet revealed that some research projects are ongoing and that such data could be available in the near future. For now, it is assumed that criteria developed for human health will also be protective for the secondary poisoning endpoint. This assumption appeared to be valid for PFOS and PFOA in water (Moermond et al., 2010; Verbruggen et al., 2017).

3.2 Health-Based Guidance Value (PFOA)

The Health-Based Guidance Value (HBGV) is defined as the highest chronic, human daily intake (from food) which produces no adverse effects. The derivation of the HBGV often relies on the extrapolation of animal toxicity to man. In this extrapolation, several uncertainties are taken into account, among them interspecies differences in Absorption, Distribution, Metabolism and Elimination (ADME, hereafter referred to as kinetics). Traditionally, an Assessment Factor (AF) of (maximally) 4 is incorporated to account for interspecies differences in kinetics, also on PFASs (EFSA, 2008). However, for some PFASs, interspecies differences in kinetics grossly exceed a factor of 4, mainly due to the much slower removal from the human body compared with removal from the animal body (see Annex II). In the case of the PFASs, therefore, PFOA and PFOS interspecies differences in elimination kinetics in the interspecies extrapolation of animal toxicity were explicitly taken into account (ATSDR, 2015; NJDWQI, 2016; US EPA, 2016a and 2016b; FSANZ, 2017; RIVM, 2016). In concordance with this approach, RIVM based its HBGV for PFOA on the extrapolation of liver toxicity as observed in rats after semi-chronic exposure, i.e. daily for a period of 90 days. For this effect, a so-called No Observed Adverse Effect Level (NOAEL)[1] of 0.06 mg/kg bw/day was found, resulting in a HBGV of 12.5 ng/kg bw/day (for details, see Zeilmaker *et al.*, 2016).

3.3 Relative Potency Factor Approach

3.3.1 Principle

In the context of mixture toxicology, the combined toxicity of two or more substances may be based on the concept of dose-addition (EFSA, 2008, 2013). Substances can be seen as dose-additive when they act in *a similar manner with the same mechanism/mode of action,* but may differ only in their potencies. The concept stipulates that the total effect after simultaneous exposure to such compounds can be estimated from the sum of the doses or concentrations of each component, i.e. the

[1] The NOAEL is defined as the highest tested dose which does not show a statistically significant effect when compared with untreated controls.

substances behave as if they were a dilution of one another. Experimental studies conducted with mixtures have demonstrated that compounds which act on the same sub-system of an organism with a similar mode of action do follow the concept of dose-addition and can result in combined effects (EFSA, 2008, 2013; Kortenkamp et al., 2009). For the purposes of grouping pesticide residues in food in order to assess cumulative toxicity, EFSA employs the dose-addition concept for substances, inducing a common, toxicologically relevant phenomenological (specific) effect on organs/tissue systems, *irrespective of whether this is a result of the same mode of action or not* (EFSA, 2013). This decision was primarily based on experimental evidence (Kortenkamp *et al*. 2009, 2012; EFSA, 2013) that showed that combination effects may also occur through different modes/mechanisms of action. Up to now, EFSA has adopted an Opinion on the grouping of pesticides that induce toxicity on the nervous system and/or the thyroid/thyroid hormone system, where the phenomenological (specific) effects and their characterization are laid down (EFSA, 2013). A proposal for cumulative assessment groups for other organs/tissue systems (such as the liver, the reproductive/developmental system and the adrenals) was also presented by a joint Consortium ANSES/ICPS/RIVM (2016).

To predict the mixture toxicity, after the grouping of pesticides, EFSA proposes using the Relative Potency Factor (RPF) method (EFSA, 2012) that was used previously for compounds such as specific pesticides (organophosphorus compounds) (EFSA, 2008) and the dioxins (Toxic Equivalency Factor –TEF- method; Van den Berg et al., 2006). The RPF method normalizes the dose of each chemical, according to its potency, to an Index (reference) Compound (IC), with the IC having a RPF equal to 1. Combining the occurrence of each mixture component with its specific RPF value then expresses each of the mixture components in terms of IC equivalents. Summing up all mixture components then leads to a mixture exposure expressed in terms of IC equivalents. The latter then can be compared with IC HBGV, in this case RIVM's HBGV of PFOA.

3.3.2 RPF for PFASs

According to previous PFOA/PFOS risk assessments, liver toxicity (specific effect: liver hypertrophy) was found to be the most sensitive toxic endpoint. For this reason, the liver was selected as the prime target organ for other PFASs as well. Phenomenological effects on the liver, among them hepatic hypertrophy, were distinguished based on the work performed by Consortium ANSES/ICPS/RIVM (2016) for pesticides (see overview below).

Phenomenological effects defined for liver system (including biliary system and gall bladder) (Consortium ANSES/ICPS/RIVM (2016):

- Hepatocellular necrosis
- Hepatocyte cell degeneration
- Cytoplasmic inclusions
- Fatty changes
- ***Hepatic hypertrophy***
- Pigment
- Pigment porphyrin
- Inflammatory cells infiltrates
- Spongiosis

- Vascular lesion/Angiectasis
- Vascular lesion/Thrombosis
- Karyomegaly
- Foci of cellular alteration
- Regenerative hyperplasia/hyperplasia
- Hepatocellular neoplasms
- Cholestasis
- le duct hyperplasia
- Cholecystitis
- Choleliths
- Gallbladder hyperplasia
- Gallbladder neoplasms
- Extramedullary haematopoiesis

It is acknowledged here that *hepatic hypertrophy* is not necessarily an indicator of adversity *per se*. Nonetheless, progression into liver toxicity, characterized by vacuolization, eosinophilic hepatocytic granules and necrosis, is often observed. Given that hepatic hypertrophy is the sensitive endpoint on which the derivation of the HBGV for PFOA and PFOS were based, it was considered as a relevant effect for the comparison of potencies between the PFASs.

RPFs were derived as follows. Firstly, for each of the PFASs for which suitable toxicity data were available, a mathematical dose-response function was fitted to the data, i.e. absolute liver weight, relative liver weight (= liver weight divided by body weight) and liver hypertrophy (for details, see Annex IV).

In concordance with EFSA guidelines (EFSA, 2017) (continuous) liver weight data were analysed using the so-called Exponential and Hill models. However, as the fit to the data did not differ between the models, for practical reasons only the Exponential model was used for further analysis. The (quantal) liver hypertrophy data were analysed using a log-logistic model.

Secondly, the fitted dose-responses were used to calculate the so-called benchmark doses (BMDs) for each of the three mentioned effects. The BMD is the dose which results in a pre-set (acceptable) effect size or response, the benchmark response (BMR). In this report, a 5% increase in absolute or relative liver weight or a 10% extra risk in liver hypertrophy were used as BMR. In this way, for each PFAS, a BMD for absolute liver weight, a BMD for relative liver weight and a BMD for liver hypertrophy were calculated.

Thirdly, for each PFAS *i,* RPFs were calculated as the ratio of the BMD of PFAS *i* and the BMD of the IC PFOA:

$$RPF_i = \frac{BMD_{PFOA}}{BMD_i} \qquad \text{eq. 1}$$

BMDs provide an excellent starting point for a RPF derivation. The reason for this is that BMDs are equipotent doses. Because equipotent doses are required to ensure that the differences in the doses in the nominator and denominator of equation 1 are not caused by differences in the effect

(size) related to these doses, so they hold for the entire dose-response relationship. Note that this is one of the reasons why NOAELs or LOAELs are not suitable for deriving RPFs. Because NOAELs/LOAELS from different substances could relate to different effect levels (even somewhere below the detectable effect size of the experiment), i.e. NOAELs/LOELs do not reflect equipotent doses (Bokkers, 2007; Slob and Pieters, 1998).

4 Results

Overall, the literature search revealed that the available toxicological information is incomplete with regard to a toxicological evaluation leading to a HBGV derivation of individual PFAS compounds, with exceptions being PFHxA and the well-studied PFOS and PFOA. Nonetheless, sub-acute and sub-chronic oral studies in rats and mice were found for quite a few of the substances listed in Table 2.1, i.e. PFBS, PFHxS, PFHpA, PFBA, PFHxA, PFNA, PFDA, PFUnDA, PFDoA, PFTA, PFHxDA and PFODA. In addition, the endpoints for reproductive and/or developmental toxicology were examined in some cases. Only one standard chronic duration /carcinogenicity study was identified (PFHxA).

Notwithstanding the incompleteness of toxicity data, they were found sufficient to apply the RPF methodology to the liver toxicity of PFASs. Because, as mentioned, the application of the RTP method necessitates a common, toxicologically relevant phenomenological (specific) effect on organs/tissue systems of the PFASs of interest, preferably observed in repeated dose toxicity studies. For quite some PFASs, toxicity on the liver, characterized by hepatic hypertrophy, i.e. enlargement of the liver in combination with histopathology, has been reported at low doses. Furthermore, liver hypertrophy, in most cases, was found to be the critical effect (PFOS/PFOA). Exceptions were the PFHxS, which induced decreased cholesterol levels at a lower dose, and the PFDOA, with haematological and biochemical changes measured prior to hypertrophy (see Annex III, Table A3). Other effects often observed at the same dose level with hepatic hypertrophy were hepatic necrosis, anaemic symptoms (decreased erythrocyte count, haemoglobin and haematocrit), and thyroid hyperplasia. Reproductive toxicity is also often observed as a result of *in utero* exposure during gestation, commonly manifested by decreased body weight and body weight gain (foetus, offspring), as well as with litter loss. Reproductive toxicity was not considered further in this report.

The selected common phenomenological effect for the application of the RPF methodology was *liver toxicity, as revealed by liver hypertrophy (hepatocellular, centrilobular) and accompanying liver enlargement, i.e. absolute and relative liver weight.* Consequently, a BMD analysis for these effects was performed for all of the above-mentioned 12 PFASs.

As an example, Figure 4.1 shows the results of the BMD analysis for relative liver weight as induced in male rats after semi-chronic exposure to PFOA and PFOS.
As shown for PFOA, a BMD of 0.33 mg/kg bw/day was found, whereas the BMD for PFOS amounted to 0.16 mg/kg bw/day. Taking the BMD of PFOA as a reference, these BMDs result in a RPF of PFOS of 0.33/0.16 = 2.1, rounded to 2 (one significant digit) for the toxic endpoint relative liver weight in the (male) rat.

The BMD analysis for absolute liver weight resulted in a RPF of PFOS of 1.8, rounded to 2, (for details, see Annex IV). Note that, in the absence of suitable data on liver hypertrophy, a RPF for this effect could not be determined for PFOS.

In concordance with PFOA and PFOS, a BMD analysis was performed for the other ten PFASs as well (for details, see Annex IV). Figure 4.2 presents an overview of the RPFs and their uncertainty obtained from this analysis.

Figure 4.2 shows that RFPs for absolute and relative liver weight were found to be quite similar, whereas RPFs based on hypertrophy were below that of liver weight.
Since the set of RPFs from relative liver weight provides the most complete data set, the RPFs for this endpoint were further used for calculating PFOA equivalents (see Table 4.1). PFBS, PFBA and PFHxA showed relatively low RPFs, i.e. RPFs being one to three orders of magnitude lower than PFOA, possibly as a result of their higher aqueous solubilities. This probably stems from the rather efficient removal of these compounds from the rat body, with elimination half-lives being in the order of several hours, as compared with an elimination half-life of around two days for PFOA. In contrast, PFASs showing accumulating properties in the rat, such as PFHxS, PFOS, PFNA and PFUnDA, have RPFs well exceeding that of PFOA. In the (male) rat, these PFAS have elimination half-lives of 20-40 days (see Annex II), as compared with, for example, 20 days for the dioxin 2378-TCDD, a well-known bioaccumulating compound in the rat. Curiously, PFTA (C14) shows a lower RPF than PFOA, with the higher chain lengths PFHxDA (C16) and PFoDA (C18) showing an even lower RPF. This effect probably stems from a low absorption level in the gastrointestinal tract.

As shown in Table 4.1, for 12 of the 19 PFASs of interest, RPFs could be derived for liver toxicity. This inevitably necessitates making assumptions concerning the RPFs of the remaining 7 PFASs (see Table 4.2). For example, in the case of PFPeS, it is assumed that, based on carbon chain length, its RPF is above the RPF of PFBS, i.e. 0.001, and below the RPF of PFHxS, i.e. 0.6.

