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Memorandum on the implementation of the EFSA sum TWI of PFASs

RIVM – Final 7 April 2021

Summary

The European Food Safety Authority (EFSA) has established a new health-based limit value for perfluoralkyl substances (PFASs) in food. This value is lower than previously used values. The limit value is expressed as a Tolerable Weekly Intake (TWI). EFSA used the latest scientific insights considering the possible health-related effects of exposure to PFASs. RIVM will use the new EFSA health-based limit value when performing risk assessments and to establish risk limits for this group of substances.

Additional step required

Before RIVM can use this new value for practical applicability, an additional step is needed because EFSA established a TWI for the sum of four PFASs, namely PFOS, PFOA, PFNA and PFHxS. There are occasions however, for example when assessing soil, drinking water or surface water, where other PFASs occur as well which can have detrimental effects on people's health, in addition to the four PFASs referred to. However, it may also be the case that just a single PFAS is present. RIVM would like to use the TWI in these cases as well, which is why an additional step is required. RIVM has investigated various possibilities to implement this additional translation.

Uniform method for using TWI

RIVM is proposing a uniform method for using the EFSA TWI which is applicable in various policy contexts. This method comprises applying the EFSA TWI in combination with so-called Relative Potency Factors (RPFs). These RPFs indicate how harmful individual PFASs are compared to PFOA. These RPFs can be used to express concentrations or exposures to various PFASs in 'PFOA equivalents' (or PEQs for short). These equivalents can be added up, and the sum of the PFOA equivalents can subsequently be compared with the EFSA TWI or risk limit under which no detrimental health effects are expected. The method allows application of the EFSA TWI or risk limits to various PFAS mixtures. This approach is comparable to the method used for dioxins and is, therefore, not new.

Relative Potency Factors (RPFs) and uncertainties

The EFSA TWI and RPFs are determined on the basis of different effects. The TWI is based on immune effects, while the RPFs are derived from liver effects. EFSA has assumed that the four PFASs considered are equally harmful. The RPF method acknowledges that the various PFASs are not all equally harmful. By applying the RPFs, it is assumed that the differences in harmfulness also apply to other effects which can be caused by PFASs, including immune effects. The RPF method also takes account of the possibility that numerous PFASs can cause an effect. The method proposed by RIVM is not perfect, but probably approximates the mutual potency differences in terms of immune effects by PFASs more effectively than the assumption that the various PFASs are equally harmful. The fact that the RPF method takes account of (a maximum of) 23 PFASs instead of four means it is less likely that the health effects will be underestimated.

Inhoud

1. Introduction and purpose of this memorandum

In its opinion on perfluoralkyl substances (PFASs), EFSA presents a health-based limit value (Tolerable Weekly Intake or TWI) for the sum of four PFASs (PFOS, PFOA, PFNA and PFHxS) (EFSA, 2020a). Following an evaluation, RIVM decided to use the EFSA TWI as a basis for the health-based assessment of PFASs (RIVM, 2020a and 2020b). This memorandum highlights a number of points to consider with regard to practical application of the EFSA TWI and indicates how RIVM is going to use the EFSA TWI and why. In this memorandum the various options are discussed and a single option is selected which is considered to be most suitable in terms of making the EFSA TWI broadly usable because the option in question enables an assessment of individual PFASs, the four PFASs assessed by EFSA and other mixtures of PFASs. RIVM will use the selected method for applying the EFSA TWI in work relating to PFASs for various policy contexts (uniform approach). A generic description of the impact of a TWI in various policy contexts is provided in the memorandum entitled 'Status of an EFSA opinion and the role of a health-based limit value in various policy contexts' (Bulder et al, 2020).

2. Three points to consider when using the EFSA sum TWI

2.1 (1) Relevance of the PFASs selected by EFSA

EFSA has opted for a health-based limit value for the sum of PFOS, PFOA, PFNA and PFHxS. These PFASs have been assessed as a sum because EFSA assumes that these four PFASs cause the same critical effect and because these are the main PFASs found in people's blood. The study on which EFSA bases its TWI shows that these four PFASs make up 90% of the PFASs found in blood serum[1]. The study reported that an association was only found between PFOA and the effect on the immune system. However, EFSA does not rule out the possibility that this effect may have been caused by the other three PFASs as well.