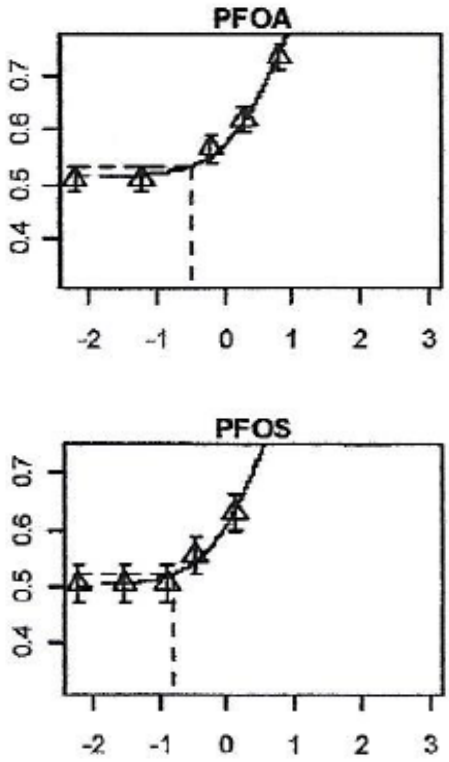

Figure 4.1 BMD analysis of the increase in relative liver weight as induced by PFOA and PFOS after semi-chronic dietary exposure in male rats.

PFOA doses: 0, 0.06, 0.64, 1.94 and 6.50 mg/kg bw/day for 91 days.
PFOS doses: 0, 0.03, 0.13, 0.34 and 1.33 mg/kg bw/day for 98 days.
X-axis: log10 daily dose (mg/kg bw/day); Y-axis: log10 relative weight;

Solid line: fitted exponential dose-response function.
Dashed line: BMR on y-axis corresponding with BMD on x-axis

BMDPFOA: 0.33 mg/kg bw/day (90% CI: 0.24 -0.46)
BMDPFOS: 0.16 mg/kg bw/day (90% CI: 0.11 -0.23)
Note the relatively small uncertainty in the BMD, resulting from the rather good dose-response information in the available data.

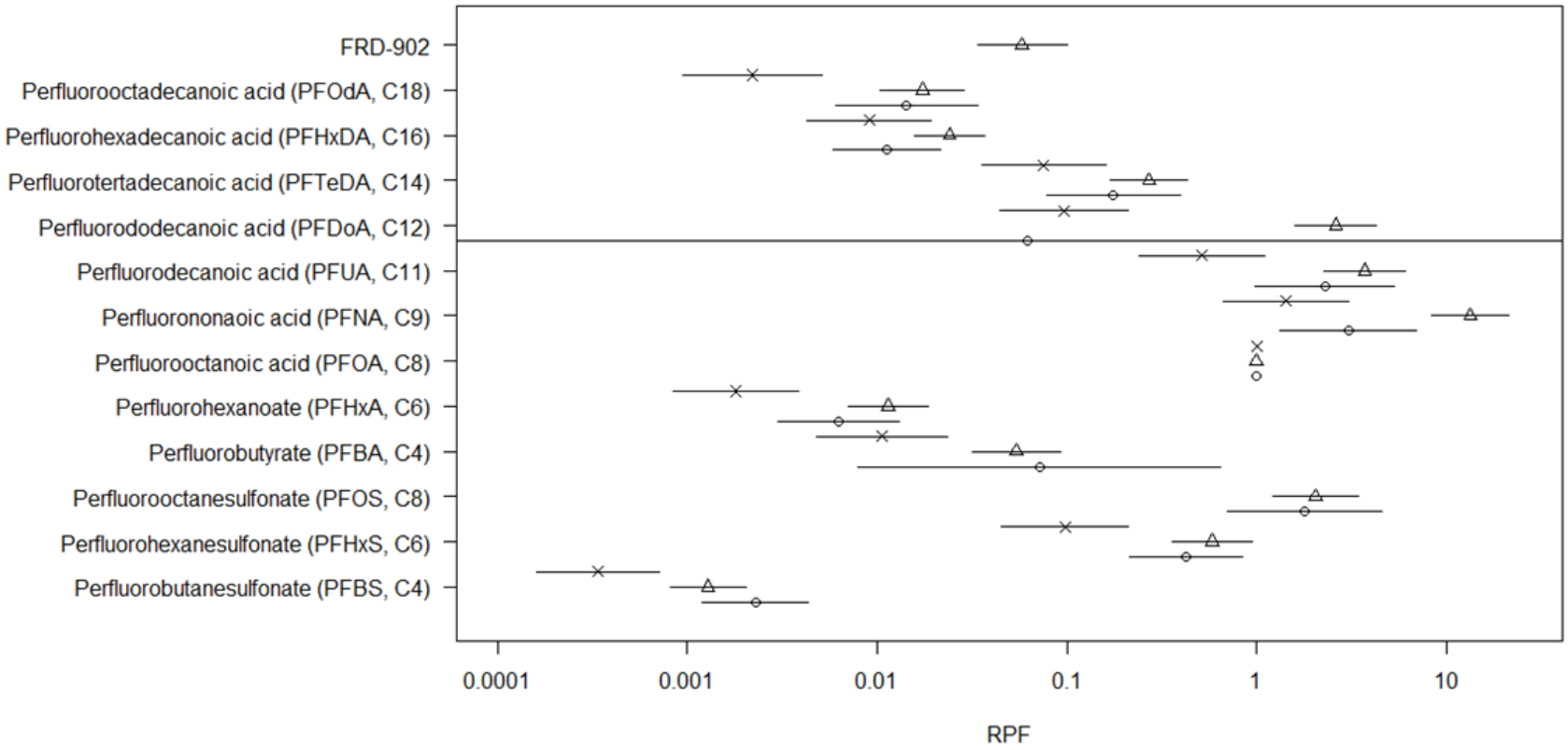

Figure 4.2 RPFs (and 90% CI) for PFASs. PFOA is used as a reference compound (RPF=1). For each PFAS, three RPFs are derived: circles, triangles, X correspond to RPFs based on absolute liver weight, relative liver weight and hypertrophy respectively. For PFOS, no suitable hypertrophy data were available. PFDoA does not show a dose-response in the absolute liver weight data, resulting in a very wide confidence interval.

Table 4.1 RPFs for 12 PFASs based on semi-chronic liver toxicity in male rats. Endpoint: relative liver weight.

Congener	**RPF**
Perfluorobutanesulfonate (PFBS, C4)	0.001
Perfluorohexanesulfonate (PFHxS, C6)	0.6
Perfluorooctanesulfonate (PFOS, C8)	2
Perfluorobutanoic acid (PFBA, C4)	0.05
Perfluoropentanoic acid (PFHxA, C6)	0.01
Perfluorooctanoic acid (PFOA, C8)	1
Perfluorononaoic acid (PFNA, C9)	10
Perfluoroundecanoic acid (PFUnDA, C11)	4
Perfluorododecanoic acid (PFDoDA, C12)	3
Perfluorotetradecanoic acid (PFTeDA, C14)	0.3
Perfluorohexadecanoic acid (PFHxDA, C16)	0.02
Perfluorooctadecanoic acid (PFODA, C18)	0.02

Table 4.2 As Table 4.1, including read across (marked bold) of seven additional PFASs.

Congener	**RPF**
Perfluorobutanesulfonate (PFBS, C4)	0.001
Perfluoropentane sulfonic acid (PFPeS, C5)	**0.001 ≤ RPF ≤ 0.6**
Perfluorohexanesulfonate (PFHxS, C6)	0.6
Perfluoroheptane sulfonic acid (PFHpS, C7)	**0.6 ≤ RPF ≤ 2**
Perfluorooctanesulfonate (PFOS, C8)	2
Perfluorodecane sulfonic acid (PFDS, C10)	**2**
Perfluorobutyrate (PFBA, C4)	0.05
Perfluoropentanoic acid (PFPeA, C5)	**0.01 ≤ RPF≤ 0.05**
Perfluorohexanoate (PFHxA, C6)	0.01
Perfluoroheptanoic acid (PFHpA, C7)	**0.01 ≤ RPF≤ 1**
Perfluorooctanoic acid (PFOA, C8)	1
Perfluorononaoic acid (PFNA, C9)	10
Perfluorodecanoic acid (PFDA, C10)	**4 ≤ RPF≤ 10**
Perfluoroundecanoic acid (PFUnDA, C11)	4
Perfluorododecanoic acid (PFDoDA, C12)	3
Perfluorotridecanoic acid (PFTrDA, C13)	**0.3 ≤ RP F≤ 3**
Perfluorotetradecanoic acid (PFTeDA, C14)	0.3
Perfluorohexadecanoic acid (PFHxDA, C16)	0.02
Perfluorooctadecanoic acid (PFODA, C18)	0.02

5 Example calculations for drinking water and soil

The RTFs mentioned in Table 4.2 can be used to convert a PFAS mixture into exposure equivalents of the IC PFOA. As examples, the occurrence of PFASs in drinking water and soil is presented here.

5.1 Drinking water

Zafeiraki *et al.* (2015) quantified 11 PFASs (PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFBS, PFHxS, PFHpS, PFOS) in drinking water in 37 locations in the Netherlands, among them the city of Dordrecht.

The applied analytic method consisted of LC-MS/MS spectroscopy with a limit of detection (LOD) of 0.2 ng/L (3 times the signal-to-noise ratio). The limit of quantification (LOQ) was accordingly determined at 0.6 ng/l (10 times the signal-to-noise ratio). Recoveries ranged between 85% and 115% for all the mass-labelled compounds except for the ^{13}C-PFUnA (60%–80%).
As shown in Table 5.1, five PFASs exceeded the LOQ, i.e. PFBS, PFPA, PFHxA, PFHpA and PFOA. When expressed in PFOA equivalents, this concentration is lower than 24.1 ng/L, as compared with the drinking water limit of PFOA in the Netherlands of 87.5 ng/L. PFOA accounted for 19 to 96% of the PFOA equivalents and PFOS for 0 to a maximum of 26%.

Table 5.1 The occurrence of PFASs in drinking water in Dordrecht (after Zafeiraki et al., 2015) and its corresponding PFOA equivalent concentration (ng/L).

Substance	**Concentration (ng/L)**	**RPF**	**PFOA equivalents (PEQ, ng/L)[1]**
PFBS	3.0	0.001	0.003
PFHxS	< 0.6	0.6	<0.36
PFHpS	< 0.6	0.6 ≤RPF≤ 2	<0.36 ≤PEQ≤1.2
PFOS	< 0.6	2	< 1.2
PFPeA	10.4	0.01 ≤RPF≤ 0.05	0.10 ≤PEQ≤0.52
PFHxA	4.1	0.01	0.041
PFHpA	1.8	0.01 ≤RPF≤ 1	0.02 ≤PEQ≤1.8
PFOA	4.5	1	4.5
PFNA	< 0.6	10	< 6
PFDA	< 0.6	4 ≤RPF≤ 10	<2.4 ≤PEQ≤ 6
PFUnDA	< 0.6	4	<2.4
Sum of PFASs	23.8-27.4		Lower bound[2]: 4.7 - 17.4 Upper bound[3]: 7.0 - 24.0

[1]values below the LOQ set at the LOQ ("worst case" approach); [2] based on the lowest RPF value for compounds within the RPF range; [3]based on highest RPF value for compounds within the RPF range

Applying improved analytics, Gebbink *et al.* (2017) recently extended the findings of the Zafeiraki study. Again, drinking water in the city of Dordrecht was measured, with the PFOA equivalent concentration being lower than 10.2 ng/L (see Table 5.2).

Table 5.2 The occurrence of PFASs in drinking water in Dordrecht (after Gebbink et al., 2017) and its corresponding PFOA equivalent concentration (ng/L).

Substance	**Concentration (ng/L)**	**RPF**	**PFOA equivalents (PEQ, ng/L)[1]**
PFBS	3.4	0.001	0.003
PFHxS	0.43	0.6	0.26
PFHpS	0.03	0.6 ≤RPF≤ 2	0.02 ≤PEQ≤0.06
PFOS	0.41	2	0.82
PFBA	< 2	0.05	< 0.10
PFPeA	5.7	0.01 ≤RPF≤ 0.05	0.06≤PEQ≤0.29
PFHxA	4.7	0.01	0.047
PFHpA	2.1	0.01 ≤RPF≤ 1	0.02 ≤PEQ≤2.1
PFOA	2.2	1	2.2
PFNA	0.25	10	2.5
PFDA	0.06	4 ≤RPF≤ 10	0.24 ≤PEQ≤0.6
Sum of PFASs	19.3- 21.3		Lower bound[2]: 6,2- 6.3 Upper bound[3]: 8.9- 9.0

[1]values below the LOQ set at the LOQ ("worst case" approach); [2] based on the lowest RPF value for compounds within the RPF range; [3]based on the highest RPF value for compounds within the RPF range

5.2 Soil

Table 5.3 and Table 5.4 show the PFASs concentrations in two soil samples as provided by Arcadis. When expressed in PFOA equivalents, these concentrations are lower than 406 resp 13.4 µg/kg d.m. These levels can be compared to risk limits for humans in soil. The current human risk limits for PFOA in soil for different land use scenarios are 86 µg/kg (for vegetable gardens) for d.m. up to 4,200 µg/kg d.m. The limitation is that the fate of the compounds, except for PFOS and PFOA, is not known. A comparison of the PFOA equivalents with the risk limits of PFOA therefore is only an indication and does not take into account the big differences, e.g. in uptake by plants (vegetable consumption) and volatilization to indoor air (inhalation).

Table 5.3 The occurrence of PFASs in soil (data supplied by Arcadis and its corresponding PFOA equivalent concentration (µg/kg dry matter).

Substance	**Concentration (µg/kg d.m.)**	**RPF**	**PFOA equivalents (PEQ, µg/kg d.m.)[1]**
PFBS	28	0.001	0.28
PFHxS	30	0.6	18
PFOS	190	2	380
PFDS	1.4	2	2.8
PFBA	< 0.10	0.06	0.006
PFPeA	1.0	0.01 ≤RPF≤ 0.05	0.01 ≤PEQ≤ 0.05
PFHxA	12	0.01	0.12
PFHpA	0.19	0.01 ≤RPF≤ 1	0.0019 ≤PEQ≤0.19
PFOA	1.3	1	1.3
PFNA	< 0.10	10	< 1.0
PFDA	< 0.10	4 ≤RPF≤ 10	0.4 ≤PEQ≤ 1.0
PFUnDA	< 0.10	4	< 0.4
PFDoDA	< 0.10	3	< 0.3
PFTrDA	< 0.10	0.3 ≤RPF≤ 3	0.03 ≤PEQ≤ 0.3
PFTeDA	< 0.10	0.3	< 0.03
PFHxDA	< 0.10	0.02	<0.002
PFODA	< 0.10	0.02	< 0.002
Sum of PFASs	263,9 - 264.8		Lower bound[2]: 402,5- 404,4 Upper bound[3]: 402,5- 405,5

[1]values below the LOQ set at the LOQ ("worst case" approach); [2] based on the lowest RPF value for compounds within the RPF range; [3]based on the highest RPF value for compounds within the RPF range

Table 5.4 The occurrence of PFASs in soil (data supplied by Aracadis) and its corresponding PFOA equivalent concentration (µg/kg dry matter).

Substance	**Concentration (µg/kg d.m.)**	**RPF**	**PFOA equivalents (PEQ, µg/kg d.m.)[1]**
PFBS	< 0.10	0.001	< 0.0001
PFHxS	< 0.10	0.6	< 0.06
PFOS	2.6	2	5.2
PFDS	< 0.10		
PFBA	< 0.10	0.06	< 0.06
PFPeA	< 0.10	0.01 ≤RPF≤ 0.05	0.001 ≤PEQ≤ 0.005
PFHxA	< 0.10	0.01	< 0.001
PFHpA	0.19	0.01 ≤RPF≤ 1	0.0019 ≤PEQ≤0.19
PFOA	4.8	1	4.8
PFNA	< 0.10	10	< 1.0
PFDA	< 0.10	4 ≤RPF≤ 10	0.4 ≤PEQ≤1
PFUnDA	< 0.10	4	< 0.4
PFDoDA	< 0.10	3	< 0.3
PFTrDA	< 0.10	0.3 ≤RPF≤ 3	0.03 ≤PEQ≤0.30
PFTeDA	< 0.10	0.3	< 0.03
PFHxDA	< 0.10	0.02	< 0.002
PFODA	< 0.10	0.02	< 0.002
Sum of PFASs	7.6- 9.0		Lower bound[2]: < 10-12.3 Upper bound[3]: < 10-13.4

[1]values below the LOQ set at the LOQ ("worst case" approach); [2] based on the lowest RPF value for compounds within the RPF range; [3]based on the highest RPF value for compounds within the RPF range

6 Discussion

Available Database
In 2016, RIVM derived a HBGV for PFOA on the basis of a semi-chronic, i.e. 91-day, dietary exposure toxicity study in the (male) rat. Clearly, to derive RPFs using PFOA as an IC warrants the availability of such studies for other PFASs as well.
A literature study revealed semi-chronic toxicity studies to be available for PFBA, PFOS, PFBA, PFHxA and PFNA, whereas 42-day exposure studies were found for PFHxS, PFUA, PFDoA, PFTeDA, PFHxDA and PFOdA. Regarding the toxicity endpoint of interest, i.e. relative liver weight, the latter studies might be used for the derivation of RPFs as well. The reason for this is that, in the rat, the maximum increase in relative liver weight by PFOA is observed already after 1 week of exposure to remain stable at longer exposure duration (Elcombe *et al.*, 2010). Furthermore, the dose-response characteristics for this effect are comparable after sub-acute, i.e. 28 day, and semi-chronic, i.e. 91-day, exposure (Loveless *et al.*, 2008; Perkins *et al.*, 2004).

Assumption of dose-addition
The RPF approach taken rests on the assumption of dose-addition, i.e. the absence of any interaction between mixture congeners in inducing liver toxicity. Verifying this assumption requires the availability of toxicity studies in which mixture toxicity is directly compared with that of its constituting congeners. Unfortunately, such studies are not available for PFASs. Therefore, for the time being, the assumption made concerning the dose addition of PFAS congeners still needs to be verified.
Nevertheless, US EPA (2016a,b) considered PFOA and PFOS equipotent, whereas this report indicates a RPF of 2 for PFOS. This difference has a methodological foundation. Whereas both US EPA and this report based their potency ranking on the extrapolation of similar animal toxicity to man, the former based this extrapolation on the observed animal NOAEL, whereas the latter used a BMD modelling approach instead. As mentioned before, using the NOAEL for this purpose introduces unnecessary uncertainty in potency-ranking. For this reason, the referred BMD method is preferred over the NOAEL in scaling the toxic potency of PFASs and, hence, PFOA and PFOS are not considered equipotent congeners.

Neglecting PFAS precursors
In this report, PFOA equivalents are calculated for a mixture of PFAS congeners, while neglecting the conversion of environmental PFAS precursors to these congeners. The extent to which this introduces uncertainty in the calculation of PFOA equivalents depends on the occurrence of the precursors in the media of interest, i.e. Dutch surface/drinking water and soil, as well as the efficiency with which such precursors are converted into PFAS congeners for which a RPF is available. However, though systematically addressing this uncertainty was not feasible within the scope of the current project, the indicative monitoring results may raise immediate concerns in some cases. For example, examining the presented soil concentrations in Table 5.4, the precursors 6:2 fluorotelomere sulfonate (6:2 FTS), 8:2 fluorotelomere sulfonate (8:2 FTS) and 10:2 fluorotelomere sulfonate (10:2 FTS) were

found to be lower than 10 µg/kg dw (data not shown). So, when complete conversion of these precursors up to the detection limit into PFOS or PFOA would occur, this would have a significant contribution to the occurrence of these compounds already present.

GenX product FRD-902/-903
Next to PFOA in 2016, RIVM derived a HBGV for the PFAS GenX product FRD-902/-903 (Beekman *et al.*, 2016). This derivation was based on a two-year chronic toxicity study (with interim kills after one year) in the rat which revealed a NOAEL of 0.1 mg/kg bw/day for the disturbance of the serum ratio Albumin/Globulin and a NOAEL of 1 mg/kg bw/day for liver toxicity (absolute and relative liver weight). Liver effects are also found in other GenX studies, among them a sub-chronic study (Haas *et al.*, 2009). Based on the latter study, a RPF of 0.06 was derived for FRD-902/-903 (see Figure 4.2).

Estimating human toxicity
The RPFs presented in this report are based on the extrapolation of animal toxicity to man. This is in concordance with several international scientific agencies that have evaluated, but not used, epidemiological/human studies for the derivation of a HBGV (ATSDR, 2015; FSANZ, 2017; NJDWQI, 2016; US EPA, 2016a; Zeilmaker *et al.,* 2016). Here it should be kept in mind that this approach is currently under review by the UmweltBundesAmt (UBA, Germany) (http://www.umweltbundesamt.de/themen/gesundheit/kommissionen-arbeitsgruppen/kommission-human-biomonitoring/beurteilungswerte-der-hbm-kommission, addressed 19-10-2017) and EFSA's Working Group of PFASs (in press).

Interspecies extrapolation of animal RPFs
The RPFs presented here are defined at the level of the external exposure in the rat, i.e. the administered dose. As shown, RPFs for PFBS, PFBA, PFHxA and GenX were found to be much, much lower than that for PFOA. In the case of PFBS, PFBA, PFHxA and GenX, this difference can be explained entirely by differences in toxicokinetics, with fast elimination kinetics leading to equipotency with PFOA at the level of the serum, i.e. equal serum levels of these compounds induce the same level of hepatic toxicity as PFOA (Gomis *et al.*, 2018; note that this observation strongly suggests that the PFBS, PFBA, PFHxA, GenX and PFOS induce hepatic toxicity via one common mechanism). Because, PFBS, PFBA and PFHxA in the rat as well as in humans are eliminated much more rapidly than and to the same extent as PFOA (see Annex II, Table A2), it has been concluded that the derived RPFs of these compounds hold for humans too.

Similarly, PFHxS, PFOS, PFNA, PFUnDA, PFDoDA and PFTeDA show a similar or even higher RPF than PFOA. This coincides with the even more persistent behaviour of these congeners in the rat when compared with PFOA (see Annex II, Table A2). Because such kinetic characteristics are paralleled in man, it has been concluded that, as a first tier, the derived RPFs of these compounds hold for humans as well.

By analogy, a quite persistent behaviour, with correspondingly high RPF, is expected for PFHxDA and PFODA. However, the contrary was found to

be the case, most probably due to the negligible absorption of these two congeners. Again, this is expected to hold for humans too.

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Annex I Information from ECHA database

Table A1 Information as collected from ECHA's database on each individual PFAS. The search was performed with the CAS and EC Numbers.

Substance	Abbreviation	CAS No	Information from ECHA's database
Perfluoroalkane sulfonic acids			
Perfluorobutane sulfonic acid	PFBS	375-73-5	under PACT-RMOA[2], Norway, evaluation is ongoing
Perfluoropentane sulfonic acid	PFPeS	375-92-8	no data
Perfluorohexane sulfonic acid	PFHxS	355-46-4	Under evaluation (or evaluated) as a substance of very high concern
Perfluoroheptane sulfonic acid	PFHpS	355-46-4	Annex III inventory, pre- registered under REACH
Perfluorooctane sulfonic acid	PFOS	375-39-8	
Perfluorodecane sulfonic acid	PFDS	335-77-3	Annex III inventory, pre- registered under REACH
Perfluoralkane carboxylic acids			
Perfluorobutanoic acid	PFBA	357-22-44	Not found
Perfluoropentanoic acid	PFPeA	2706-90-3	Annex III inventory, pre- registered under REACH
Perfluorohexanoic acid	PFHxA	307-24-4	Annex III inventory, pre-registered under REACH, PACT (under evaluation by Germany)
Perfluoroheptanoic acid	PFHpA	375-85-9	Annex III inventory, pre-registered under REACH
Perfluorooctanoic acid	PFOA	335-67-1	SVHC, Candidate list, Annex III inventory
Perfluorononanoic acid	PFNA	375-95-1	SVHC, Candidate list, Annex III inventory
Perfluorodecanoic acid	PFDA	335-76-2	SVHC, Candidate list, Annex III inventory
Perfluoroundecanoic acid	PFUnDA	2508-94-8	Substance of very high concern (SVHC) and included in the candidate list for authorization
Perfluorododecanoic acid	PFDoA	307-55-1	SVHC, Candidate list, Annex III inventory
Perfluorotridecanoic acid	PFTrA	72629-94-8	Substance of very high concern (SVHC) and included in the candidate list for authorization.

[2] The Public Activities Coordination Tool (PACT) lists the substances for which a risk management option analysis (RMOA) or an informal hazard assessment for PBT/vPvB (persistent, bioaccumulative and toxic/very persistent and very bioaccumulative) properties or endocrine disruptor properties is either under development or has been completed since the implementation of the SVHC Roadmap commenced in February 2013.