The four PFASs which EFSA considers in its opinion for exposure via food are not, by definition, also the most relevant PFASs for other exposure routes, environmental compartments and policy contexts. We know, for example, that also other PFASs occur in soil, drinking water, surface water and groundwater. Using the current analysis methods, a maximum of between 10 and 20 different PFAS can be found in these matrices and in food (Brandsma et al., 2019; Gebbink et al., 2017; Wintersen et al., 2020; EU, 2020). Hence, besides the four PFASs assessed by EFSA there is, in practice, a need for the assessment of a broader group of PFASs. This is important to enable estimation of the risks of PFAS mixtures properly. Conversely, individual risk limits or standards may actually be needed in certain situations, for example if the four PFASs do not all occur or have been measured.

2.2 (2) Mixture ratio between various PFAS and (3) assumption of equipotency

In order to be able to interpret the EFSA TWI and translate it to national risk limits, it is important to gain an insight into how the EFSA TWI has been established. An overview of the EFSA procedure can be found in Appendix A. The main message from the overview is that EFSA calculated the amount of the four PFASs that can be ingested by adults on a daily basis for long periods of time. Exposure below this daily amount ensures that the blood serum concentration of breastfed children stays below the critical concentration leading to immune effects. In doing so, EFSA assumes that, at serum concentration level, the four selected PFASs are each equally potent (equipotent) and that their effects are cumulative.

[1] The Abraham et al. (2020) study used blood samples taken at the end of the 1990s. Although other PFASs were analysed, they were not found (<LOD).

These assumptions by EFSA largely determine the way in which the EFSA TWI can be used (Appendices A1 and A2). One of the most important observations made is that the EFSA TWI is dependent on the ratio between PFOA&PFNA on the one hand and PFOS&PFHxS on the other. This means that the EFSA TWI is, in principle, only applicable to mixtures with the same mixture ratio as the ratio in the study by Abraham et al. (2019) on which the EFSA TWI is based. EFSA also assumes that the four PFASs can cause immunotoxicity to the same extent. In other words, the four PFAS are considered to be equipotent at serum level. However, EFSA also assumes differences in toxicokinetics between PFOA&PFNA and PFOS&PFHxS (see Appendix A). This therefore means that external dosages are not equipotent. Nevertheless, EFSA recommends using the sum of the four PFASs without taking (internal or external) potency differences into account. EFSA states that it was not able to establish Relative Potency Factors (RPFs) for immune effects due to a lack of suitable studies and that it therefore took the pragmatic decision to assume equipotency.
It is known that PFASs are not equipotent with regard to the other effects of PFASs (for example liver effects). Therefore, RIVM considers it plausible that various PFASs are not equipotent with regard to immune effects either. If this is the case, the EFSA TWI will once again not be applicable to other mixture ratios. This is an important point which will be discussed in more detail later on in this memorandum. Another observation is that EFSA did not consider a large number of other PFASs due to a lack of information about the effects of these substances on the immune system, as well as because the four PFASs make up 90% of the PFAS concentrations in blood serum. However, other PFASs do contribute to the (external) exposure. For example, half the exposure via food is down to PFASs other than the four EFSA-PFASs[2]. It cannot be ruled out that these PFASs also contribute to the (toxicological) effects. The extent of this contribution depends on the potency of the other PFASs. A focus on just the four EFSA PFASs may lead to an underestimation of the risk in situations in which there is exposure to other PFASs as well.

3. Possible options for using the EFSA TWI

Using the EFSA opinion as a starting point, Appendix B describes a number of options for assessing individual PFASs and PFAS mixtures on the basis of the EFSA TWI. A broadly applicable method is needed to allow the uniform assessment of the different situations in various policy contexts. Ideally the method will take the mentioned three criteria into account which, according to RIVM, result from the EFSA opinion, and the desire to achieve a broadly applicable method, namely:

1) that the method is applicable to individual PFASs, the four PFASs studied by EFSA and other PFASs;
2) that it is desirable to be able to assess PFAS mixtures in different compositions;
3) that it preferably accounts for differences in potency between PFASs.

A fourth criterion to consider is that it is important that

4) the method is conceptually simple and practically applicable.

RIVM has drawn up six options on how the EFSA TWI could be used (Appendix B). The six options differ in the extent to which they take account of the four criteria. Eventually RIVM selected the Relative Potency Factor method (option e in Appendix B) as the option that best fulfils the above-mentioned criteria. This choice is substantiated and clarified in more detail below.