Substance	Abbreviation	CAS No	Information from ECHA's database
Perfluorotetradecanoic acid	PFTA	376-06-7	Substance of very high concern (SVHC) and included in the candidate list for authorization.
Perfluorohexadecanoic acid	PFHxDA	67905-19-5	Annex III inventory, pre-registration
Perfluorooctadecanoic acid	PFODA	16517-11-6	Annex III inventory
Fluorotelomer sulfonamide	6: 2 FTS	27619-97-2	Annex III inventory, pre-registration
Fluorotelomer sulfonamide	8: 2 FTS	39108-34-4	Annex III inventory, pre-registration

Annex II Kinetic terminal half-lives for PFAS

Table A2 Kinetic terminal half-lives for PFASs.

Substance	Species/Terminal half- life				
	Rat	**Mouse**	**Pig**	**Monkey**	**Humans**
PFBS	4.51 h (males) 3.96 h (females) (Olsen *et al*., 2009)	4.51 h (Olsen *et al*., 2009)	Pig: 43 d (Numata *et al*., 2014)	4 d (males) 3.5 d (females) (Olsen *et al*., 2009)	GM 25.8 d (6 subjects) (Olsen *et al*., 2009)
PFHxS	29.1 d (males) 1.64 d (females) (Sundström *et al*., 2012)	31 d (males) 25 d (females) (Sundström *et al*., 2012)	713 d (Numata *et al*., 2014)	141 d (males) 87 d (females) (Sundström *et al*., 2012)	Occupational workers: AM 8.5 y (males) GM 7.3 y (females) (Olsen *et al*. 2007)
PFHpS	-	-	411 d (Numata *et al*., 2014)	-	-
PFOS	27.8 days (males) 24.8 days(females) (Kim *et al*., 2016)	42.8 d (males) 37.8 d (females) (Chang *et al*., 2012)	634 d (Numata *et al*., 2014)	132 days (males) 110 days (females) (Chang *et al*., 2012)	Occupational workers: 5.4 years (Olsen *et al*. 2007)
PFBA	6.4-9.2 h (males) 1.0-1.8 h (females) (Chang *et al*., 2008)	5.2-16 h (males) 2.8-3.1 h (females) (Chang *et al*., 2008)	-	40.3 h (males) 41.0 h (females) (Chang *et al*., 2008)	Occupational workers: AM 64.8 h (males) AM 81.6 h (females) (Chang *et al*., 2008)
PFHxA	1.0 - 2.8 h (males) 0.4-2.7 h (females) (Chengelis *et al*., 2009)	-	4.1 d (Numata *et al*., 2014)	14-47 h (Russel *et al*., 2013)	GM 32 d (Russel *et al*., 2013)
PFHpA		2.4 h (males) 1.2 h (females) (Ohmori *et al*., 2003)	74 d (Numata *et al*., 2014)	-	GM 0.82 y (Zhang *et al*., 2013)

Substance	Species/Terminal half- life				
	Rat	**Mouse**	**Pig**	**Monkey**	**Humans**
PFOA	1.6-1.8 d (males) 0.15-0.19 d (females) (Kim *et al*., 2016)	21.7 d (males) 15.6 d (females) (Lou *et al*., 2009)	236 d (Numata *et al*., 2014)	21 d (males) 30 d (females) (Butenhoff *et al*., 2004)	Occupational workers: AM 3.8 y (Olsen *et al*. 2007) Adults (contaminated drinking water): 2.3 y (Bartell *et al*., 2010), 3.3 y (Brede *et al*., 2010)
PFNA	24 d (males) 32 d (females) (Tatum-Gibbs *et al*., 2011)	131.2 d (males) 47.3 d (females) (Tatum-Gibbs *et al*., 2011)	-	-	GM 1.7-3.2 y (Zhang *et al*. 2013)
PFDA	40 d (males) 59 d (females) (Ohmori *et al*., 2003)	-	-	-	GM 4-7.1 y (Zhang *et al*. 2013)
PFUnDA	-	-	-	-	GM 4-7.4 y (Zhang *et al*. 2013)

Annex III. Summary of toxicity effects of PFAS

Table A3 Summary of toxicity effects of PFASs

PFASs	Guideline	Exposure duration	Animal	NOAEL adult	Critical specific effect (adult)	NOAEL F1	Critical specific effect (F1)	Remarks	Reference
PFBS	OECD 416	7 w prior mating, mating, + for females also gestation + lactation. F1 same exposure but starting at weaning (except the indirect). F2 in utero till lactation.	rat	100	hepatic hypertrophy, kidney hyperplasia	>1000	No effects; highest dose tested	-	Lieder et al., 2009b
	OECD 408	13 w	rat	60	anaemic effects, ↓ spleen weights	-	-	no effects on the liver	Lieder et al., 2009a
PFPeS	no relevant studies identified								
PFHxS	OECD 422	M: 43 d, F: 43 d + GD till PND21	rat	< 0.3	decreased serum cholesterol		-	-	Butenhoff et al., 2009b
				1	hepatic hypertrophy, thyroid hypertrophy/hyperplasia	> 10	No effects; highest dose tested	-	
PFHpS	no relevant studies identified								
PFOS	no guide-line followed	98 d (14 w)	rat	0.34	hepatic hypertrophy, hepatic centrilobular cytoplasmic vacuolisation, decreased serum cholesterol	-	-	-	Seacat et al., 2003

PFASs	Guideline	Exposure duration	Animal	NOAEL adult	Critical specific effect (adult)	NOAEL F1	Critical specific effect (F1)	Remarks	Reference
	no guide-line followed	182 d (26 w)	monkey	0.15	↓ survival, ↓ BW gain, ↑ liver wt; hepatic hypertrophy, ↓T3 and ↑TSH	-	-	-	Seacat et al., 2002
	no guide-line followed	6 w prior mating, mating, and for females also gestation/lactation/parturition for two generations	rat	0.1	decreased BW gain	0.1	effects on postnatal growth: decreased pup BW	-	Luebker et al. 2005b
	no guide-line followed	728 d (104 w)	rat	0.024	hepatic hypertrophy			-	Butenhoff et al. 2012b
PFDS	no relevant studies identified								
PFBA	no guide-line followed	13 w	rat	6	hepatic hypertrophy, thyroid hypertrophy, anaemic effects, ↓ cholesterol	-	-	no effects on females	Butenhoff et al., 2012
	no guide-line followed	GD 1-17	mouse	35	effects on gestation: ↑ full litter resorption	<35	effects on postnatal growth: delayed eye opening	-	Das et al., 2008
PFPeA	no relevant studies identified								
PFHxA	OECD 415	F: 70 d prior mating, GD + lactation (126 d); M: 110 d	rat	-	no sufficient information provided	100	effect on pre- and postnatal growth: ↓ pup weight	-	Loveless et al., 2009
	OECD 408	13 w	rat	20	hepatic hypertrophy, nasal lesions, ↑ liver & kidney weight	-	-	-	Loveless et al., 2009
	no guideline	13 w	rat	10	↓ cholesterol,	-	-	-	Chengelis et

PFASs	Guideline	Exposure duration	Animal	NOAEL adult	Critical specific effect (adult)	NOAEL F1	Critical specific effect (F1)	Remarks	Reference
	followed			50	hepatic hypertrophy, hepatic necrosis, ↑ liver weight, anaemic effects, ↑ ALT and ALP				al., 2009
	no guideline followed	104 w	rat	30	hepatic necrosis, kidney tubular degeneration & necrosis, anaemic effects	-	-	-	Klaunig et al., 2015
	OECD 414	GD 6-20	rat	-	-	100	Effect on prenatal development/ growth: ↓ foetal weight	-	Loveless et al., 2009
	no guide-line followed	GD 6-18	mouse	100	effects on bw (mortality?)	100	effects on pre- and postnatal growth: ↓ pup weight	combined dose level between 2 experi-mental phases; effects seen at maternal-ly toxic doses	Iwai et al., 2014
PFHpA	no relevant studies identified								
PFOA	no guide-line followed	13 w	rat	0.06	hepatic hypertrophy, ↑ liver weight		-	-	Perkins et al., 2004
PFNA	no guide-line followed	GD 1-17	mouse	5	↓ body weight gain	< 1	Effect on postnatal development/ growth: ↑ pup liver	-	Das et al., 2015

PFASs	Guideline	Exposure duration	Animal	NOAEL adult	Critical specific effect (adult)	NOAEL F1	Critical specific effect (F1)	Remarks	Reference
							weight		
Mixture : C6-C18; mainly C9	OECD 416	70 d prior mating, mating, + for females also gestation + lactation. F1 same exposure but starting at weaning (except the indirect). F2 in utero till lactation.	rat	< 0.025	hepatic hypertrophy, hepatic necrosis, liver fatty changes	F1 0.025 F2 0.125	Effect on postnatal development/ growth: ↑ pup liver weight	-	Stump et al., 2008
	OECD 408	13 w	rat	0.025	hepatic hypertrophy and liver foci of cellular alteration	-	-	-	Mertens et al., 2010
PFDA	no guide-line followed	GD 10-13 or GD 6-15	mouse	3	↓ body weight gain	0.3	Effect on prenatal development/growth: ↓ foetal weight	-	Harris et al., 1989
PFUnA	OECD 422	M 43 d, F 43 d + GD till PND21	rat	0.1	hepatic hypertrophy and necrosis, liver fatty changes, ↑ liver weight	0.3	effects on postnatal growth: ↓ pup weight	-	Takahashi et al., 2014
PFDoA	OECD 422	M 43 d, F 43 d + GD till PND21	rat	0.1	↑ liver weight, biochemistry, haematological changes	0.5	effect on postnatal general growth: ↓ pup	liver effects seen also	Kato et al., 2014

PFASs	Guideline	Exposure duration	Animal	NOAEL adult	Critical specific effect (adult)	NOAEL F1	Critical specific effect (F1)	Remarks	Reference
				0.5	hepatic hypertrophy and necrosis, liver inflammatory cell infiltrates and bilirubin deposition, adrenal and thymus cortex atrophy, bile duct hyperplasia, several organ weight changes, decreased haematopoiesis in the bone marrow, stomach/fore-stomach histopathology		weight	at 0.1, but not SS	
				0.5	effects on the reproductive system			-	
PFTeDA	OECD 422	M 43 d, F 43 d + GD till PND21	rat	1	hepatic hypertrophy, thyroid hypertrophy	3	effect on postnatal general growth/develop-ment: ↓ pup weight	-	Hirata-Koizumi et al., 2015
PFHxDA	OECD 422	M 43 d, F 43 d + GD till PND21	rat	4	hepatic hypertrophy, liver fatty changes, ↑ liver and thyroid weight	100/ no LOAEL	No effects; highest dose tested	-	Hirata-Koizumi et al., 2015
PFOcDA	OECD 422	M 43 d, F 43 d + GD till PND21	rat	40	hepatic hypertrophy, hepatic necrosis	200	Effect on prenatal and postnatal development/ growth	-	Hirata-Koizumi et al., 2012

Annex IV BMD analysis: Derivation of RPFs for PFAS

Introduction

The RPF approach was used to assess the cumulative risk of a mixture of per- and polyfluoroalkyl substances (PFASs). The RPF approach assumes that the mixture components (1) display similar toxicity; (2) differ only in potency (if so, their individual dose-response curves should be parallel on log-dose scale, because when the (log) dose-response curves are not parallel, differences in potency are not constant over the range of effects); (3) do not interact (Bosgra, 2009, van den Berg, 2000, 2006). Note that if biochemical interactions occur between the substances, the dose-response information of the individual substances is not, by definition, sufficient to predict their combined effects. No mixture experiments with PFASs are available and, hence, no information is available on the question of whether the combinations follow dose addition. However, in the absence of data, it was assumed that no interactions between PFASs take place. In that case, dose addition gives an accurate prediction of the cumulative effects if dose-response relationships can be described by parallel curves.
Here it is assumed that the mentioned requirements apply to (a mixture of) PFASs.

RPFs convert concentrations of each PFAS to the equivalent concentrations of one index substance. The choice of the index substance is arbitrary and should not influence the outcome of the risk assessment. Because RIVM has derived a HBGV for PFOA, this chemical was chosen as the index substance for a PFAS mixture (Zeilmaker et al., 2016).
By definition, the RPF of PFAS i is defined as the ratio of its benchmark dose (BMD, EFSA 2017) and that of the index PFAS (i.e. PFOA):

$$〖RPF〗_i = 〖BMD〗_{PFOA} / 〖BMD〗_i \qquad \text{eq. 1}$$

To derive RPFs, BMDs provide an excellent starting point. The reason for this is that BMDs are equipotent doses. Equipotent doses are required to ensure that the differences in the doses in the nominator and denominator of equation 1 are not caused by differences in the effect related to these doses. Note that this is one of the reasons why NOAELs or LOAELs are not suitable for deriving RPFs. This is because NOAELs from different substances could relate to different effect levels (somewhere below the detectable effect size of the experiment), i.e. NOAELs may not reflect equipotent doses (Bokkers, 2007; Slob and Pieters, 1998).

The BMDs and relative potencies of the PFASs are ideally obtained from the dose-response modelling of experimentally induced toxicity. In this context, PFASs are known to cause effects on the liver (though the mode of action remains unknown). Furthermore, in concordance with other international regulatory agencies (US EPA, ATSDR, FSANZ), in its derivation of a HBGV, RIVM selected liver toxicity as the most sensitive toxic effect for PFOA in experimental animals (NOAEL for increased absolute and relative liver weight after oral exposure: 0.06 mg/kg bw/day, Zeilmaker et al., 2016). For this reason, a database of liver

endpoints affected after oral exposure was created for all relevant PFASs (Table A1). To ensure that differences which are found are not related to differences in experimental setup, data were obtained from studies with similar experimental setups, e.g. same species, sex, comparable exposure duration (42-98 days), exposure route (van den Berg, 2006; Zeilmaker et al., 2016). The database includes summary data (mean and standard deviation per dose group) for absolute and relative liver weight on 12 PFASs. Incidence data on liver hypertrophy is included in the table for 11 PFASs. Unfortunately, quantitative data on hypertrophy are not available for a key substance, i.e. PFOS. After exposure to PFOS, histomorphologic changes were observed only in the livers of males given 0.34 or 1.33 mg/kg bw and in females at 1.6 mg/kg bw. The changes consisted of centrilobular hepatocytic hypertrophy and midzonal to centrilobular vacuolation. The incidence and severity of the changes tended to be greater in the 1.5 mg/kg bw dose-group of males (Seacat et al., 2003). For some PFASs, the severity of incidence (e.g. mild, moderate severe) was reported. This information was reduced to incidence data because not all studies reported severity and because the definition of the severity levels may differ between studies.

Dose-response modelling was used to verify whether the dose-response data of the PFASs considered can be described by parallel curves. In this, the exponential and Hill models (EFSA, 2017) were used to describe the (continuous) absolute and relative liver weight data. A set of models (EFSA, 2017) was fitted to the (quantal) liver hypertrophy data. The models were fitted to the data of all PFASs simultaneously by forcing the dose-response curves on log-dose scale to be parallel and by allowing the background (parameter a) and the potency (parameter b) to differ between PFASs. The BMDs corresponding to a benchmark response (BMR) of a 5% increase in the absolute and relative liver weight and to a 10% extra risk of liver hypertrophy were derived. It should be noted that the ratio of BMDs does not depend on the value of BMR in this model approach (Bokkers, 2005). The ratio can also be calculated by the ratio of the parameters b for both substances. Thus, the difference in potency between two substances can be expressed by a single factor that holds for any effect size in these models. The choice of BMR does influence the uncertainty of the BMD and, as a consequence, the uncertainty in the RPF. In general, the uncertainty in the BMD will be larger (i.e. larger BMDU/BMDL ratio) when the BMD lies relatively far outside the dose range or the BMR is too small. The analyses below show that, by using the chosen BMRs, large uncertainties in the BMDs are avoided.

Table A1: Rat dose-response data obtained from the literature.

Chemical	Reference[1]	Exposure Duration	Sex	External dose (mg/kg bw/day)	Liver hypertrophy		Absolute liver weight			Relative liver weight		
					Inci-dence	Total	Mean (g)	SD	Sample size	Mean (% of bw)	SD	Sample size
Perfluorobutanesulfonate (PFBS, C4).	Lieder, 2009b	70 days	male	0	0	30	19.2	2.4	30	3.4	0.3	30
				30	n.a.	n.a.	20.0	2.9	30	3.5	0.4	30
				100	n.a.	n.a.	20.5	2.4	30	3.6	0.3	30
				300	3	30	21.5	2.9	30	3.8	0.3	30
				1000	26	30	27.0	2.8	29	4.1	0.4	30
Perfluorohex-anesulfonate (PFHxS, C6)	Butenhoff, 2009b	42 days	male	0	0	10	15.30	0.15	10	3.12	0.03	10
				0.3	0	10	15.20	1.09	10	3.20	0.23	10
				1.0	0	10	16.41	2.02	10	3.42	0.42	10
				3.0	9	10	18.15	1.12	10	3.73	0.23	10
				10.0	10	10	24.26	3.33	10	5.25	0.72	10
Perfluorooctanesulfonate (PFOS, C8)	Seacat, 2003	98 days	male	0	NA		15.5	1.1	5	3.2	0.3	5
				0.03	NA		15.5	2.7	5	3.2	0.2	5
				0.13	NA		14.0	1.4	5	3.2	0.2	5
				0.34	NA		18.8	3.0	5	3.6	0.3	5
				1.33	NA		20.3	2.2	5	4.3	0.4	5
Perfluorobutyrate (PFBA, C4)	Butenhoff, 2012c	90 days	male	0	0	10	10.92	1.17	10	2.1	0.23	20
				1.2	0	10	11.40	9.75	10	2.1	0.14	10
				6	0	10	11.39	1.36	10	2.2	0.27	10
				30	9	10	13.41	2.01	10	2.6	0.39	20
Perfluorohexanoat	Loveless,	90 days	male	0	0	10	15.09	1.59	10	2.69	0.17	10

Chemical	Reference[1]	Exposure Duration	Sex	External dose (mg/kg bw/day)	Liver hypertrophy		Absolute liver weight			Relative liver weight		
					Inci-dence	Total	Mean (g)	SD	Sample size	Mean (% of bw)	SD	Sample size
e (PFHxA, C6)	2009											
				20	0	10	15.18	2.07	10	2.70	0.26	10
				100	4	10	16.49	2.38	10	3.00	0.23	10
				500	10	10	21.98	2.35	10	4.38	0.49	10
Perfluorooctanoate (PFOA, C8)	Perkins, 2004	91 days	male	0	0	15	16.46	1.42	15	3.24	0.28	15
				0.06	0	15	17.76	1.27	15	3.24	0.23	15
				0.64	15	15	20.33	1.76	15	3.69	0.32	15
				1.94	15	15	22.36	1.38	15	4.21	0.56	15
				6.50	15	15	27.17	4.15	15	5.50	0.84	15
Perfluorononaoic acid (PFNA, C9)	Mertens, 2010	91 days	male	0	0	10	13.03	1.80	15	2.50	0.10	15
				0.025	0	10	13.92	2.59	10	2.63	0.19	10
				0.125	4	10	16.14	3.19	10	3.12	0.31	10
				0.6	10	10	17.45	2.31	15	4.51	0.43	15
Perfluoroundecanoic acid (PFUA, C11)	Takahashi, 2014	42 days	male	0	0	7	15.12	2.14	5	2.88	0.27	5
				0.1	0	12	16.45	2.06	5	3.02	0.19	5
				0.3	3	12	17.54	0.73	5	3.39	0.16	5
				1.0	7	7	20.95	2.56	5	4.18	0.19	5
Perfluorododecanoic acid (PFDoA, C12)	Kato, 2014	42 days	male	0	0	7	12.0	1.3	5	2.51	0.14	5
				0.1	0	12	13.5	2.1	5	2.67	0.21	5
				0.5	0	12	14.7	2.6	5	3.00	0.30	5

Chemical	Reference[1]	Exposure Duration	Sex	External dose (mg/kg bw/day)	Liver hypertrophy		Absolute liver weight			Relative liver weight		
					Inci-dence	Total	Mean (g)	SD	Sample size	Mean (% of bw)	SD	Sample size
				2.5	5	7	13.6	3.1	5	4.30	0.27	5
Perfluorotetradecanoic acid (PFTeDA, C14)	Hirata-Koizumi, 2015	42 days	male	0	0	7	11.95	1.53	7	2.41	0.11	7
				1	0	12	12.09	0.73	7	2.49	0.12	7
				3	8	12	14.52	1.82	7	2.87	0.23	7
				10	7	7	15.21	0.53	7	3.25	0.07	7
Perfluorohexadecanoic acid (PFHxDA, C16)	Hirata-Koizumi, 2015	42 days	male	0	0	7	12.15	1.27	7	2.50	0.04	7
				4	0	12	11.81	0.55	7	2.45	0.10	7
				20	5	12	12.12	0.85	7	2.49	0.15	7
				100	7	7	14.50	0.61	7	3.26	0.07	7
Perfluorooctadeca-noic acid (PFOdA, C18)	Hirata-Koizumi, 2012	42 days	male	0	0	7	10.9	1.8	5	2.36	0.28	5
				40	0	12	11.3	1.6	5	2.48	0.25	5
				200	12	12	15.8	1.8	5	3.35	0.14	5
				1000	7	7	18.2	1.2	5	5.00	0.13	5
Ammonium 2,3,3,3-tetrafluoro-2-(hep-tafluoropropoxy)-propanoate (FRD-902)	Haas, 2009	90 days	male	0						2.716	0.1319	10

Chemical	Reference[1]	Exposure Duration	Sex	External dose (mg/kg bw/day)	Liver hypertrophy		Absolute liver weight			Relative liver weight		
					Inci-dence	Total	Mean (g)	SD	Sample size	Mean (% of bw)	SD	Sample size
				0.1						2.727	0.2125	10
				10						3.556	0.4752	10
				100						4.535	0.5144	10

[1]for references, see main text.

Results & discussion

ABSOLUTE LIVER WEIGHT (aLW)

Fitting models 1, 3 and 5 of the exponential model family and Hill model family shows that, for both model families, model 5 with covariates for a, b and the residual variance (v) provides a good description of the data, i.e. these models result in the lowest AICs (Tables A2 and A3). Chemical was used as the covariate. The significant improvement of the model fits by adding this covariate for parameters a and b, and the residual variation indicates that the studies with the various chemicals differ in background (parameter a), the chemicals differ in potency (parameter b) and the residual variance of the studies also differs. Figure 1 shows the fitted dose-response curves. Figure A2 shows the individual fits of the PFASs with the exponential model m5-abv, which shows a good description of the measured data. The Hill model resulted in similar individual plots (not shown). Figure A3 shows the fit of the exponential model after normalizing to background (parameter a). This figure and Figure 2 illustrate that fitting parallel curves to the PFAS data results in a good description of the data. The right panel of Figure 3 shows the resulting BMD_{05} confidence intervals, illustrating the good correspondence between the Hill and exponential model results. The actual BMD, BMDL and BMDU values are reported in the summary Table A7. The BMD confidence interval of PFDoA is rather wide (Figure A3, right panel and Table A7) because in none of the dose groups does the response seem to increase compared with the background response (Figure A2).

Table A2: ANALYSIS WITH EXPONENTIAL MODELS, Best model with covariates is the exponential model m5-abv.

Model	converged	npar	loglik	aic
full	1	53	266.72	-427.44
full-v	1	64	383.1	-638.2
m1-v	1	13	-48.02	122.04
m1-av	1	24	138.75	-229.5
m3-av	1	26	233.05	-414.1
m3-abv	1	37	354.39	-634.78
m5-av	0	27	232.03	-410.06
m5-abv	1	38	359.88	-643.76

Table A3: ANALYSIS WITH HILL MODELS, Best model with covariates is: Hill model m5-abv

model	converged	npar	loglik	aic
m3-av	1	26	234.02	-416.04
m3-abv	1	37	353.34	-632.68
m5-av	1	27	232.03	-410.06
m5-abv	1	38	359.49	-642.98

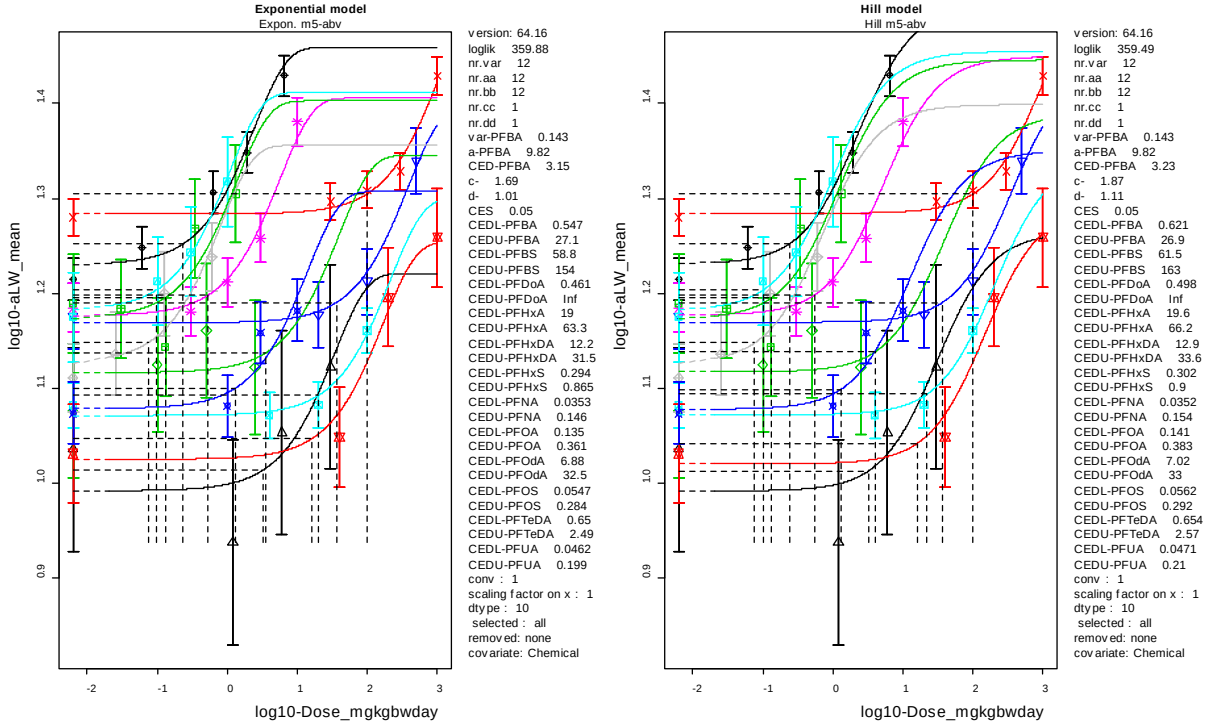

Figure A1. Fitted dose-response curves for each PFAS. Left and right panels show the fits with the exponential and Hill models, respectively.