[2] EFSA studied a total of 28 PFASs. The 13 PFASs which make up the other half of the exposure were not considered and 11 other PFASs were not detected in food (EFSA, 2020a).

RIVM regards other options for applying the EFSA TWI as less suitable than the RPF method (see Appendix B). This is mainly because they are only applicable to the four EFSA PFASs. In addition, several methods result in multiple TWIs or risk limits for various combinations of PFASs, which is difficult to interpret conceptually and implement in practice.

4. Selected method: RPF

The proposed uniform approach for the assessment of individual PFASs and PFAS mixtures uses Relative Potency Factors (RPFs) which can be applied to (external) exposure and concentrations of various PFASs (e.g. in drinking water or food products). The RPFs portray the toxic potency of individual PFASs in terms of PFOA and adjust for the differences in toxic potency between PFASs (caused by differences in toxicokinetics and toxicodynamics). Currently, RPFs based on liver effects are available for 23 PFASs (Bil et al. 2021, Appendix C). To ensure broad applicability, the assumption is that the RPFs apply to both the linear and branched isomers of these 23 PFASs [3]. The RPFs cannot be applied to internal (e.g. serum) concentrations because the values of the RPFs (partially) depend on the kinetics of the PFASs. In order to assess PFAS mixtures in human blood, internal RPFs are currently derived for a limited number of PFASs (Bil et al., in prep). The RPFs can be used to express concentrations (in e.g. food, water, or soil) or exposures to various PFASs as the sum of PFOA equivalents (PEQ), which can then be compared to a human toxicological risk limit or health-based limit value established for PFOA (for example the TDI/TWI as a criterion of the maximum tolerable daily intake). We use the EFSA TWI as a health-based limit value for PFOA (equivalents)[4]. We assume that PFOA is a logical choice of index substance (with RPF of 1) given that the authors (Abraham et al. 2020) of the underlying study on which the EFSA TWI is based conclude that there is only an association of immune effects with PFOA, and not with the other three PFASs. The application of the RPF method is illustrated using a number of examples in the text box below.

5. Advantages of the RPF method

Key advantages of the RPF method are that it is applicable to individual PFASs and to the four PFASs studied by EFSA, as well as to more PFASs than the four EFSA PFASs, and that the TWI used is not dependent on the mixture ratio between various PFASs. Other advantages are that the concept is simple and already features in the risk assessment for other chemical substances. The approach is, in fact, already being used with the comparable Toxic Equivalents Factors (TEFs) for dioxins. The practical application of the EFSA TWI as PFOA TWI means that (in contrast to some other options) only a single TWI, TDI or risk limit is necessary for a comparison with the sum of PFOA equivalents (PEQ) and this considerably simplifies usage. A final advantage of the RPF method is that additional substance-specific information can be included at a later stage (provided it is available). In the event of indirect exposure to PFASs in soil and water, for example via fish, vegetables or fruit, substance-specific bioaccumulation factors (BAFs) can be used to assess the risks of PFASs in surface water, groundwater or soil. What is more, adjustments can be made for differences in the distribution across milk and serum if additional information becomes available.

[3] Reports of PFAS concentrations do not always distinguish between the various isomers. Neither is any distinction made between the isomers when establishing the RPFs.
[4] It should be noted that the EFSA TWI is therefore not adopted one on one, but is used as a basis to assess individual PFASs and mixtures of PFASs in combination with RPFs.

6. Disadvantages of the RPF method

One disadvantage of the RPF method is that the available RPFs were established for liver effects in rats, while the EFSA TWI is based on effects on the immune system. Bil et al. (2021) discuss the fact that, in the absence of immune-specific factors from human studies, the RPFs could also be applied to other effects and to humans, but the validation of broad application of the current RPF values is desirable. RIVM has made an initial attempt at this validation. This revealed that comparable potency differences exist between PFASs in numerous endpoints such as different organ weights, hormone levels, clinical chemistry, white blood cell parameters and pathology endpoints. Consequently, it is not the case that these potency differences are only observed in conjunction with liver effects. Generally speaking these PFAS potencies are comparable with the findings in Bil et al. (2021). With regard to the four EFSA PFASs, the sequence (from low to high potency) is: PFHxS < PFOA < PFOS < PFNA. EFSA is of the opinion that the available RPFs cannot be used for immune effects given the uncertainty about a common mechanism of action of immune and liver effects (EFSA, 2020b). In addition, the RPFs take no account of the possible differences between PFASs in terms of their distribution across milk and serum. Although RIVM acknowledges these points, RIVM considers that, in view of the available scientific information referred to above, it is better justified to account for relative potencies of PFASs than the assumption of equipotency made by EFSA. As already mentioned it is, however, recommended that the RPFs are validated for immune effects in due course.