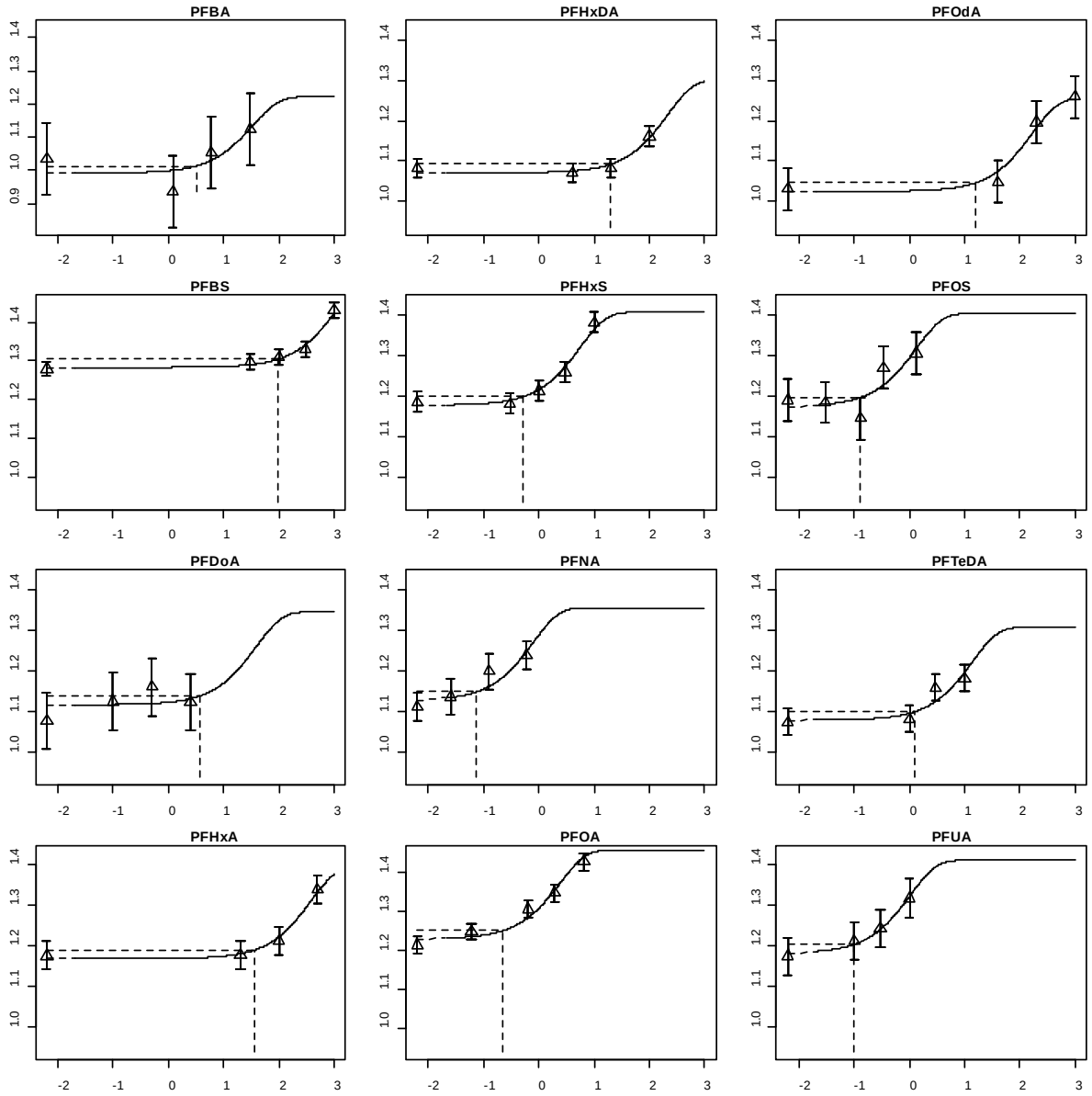

Figure A2. Individual fits of all PFASs with the exponential model m5-abv

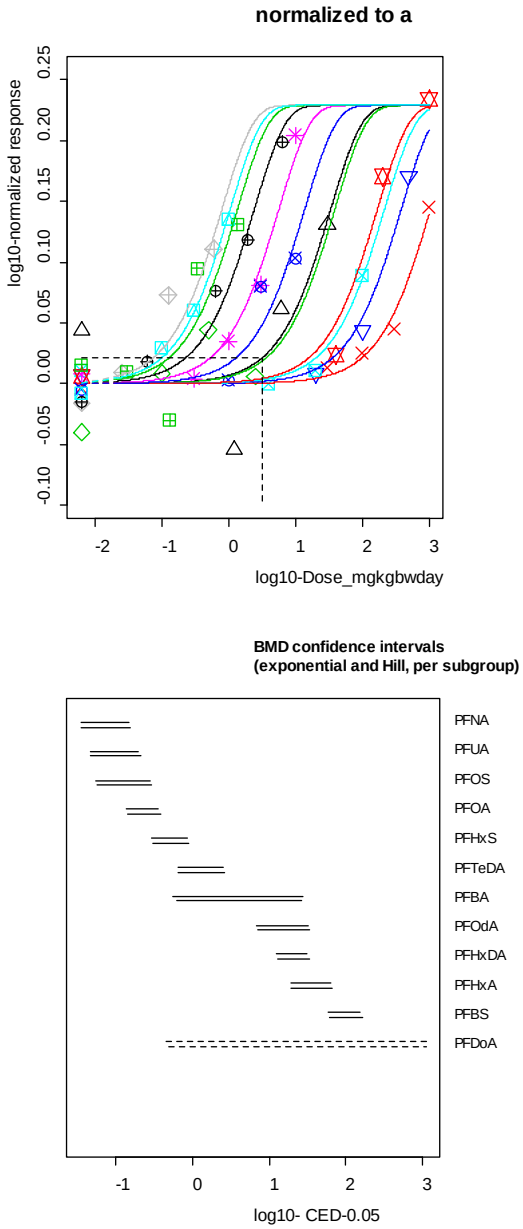

Figure A3. Top panel: fit of the exponential model after normalizing to background (parameter a). Lower panel: log10 BMD (95%-)confidence intervals, i.e. range BMDL to BMDU for each PFAS. BMR=5%. Within each PFAS, the upper line relates to the exponential model results and the bottom line corresponds to the Hill model results.

RELATIVE LIVER WEIGHT (rLW)

Fitting models 1, 3 and 5 of the exponential model family and Hill model family shows that, for both model families, model 5 with covariates for a and b, and the residual variance (v) provide a good description of the data, i.e. these models result in the lowest AICs (Table A4 and A5). Chemical was used as the covariate. The significant improvement of the model fits by adding this covariate for parameters a and b, and the residual variation indicates that the studies with the various chemicals differ in background (parameter a), the chemicals differ in potency (parameter b) and the residual variance of the studies also differs. Figure 4 shows the fitted dose-response curves. Figure 5 shows the individual fits of the PFASs with the exponential model m5-abv, which shows a good description of the measured data. The Hill model resulted in similar individual plots (not shown). Figure 6 shows the fit of the exponential model after normalizing to background. This figure and Figure A5 illustrate that fitting parallel curves to the PFAS data results in a good description of the data. The right panel of Figure A6 shows the resulting BMD_{05} confidence intervals, illustrating the good correspondence between the Hill and exponential model results. The actual BMD, BMDL and BMDU values are reported in Table A7. In contrast to the results of absolute liver weight, for relative liver weight the BMD confidence interval of PFDoA can be established and is relatively small (Fig 6, right panel and Table A7) because a clear dose-response is present (Figure A5).

Table A4. ANALYSIS WITH EXPONENTIAL. MODELS Best model with covariates is: Expon. m5-abv

Model	converged	npar	loglik	aic
Full	1	57	610.65	-1107.3
full-v	1	69	646.69	-1155.38
m1-v	1	14	68.07	-108.14
m1-av	1	26	217.35	-382.7
m3-av	1	28	314.15	-572.3
m3-abv	1	40	613.57	-1147.14
m5-av	1	29	319.03	-580.06
m5-abv	1	41	618.23	-1154.46

Table A5. ANALYSIS WITH HILL MODELS. Best model with covariates is: Hill m5-abv

Model	converged	npar	loglik	aic
m3-av	1	28	311.53	-567.06
m3-abv	1	40	610.77	-1141.54
m5-av	1	29	327.33	-596.66
m5-abv	1	41	618.21	-1154.42

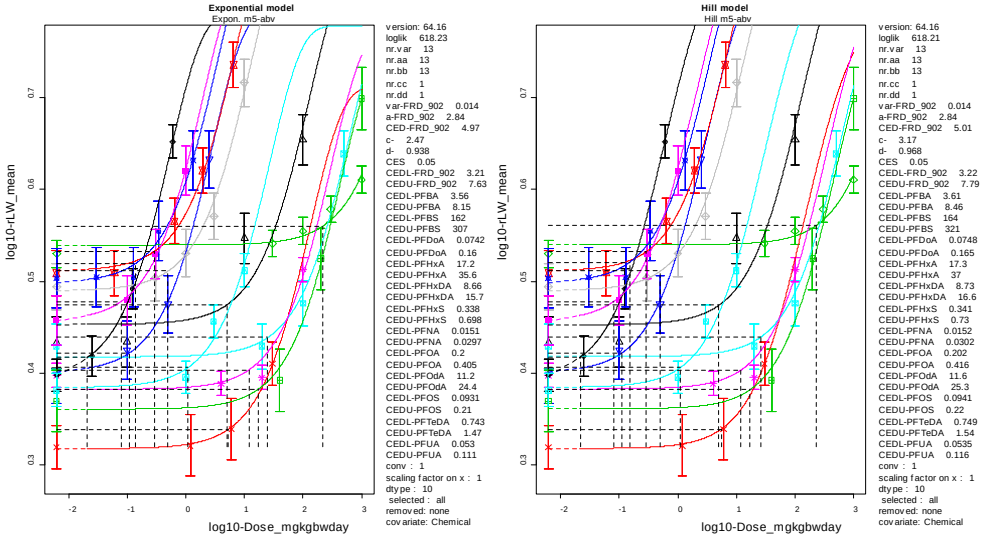

Figure A4. Fitted dose-response curves for each PFAS. Left and right panels show the fits with the exponential and Hill models, respectively.

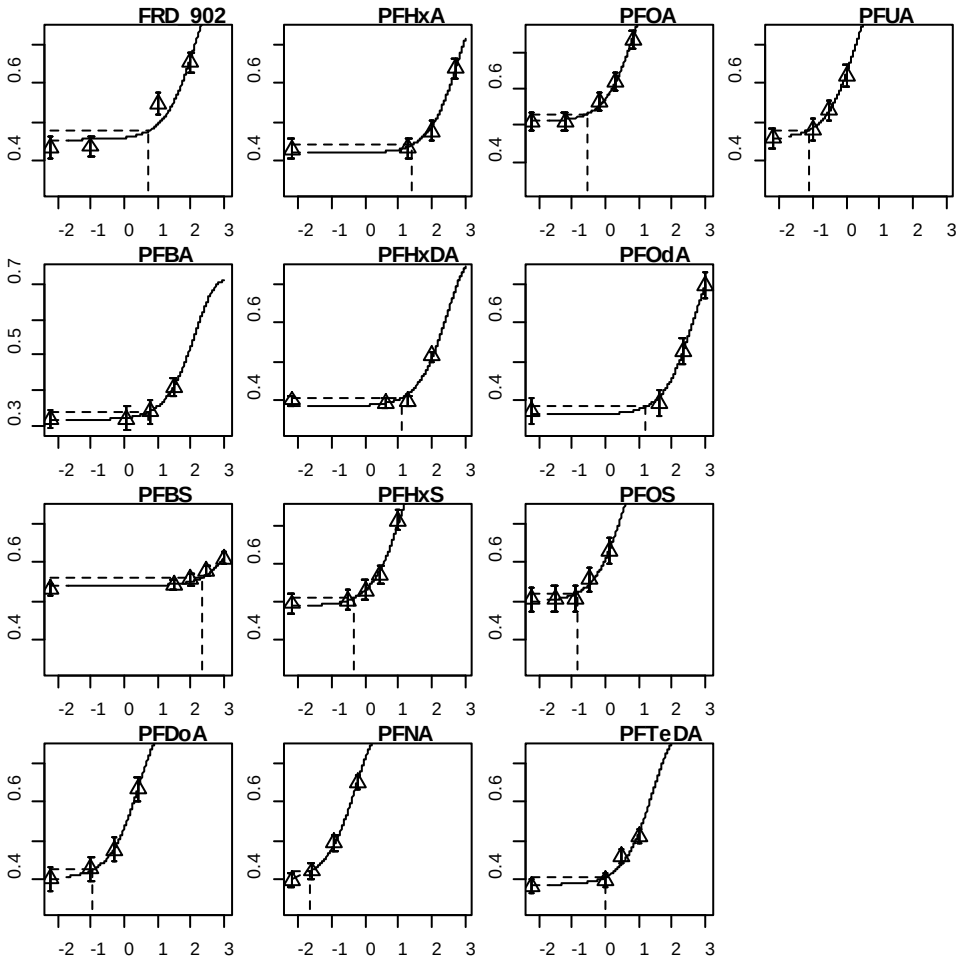

Figure A5. Individual fits of all PFASs with the exponential model m5-abv

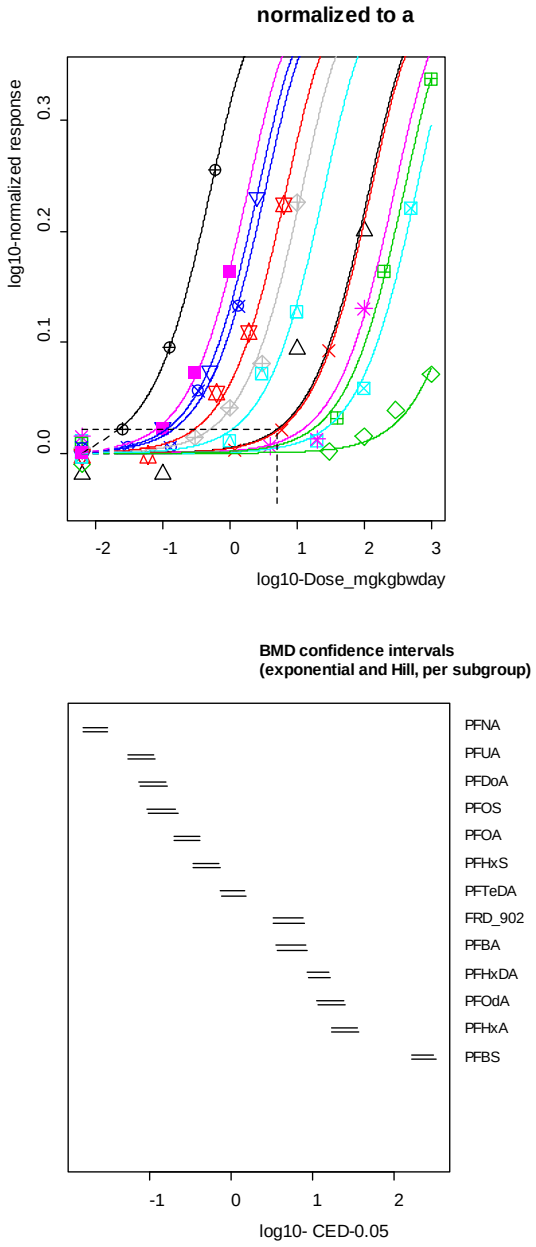

Figure A6. Top panel: fit of the exponential model after normalizing to background (parameter a). Lower panel: log10 BMD (95%-)confidence intervals, i.e. range BMDL to BMDU for each PFAS. BMR=5%. Within each PFAS, the upper line relates to the exponential model results and the bottom line corresponds to the Hill model results.

LIVER HYPERTROPHY

A set of models were fitted to the hypertrophy data, including testing for differences between chemicals (covariate) (EFSA, 2017). Three models were accepted according to the AIC: the log-logistic, Weibull and log-probit models (Table A6). Chemical was used as the covariate. The significant improvement of the model fits by adding this covariate for parameter b, indicates that the various chemicals differ in potency (parameter b). Figure 7 shows the fitted dose-response curves for the accepted models. The actual BMD, BMDL and BMDU values of the accepted models are reported in Table A7 and are very similar between the three accepted models. No BMDs are derived for PFOS for this endpoint because no quantitative information on liver histopathology suitable for a BMD analysis is presented in Seacat *et al.* (2003).

Table A6. Liver hypertrophy

model	Number of parameters	Log-likelihood	AIC	accepted	converged
null	11	-325.60	673.20	NA	
full	45	-117.42	324.84	NA	
Two stage-b	13	-77.87	181.74	no	yes
Log-logistic-b	13	-71.09	168.18	yes	yes
Weibull-b	23	-71.0	168.0	yes	yes
Log-probit-b	13	-70.55	167.10	yes	yes
Gamma-ab	23	-104.95	255.90	no	yes
Logistic-b	12	-93.38	210.76	no	yes
LVM: Expon m3-ab	23	-68.32	182.64	no	yes
LVM: Hill m3-ab	23	-68.51	183.02	no	yes

BMR: 10% extra risk, PROAST version: 64.16

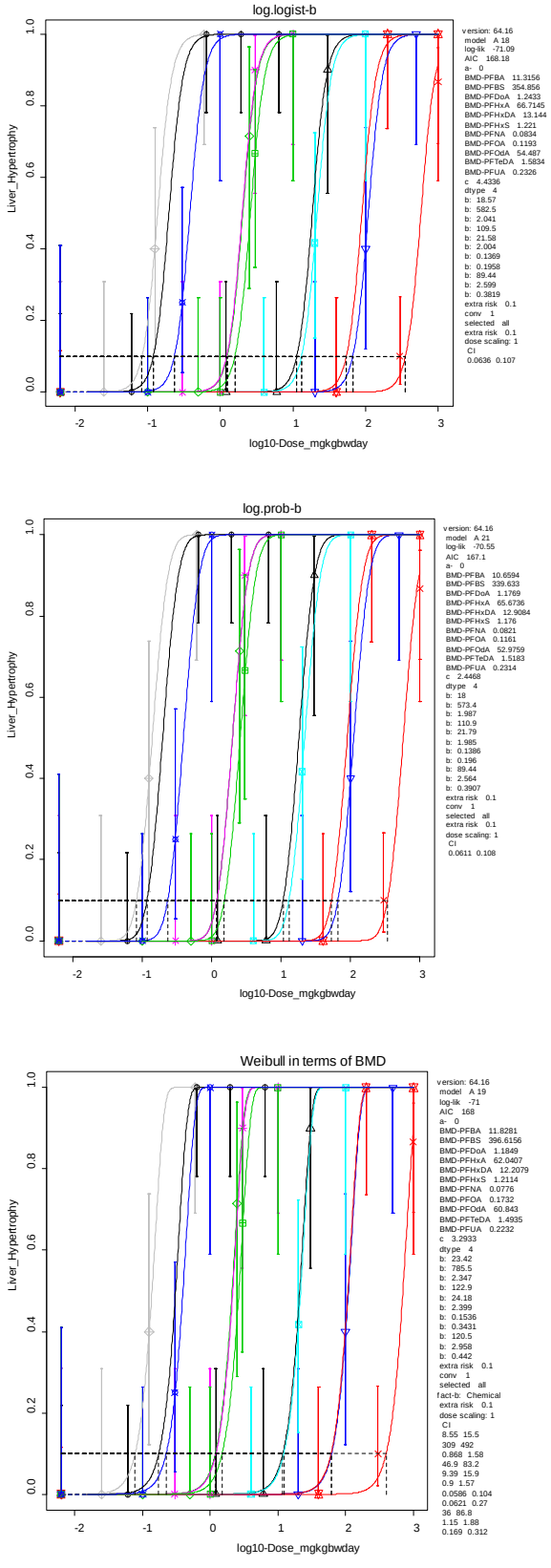

Figure A7. Dose-responses using the accepted log-logistic, log-probit and Weibull models.

DERIVING RPFs

In Table A7, an overview of the derived BMD and (BMDL – BMDU) is presented for the three end points and various models. For practical reasons, the RPFs were derived from the BMDs obtained from the exponential model and the log-logistic model. It is assumed that the RPFs will not depend on the choice of model because the BMD results are very similar between the exponential and Hill models and between the log-logistic, log-probit and Weibull models.

Using equation 1, the confidence intervals of the RPFs were derived by assuming the BMD confidence intervals are approximately lognormally distributed. For all endpoints and chemicals, except PFDoA in absolute liver weight, the ratio BMD/BMDL was equal to BMDU/BMD, indicating that the lognormality assumption is plausible. The standard deviation on log-scale is then derived for chemical *i* as:

$$SD_i = \frac{ln\left(\frac{BMDU_i}{BMD_i}\right)}{z(p)}$$

whereby z(p) is the (p x 100)th percentile of the standard normal distribution, here z(0.95) = 1.645, because the BMDU corresponds to the 95th percentile of the BMD CI. The lower 5th and upper 95th confidence bounds (RPF LB and RPF UB) of the RPF are derived as (Weisstein, 2017):

$$RPF\ LB_i = \exp\left(ln(RPF_i) - 1.645\sqrt{SD_i^2 + SD_{PFOA}^2}\right)$$

and

$$RPF\ UB_i = \exp\left(ln(RPF_i) + 1.645\sqrt{SD_i^2 + SD_{PFOA}^2}\right)$$

The resulting RPFs and their 90% confidence intervals are reported in Figure 8 and Table A8. In general, the RPFs based on absolute and relative liver weight are similar, and the RPFs based on hypertrophy are below those based on liver weight. For PFDoA, no RPF could be derived based on the absolute liver weight, and for PFOS no RPF could be derived based on hypertrophy. Since the set of RPFs derived from relative liver weight is the most complete set, the use of the RPFs derived from this endpoint is suggested. Due to the uncertainties in the RPFs, it is considered appropriate to round them off to one significant digit:

Perfluorobutanesulfonate (PFBS):	0.001
Perfluorohexanesulfonate (PFHxS):	0.6
Perfluorooctanesulfonate (PFOS):	2
Perfluorobutyrate (PFBA):	0.05
Perfluorohexanoate (PFHxA):	0.01
Perfluorooctanoic acid (PFOA):	1
Perfluorononaoic acid (PFNA):	10
Perfluorodecanoic acid (PFUA):	4
Perfluorododecanoic acid (PFDoA):	3
Perfluorotertadecanoic acid (PFTeDA):	0.3

Perfluorohexadecanoic acid (PFHxDA):	0.02
Perfluorooctadecanoic acid (PFOdA):	0.02
FRD-902	0.06

These RPFs are obtained from external doses in oral studies. Part of the differences in potency between PFASs may be due to differences in bioavailability and/or oral absorption. Therefore, the RPFs can only be applied to orally administered external doses. Absorption may be different for the individual PFASs when the exposure route changes, which would require other (route-specific) RPFs. When considering internal exposure (e.g. blood serum levels), possible differences in absorption are already accounted for, which also warrants other ("internal") RPFs (van Ede, 2016).