7. Implications of using the RPF method

By using the RPFs in combination with the EFSA TWI it is assumed that other PFASs can also have an effect on the immune system and an estimate is given of the degree of this effect per substance. This consequently takes account of the possibility that exposure to different PFASs can add up and have a cumulative effect. This is important to enable risk estimation of several PFASs which occur simultaneously in practice. The desire to take account of cumulative exposure to substances has been expressed at both national and European level (RLI, 2020; EU, 2020).

On the other hand, as already mentioned it should be realised that the available RPFs require validation and that they do not make allowances for all differences between individual PFASs[5]. Furthermore, RPFs are only available for a limited number of PFASs and, as a consequence, this method can only be used to assess the PFASs in question. For the time being, PFASs for which no RPF is available cannot be assessed using this method. If assessment of these PFASs is desirable, an RPF for the PFASs in question will first have to be established, or an individual assessment will have to be carried out based on a substance-specific risk limit or health-based limit value.

The proposed RPF method is not perfect, but we think that the actual risk can be estimated more accurately if potency differences between PFASs are taken into account and if several PFASs can be included in the risk calculation. The method justifies EFSA's basic principle that children must be protected against immune effects and that several PFASs can cause this effect.

In view of the differences in potency between PFASs and the almost infinite number of different compositions in which PFASs can occur it is, by definition, impossible to determine whether the chosen method is stricter or less strict than the other

[5] For example because they are based on liver effects and make no distinction between a difference in distribution across breast milk and blood serum.

available methods. Using a method which takes account of 23 instead of 4 PFASs will, however, makes an underestimation of the risk less likely if members of this group of 23 PFASs actually occur simultaneously.

Examples of calculations using the RPF method
It should be noted that the examples relate to direct oral exposure of humans to PFASs. This may involve ingestion via food or drinking water, or direct ingestion of surface water or soil. In the event of indirect exposure, e.g. to contaminated soil via vegetables or fruit, (substance-specific) bioaccumulation factors (BAFs) will be necessary.

1—Mixture of several PFASs in drinking water
The table below assumes fictitious concentrations in drinking water of a number of PFASs. The concentration of each PFAS is expressed as PFOA equivalents (PEQ) by multiplying the concentrations measured in water by the corresponding RPF. The sum of the PFOA equivalents can be compared with a PFOA risk limit or standard derived from the EFSA TWI. Similar calculations can be made for concentrations in fish (measured in ng/g). See Appendix C for the abbreviations of the PFASs.

PFASs	Fictitious concentration (ng/L)	RPF	PFOA equivalents (PEQ, ng/L)
PFBS	5	0.001	0.005
PFHxS	1	0.6	0.6
PFHpS	1	2	2
PFOS	1	2	2
PFBA	5	0.05	0.25
PFPeA	5	0.05	0.25
PFHxA	6	0.01	0.06
PFHpA	3	1	3
PFOA	4	1	4
HFPO-DA (~GenX)	6	0.06	0.36
Sum of PFOA equivalents			12.5

2—Mixture of the four EFSA PFASs in food products
Similarly to drinking water, concentrations in food products (categories) are the starting point for the ingestion of a mixture of PFASs from food. For example, EFSA reports (2020a, table 4) the highest (average) concentrations for the category egg and egg products as follows:

- 0.35 µg PFOS/g product;
- 0.21 µg PFOA/g product;
- 0.098 µg PFNA/g product;
- 0.06 µg PFHxS/g product.

When multiplied by the RPFs corresponding to these PFASs (PFOS: 2; PFOA: 1; PFNA: 10; PFHxS: 0.6) and added up, this results in a PFOA equivalent of 1.93 µg PFOA eq/g product. This sum of the PFOA equivalents can be compared with a PFOA risk limit or standard derived from the EFSA TWI.