Table A7. Overview of the derived BMD and, between brackets, its BMDL and BMDU in mg/kg bw/day for the three end points and various models

	Absolute liver weight [a]		**Relative liver weight** [a]		**Hypertrophy** [b]		
Chemical	**Exponential**	**Hill**	**Exponential**	**Hill**	**Log-logistic**	**Log-probit**	**Weibull**
Perfluorobutane-sulfonate (PFBS, C4)	98.82 (58.8 - 154)	104.9 (61.5 - 163)	224.8 (162 – 307)	232 (164 – 321)	354.9 (282 - 443)	339.6 (279 – 406)	396.62 (309 – 492)
Perfluorohexane-sulfonate (PFHxS, C6)	0.5262 (0.294 - 0.865)	0.5472 (0.302 - 0.9)	0.4962 (0.338 - 0.698)	0.5087 (0.341 - 0.73)	1.221 (0.879 - 1.63)	1.176 (0.866 - 1.57)	1.2114 (0.9 - 1.57)
Perfluorooctane-sulfonate (PFOS, C8)	0.1261 (0.0547 - 0.284)	0.1282 (0.0562 - 0.292)	0.1409 (0.0931 - 0.21)	0.1452 (0.0941 - 0.22)	NA	NA	NA
Perfluorobutyrate (PFBA, C4)	3.15 (0.547 – 27.1)	3.227 (0.621 - 26.9)	5.345 (3.56 - 8.15)	5.515 (3.61 – 8.46)	11.32 (7.06 - 16)	10.66 (7.1 - 15.1)	11.828 (8.55 - 15.5)
Perfluorohexanoate (PFHxA, C6)	35.91 (19 - 63.3)	37.43 (19.6 - 66.2)	25.24 (17.2 – 35.6)	25.82 (17.3 - 37)	66.71 (50.9 - 86.1)	65.67 (48.9 - 86.4)	62.041 (46.9 - 83.2)
Perfluorooctanoic acid (PFOA, C8)	0.2251 (0.135 - 0.361)	0.2389 (0.141 - 0.383)	0.2888 (0.2 - 0.405)	0.2938 (0.202 - 0.416)	0.1193 (0.0606 - 0.244)	0.1161 (0.0615 - 0.227)	0.17322 (0.0621 - 0.27)
Perfluorononaoic acid (PFNA, C9)	0.07373 (0.0353 - 0.146)	0.07573 (0.0352 - 0.154)	0.02146 (0.0151 - 0.0297)	0.02176 (0.0152 - 0.0302)	0.08337 (0.0636 - 0.107)	0.08208 (0.0611 - 0.108)	0.077551 (0.0586 - 0.104)
Perfluorodecanoic acid (PFUA, C11)	0.0982 (0.0462 - 0.199)	0.1016 (0.0471 - 0.21)	0.07781 (0.053 - 0.111)	0.07989 (0.0535 - 0.116)	0.2326 (0.182 - 0.302)	0.2314 (0.177 - 0.3)	0.2232 (0.169 - 0.312)
Perfluorododecanoic acid (PFDoA, C12)	3.626 (0.461- Inf)	4.028 (0.498 – Inf)	0.1106 (0.0742 - 0.16)	0.1126 (0.0748 - 0.165)	1.243 (0.838 - 1.68)	1.177 (0.797 - 1.65)	1.1849 (0.868 - 1.58)
Perfluorotertad	1.286	1.308	1.061	1.09	1.583	1.518	1.4935

ecanoic acid (PFTeDA, C14)	(0.65 - 2.49)	(0.654 - 2.57)	(0.743 - 1.47)	(0.749 – 1.54)	(1.2 - 1.99)	(1.16 - 1.95)	(1.15 - 1.88)
Perfluorohexadecanoic acid (PFHxDA, C16)	20.13 (12.2 - 31.5)	21.51 (12.9 - 33.6)	11.98 (8.66 – 15.7)	12.38 (8.73 – 16.6)	13.14 (10.2 - 16.5)	12.91 (9.81 - 16.6)	12.208 (9.39 - 15.9)
Perfluorooctadecanoic acid (PFOdA, C18)	15.79 (6.88 - 32.5)	15.69 (7.02 - 33)	16.7 (11.2 – 24.4)	17.36 (11.6 – 25.3)	54.49 (35.7 - 84.7)	52.98 (35.9 - 78.9)	60.843 (36 - 86.8)
FRD-902	NA	NA	4.968 (3.21 – 7.63)	5.008 (3.22 – 7.79)	NA	NA	NA

[a] BMR is 5% increase in absolute or relative liver weight compared to background
[b] BMR is 10% extra risk

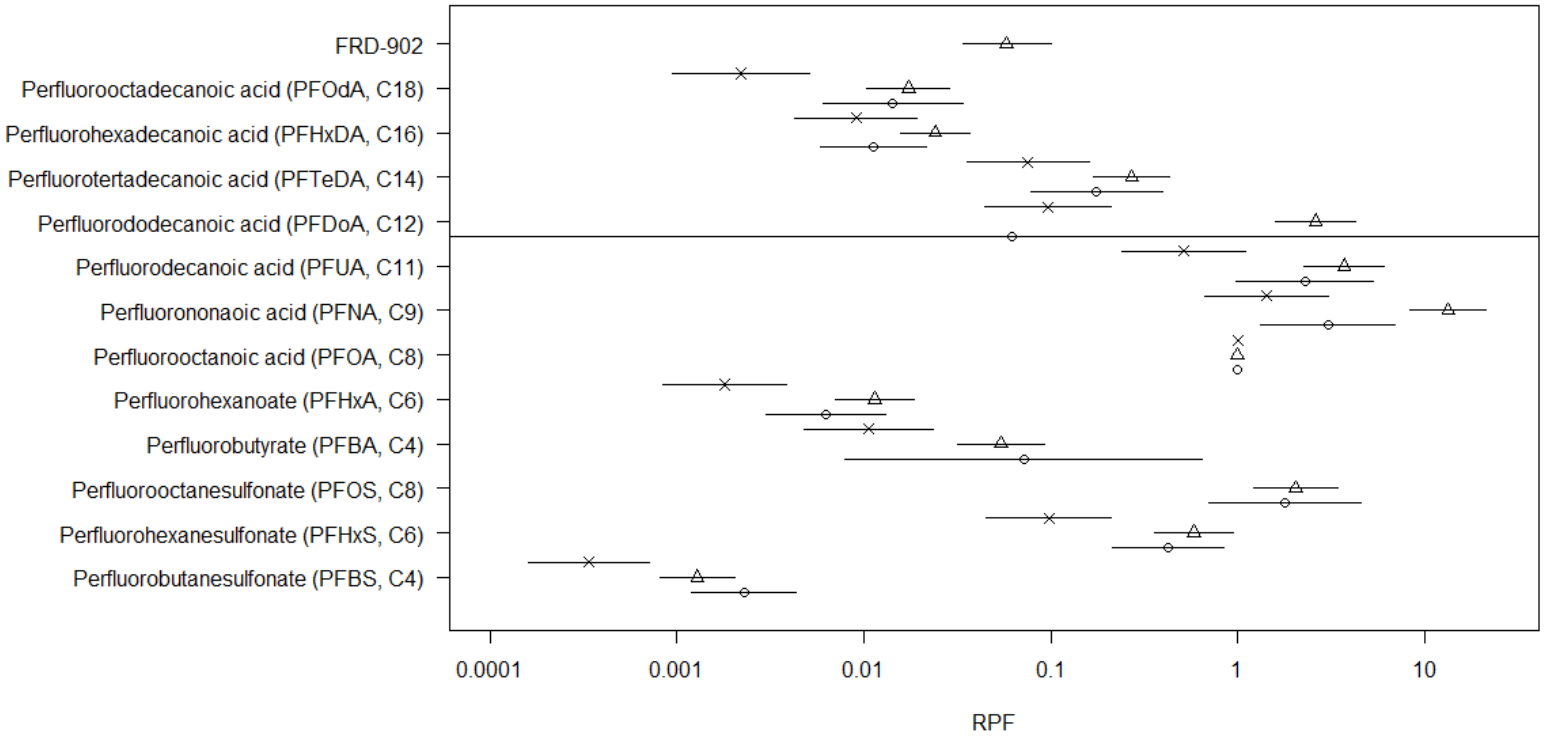

Figure A8. RPFs (and 90% CI) for PFAS. PFOA is used as the reference compound (RPF=1). For each PFAS, three RPFs are derived: circles, triangles, X correspond to RPFs based on absolute liver weight, relative liver weight and hypertrophy, respectively. For PFOS, no suitable hypertrophy data were available. PFDoA does not show a dose-response in the absolute liver weight data, resulting in a very wide confidence interval.

Table A8. RPF values.

	RPF LB	RPF UB	RPF	proposed RPFs
Absolute liver weight, exponential model				
Perfluorobutanesulfonate (PFBS, C4)	0.00119152	0.00435473	0.0023	
Perfluorohexanesulfonate (PFHxS, C6)	0.21549545	0.84920238	0.4278	
Perfluorooctanesulfonate (PFOS, C8)	0.69779677	4.56658833	1.7851	
Perfluorobutyrate (PFBA, C4)	0.00789152	0.64709689	0.0715	
Perfluorohexanoate (PFHxA, C6)	0.00299717	0.0131102	0.0063	
Perfluorooctanoic acid (PFOA, C8)			1.0000	
Perfluorononaoic acid (PFNA, C9)	1.33050546	7.00560848	3.0530	
Perfluorodecanoic acid (PFUA, C11)	0.98005985	5.3613655	2.2923	
Perfluorododecanoic acid (PFDoA, C12) [a]	0	Inf	0.0621	
Perfluorotertadecanoic acid (PFTeDA, C14)	0.07769561	0.39434158	0.1750	
Perfluorohexadecanoic acid (PFHxDA, C16)	0.00583274	0.02143834	0.0112	
Perfluorooctadecanoic acid (PFOdA, C18)	0.0060165	0.03377867	0.0143	
Relative liver weight, exponential model				
Perfluorobutanesulfonate (PFBS, C4)	0.000811128	0.002034756	0.0013	**0.001**
Perfluorohexanesulfonate (PFHxS, C6)	0.35999944	0.940977054	0.5820	**0.6**
Perfluorooctanesulfonate (PFOS, C8)	1.214851736	3.458192089	2.0497	**2**
Perfluorobutyrate (PFBA, C4)	0.031466259	0.092779888	0.0540	**0.05**
Perfluorohexanoate (PFHxA, C6)	0.007063863	0.018534182	0.0114	**0.01**
Perfluorooctanoic acid (PFOA, C8)			1.0000	**1**
Perfluorononaoic acid (PFNA, C9)	8.41959714	21.5101595	13.4576	**10**
Perfluorodecanoic acid (PFUA, C11)	2.272767566	6.061338303	3.7116	**4**
Perfluorododecanoic acid (PFDoA, C12)	1.58267945	4.308153416	2.6112	**3**

	RPF LB	RPF UB	RPF	proposed RPFs
Perfluorotertadecanoic acid (PFTeDA, C14)	0.170167289	0.435399103	0.2722	**0.3**
Perfluorohexadecanoic acid (PFHxDA, C16)	0.015634994	0.037169185	0.0241	**0.02**
Perfluorooctadecanoic acid (PFOdA, C18)	0.01040483	0.028742626	0.0173	**0.02**
FRD-902	0.033663443	0.10038589	0.0581	**0.06**
Hypertrophy, Log-logistic model				
Perfluorobutanesulfonate (PFBS, C4)	0.00015889	0.00071109	0.00034	
Perfluorohexanesulfonate (PFHxS, C6)	0.04515023	0.21137028	0.09769	
Perfluorooctanesulfonate (PFOS, C8) [b]	NA	NA	NA	
Perfluorobutyrate (PFBA, C4)	0.00475954	0.02334449	0.01054	
Perfluorohexanoate (PFHxA, C6)	0.00083632	0.00382224	0.00179	
Perfluorooctanoic acid (PFOA, C8)			1.00000	
Perfluorononaoic acid (PFNA, C9)	0.67045033	3.05279019	1.43064	
Perfluorodecanoic acid (PFUA, C11)	0.23935763	1.09829552	0.51272	
Perfluorododecanoic acid (PFDoA, C12)	0.0441362	0.20853956	0.09594	
Perfluorotertadecanoic acid (PFTeDA, C14)	0.03553772	0.1596851	0.07533	
Perfluorohexadecanoic acid (PFHxDA, C16)	0.00428259	0.01922976	0.00907	
Perfluorooctadecanoic acid (PFOdA, C18)	0.00094438	0.00507459	0.00219	

[a] PFDoA does not show a dose-response in the absolute liver weight data, resulting in a very wide confidence interval.
[b] No hypertrophy data available

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