3—Risk assessment of exposure to the four EFSA PFASs via food

A high exposure from food (adults) to PFOA, PFNA, PFHxS and PFOS amounts to (table 10. EFSA 2020a):

- 16.3 ng PFOS/kg bw/day;
- 15.9 ng PFOA/kg bw/day;
- 15.4 ng PFNA/kg bw/day;
- 15.2 ng PFHxS/kg bw/day.

When multiplied by the RPFs (PFOS: 2; PFOA: 1; PFNA: 10; PFHxS: 0.6), added and expressed as a quantity per week, this produces a PFOA equivalent of 1481 ng/kg bw/week. This exposure can be compared with the EFSA TWI of 4.4 ng/kg bw/week.

4—Risk assessment of exposure to a single (random) PFAS

If only one PFAS is assessed, the exposure of that PFAS is expressed in PFOA equivalents by multiplying the exposure by the corresponding RPF. The PFOA equivalents can then be compared with the EFSA TWI.

Example 4a:

A high exposure from food (adults) to PFHxA in Europe is approximately 16 ng/kg bw/day (table 10. EFSA 2020a). Multiplication by the RPF (0.01) of PFHxA produces a PFOA equivalent of 0.16 ng/kg bw/day, or 1.12 ng/kg bw/week. This exposure can be compared with the EFSA TWI of 4.4 ng/kg bw/week.

Example 4b:

Let us suppose that the exposure from food and drinking water to HFPO-DA (~GenX) is 21 ng/kg bw/day. Multiplication by the RPF (0.06) of HFPO-DA produces a PFOA equivalent of 1.26 ng/kg bw/day, or 8.8 ng/kg bw/week. This exposure can be compared with the EFSA TWI of 4.4 ng/kg bw/week.

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Appendix A1: EFSA procedure for establishing the TWI

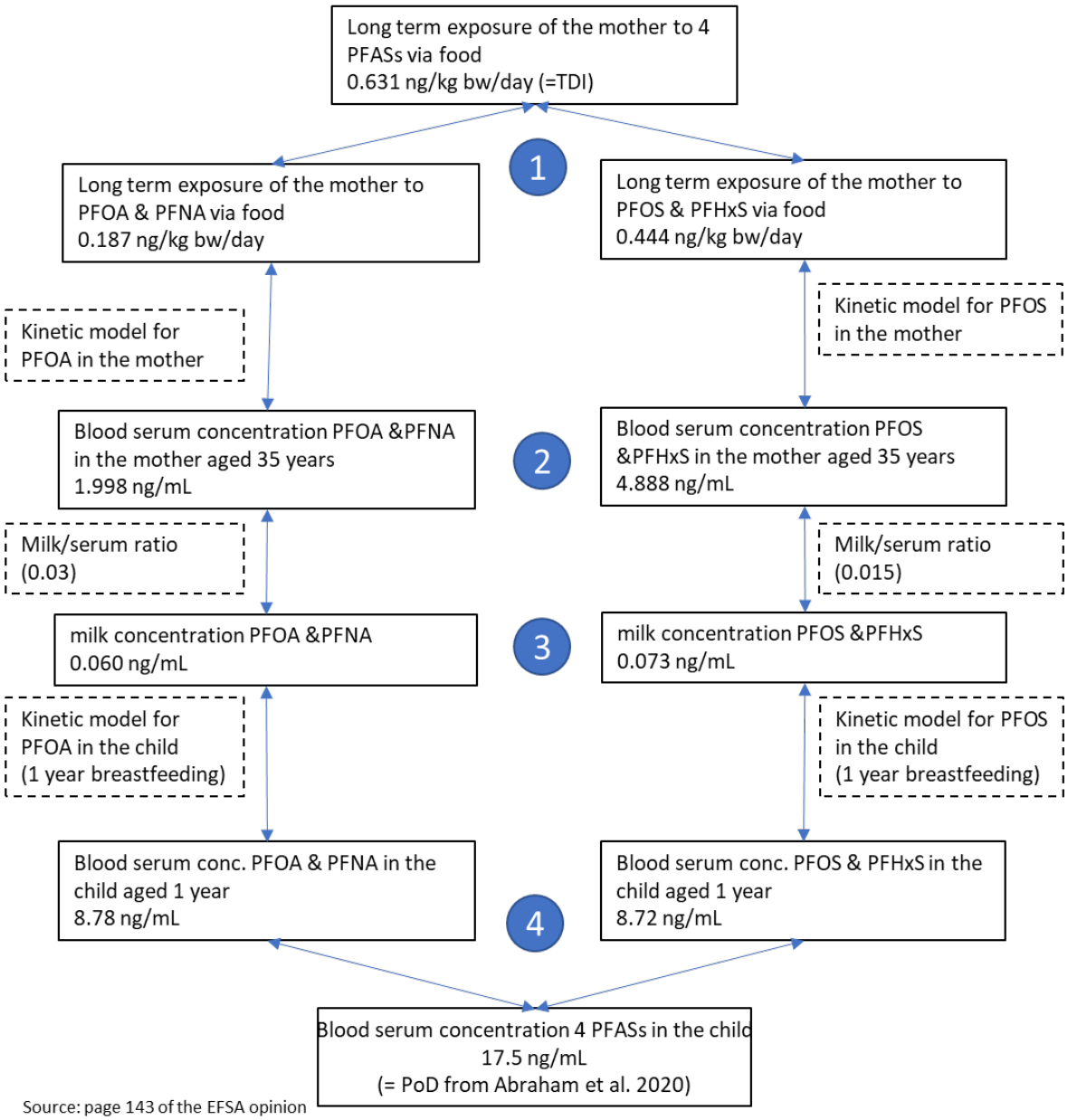
In its calculations EFSA (2020a) establishes a Tolerable Daily Intake (TDI) which is then converted into a Tolerable Weekly Intake (TWI). Both terms are used interchangeably below given that they are directly linked to each other. EFSA's point of departure (PoD) is a blood serum level of 17.5 ng for the sum of four PFASs per mL blood serum in children who have been exposed via breastfeeding over a period of 1 year. If this blood serum concentration is exceeded (detrimental) effects on the children's immunity cannot be excluded. The TWI is constructed in such a way (using kinetic modelling) that if the mothers' exposure via food is below the TWI throughout their entire lives up to and including the period during which they breastfeed their child, their breast milk will contain sufficiently low concentrations of the four PFASs. If their child is then breastfed over a period of 1 year, the exposure via the breast milk will result in a blood serum concentration of the sum of four PFASs which remains below the level of 17.5 ng/mL. In other words, the TWI is the quantity that adults can ingest daily for long periods of time without the blood serum of breastfed children reaching the critical value for immune effects.
It is assumed that the four PFASs in the child's blood are equally potent when it comes to causing immune effects and that PFOA&PFNA and PFOS&PFHxS each make up half the PoD. EFSA regards PFOA and PFNA as being one and the same substance (referred to here as PFOA&PFNA), because the assumption is that both substances are equally potent and have the same kinetics. Kinetics are the processes which describe the intake, distribution, degradation and excretion of substances in or out of the body. The same applies to PFOS and PFHxS. The assumption that PFOA&PFNA and PFOS&PFHxS each make up half of the PoD is based on the corresponding concentrations of PFOA&PFNA (17.4 ng/mL) and PFOS&PFHxS (17.3 ng/mL) found in the blood of children in the study by Abraham et al. (2020) on which basis the PoD was established. In the case of the breastfed children in the study by Abraham et al. (2020) the average blood concentrations were: PFOA = 16.8 ng/mL, PFOS =15.2 ng/mL, PFHxS = 2.1 ng/mL and PFNA = 0.6 ng/mL.

Figure A1.1 contains a diagram of this process. Note: Because the kinetics and blood/milk ratio differ between PFOA&PFNA and PFOS&PFHxS, the route from TDI to blood serum concentration in the child (and vice versa) splits.

Steps in the argumentation for establishing TWI (or TDI):

1. The mother is exposed throughout her life and
2. builds up a serum concentration as a result.
3. When the child is born, a fraction of the 4 PFASs transfers from the mother's body to the milk
4. The child is breastfed and also builds up a serum concentration.

These steps were followed in reverse to calculate the TDI (in a potential mother) from the PoD (BMDL in the child).



Source: page 143 of the EFSA opinion

Figure A1.1 Diagram showing the steps in the argumentation establishing the EFSA TWI for PFASs. See the above text for a clarification.

First and foremost it can be deduced from the diagram in Figure A1.1 that the TWI consists of the sum of a PFOA&PFNA and a PFOS&PFHxS part, which parts are interdependent. If one of the two parts is high, the other part must be low in order to remain below the PoD. When added together they should not lead to the PoD being exceeded, in other words 17.5 ng of the sum of the PFASs/mL in the serum of 1--year-old infants. Secondly, it is noticeable that a different exposure to PFOA&PFNA and PFOS&PFHxS in the mother (of 0.187:0.444, so approximately 1:2) leads to an identical serum concentration in the child. In other words, if the mother is exposed to 2 parts of PFOS&PFHxS, that will result in the same serum concentration in the child as after exposure to a single part of PFOA&PFNA. This can largely be explained by the different milk/serum ratios for PFOA&PFNA and PFOS&PFHxS, and to a lesser extent by the differences in toxicokinetics in the PBK models applied. The milk/serum ratio indicates the proportions of the substances found in the mother's milk and blood. In the case of PFOA, for example, 0.03 ng of PFOA can be found in 1 mL of breast milk if the mother's serum contains 1 ng of PFOA/mL, while 0.015 ng PFOS/mL of breast milk corresponds to a serum concentration of 1 ng of PFOS/mL.

These two findings relating to the EFSA calculation show that the blood serum concentration of breastfed children exceeds the critical value (PoD of 17.5 ng/mL) for immune effects if PFOA&PFNA completely make up the (sum) TWI. In other words, the TWI is dependent on the mixture ratio of PFOA&PFNA and PFOS&PFHxS. See Appendix A2 for details. The TWI does not suffice for other mixture ratios of the external exposure (1:2 for PFOA&PFNA : PFOS&PFHxS).

Other assumptions made by EFSA in order to establish a TWI from the human study in which children were exposed to a mixture of PFASs are that the four PFASs (external dosage) are equipotent and that other PFASs (other than the four EFSA PFASs) do not contribute to the effects on the immune system. The latter is not based on the demonstrated absence of immune effects from studies with other PFASs, but on the absence of relevant information. Such required studies were actually not carried out.
Given that perfluoralkyl acids (PFAAs such as PFOA and PFOS, but also PFAAs with longer or shorter chains) generally cause similar effects (ATSDR, 2018; EFSA, 2020a; Bil et al. 2021), it cannot be excluded that PFASs other than the four EFSA PFASs can also cause immune effects. The potency to cause immune effects could differ between the different PFASs. This is likely in view of the different potencies between PFASs which are seen in one of the general effects caused by PFASs, namely liver effects (Bil et al. 2021). RIVM's provisional analysis of NTP studies (NTP 2019a and 2019b) revealed that potency differences exist between PFASs in, for example, different organ weights, hormone levels, clinical chemistry, white blood cell parameters and pathology endpoints. Generally speaking the PFASs potencies are classified in the same way as found in Bil et al. (2021). With regard to the four EFSA PFASs, the sequence (from low to high potency) is: PFHxS < PFOA < PFOS < PFNA. The order of magnitude of the potencies (compared with PFOA) is also comparable to the RPFs found in Bil et al. (2021).

Appendix A2: Mixture ratio of PFOA&PFNA and PFOS&PFHxS

It can be deduced from the EFSA TWI diagram that the TWI consists of the sum of a PFOA&PFNA and a PFOS&PFHxS part, which parts are interdependent. When added together they should not, in fact, lead to the PoD being exceeded, in other words 17.5 ng of the sum of the PFASs/mL in the serum of 1-year-old infants.
On the basis of the existence of the sum of the PFASs in the Abraham et al (2020) study – namely 50.1% PFOA&PFNA and 49.9% PFOS&PFHxS – EFSA provides the following PBK conversion factors for extrapolating maternal exposure to the serum level in a 1-year-old child. It should be noted that the (almost) equipotency assumption is introduced here at the serum level of the 1-year-old child, and not at the level of the (external) intake of the mother:

PFOA&PFNA conversion factor: 8.78 ng/mL/0.187 ng/kg bw/day ≈ 50
PFOS&PFHxS conversion factor: 8.72 ng/mL/0.444 ng/kg bw/day ≈ 20.

Put briefly, the serum level of the 1-year-old infant = 50*maternal PFOA&PFNA intake + 20*maternal PFOS&PFHxS intake.

These conversion factors are not exact, given that the kinetics models are not linear. However, the factors do provide an indication and make it easy to estimate whether exposure to a PFASs sum mixture constitutes a health risk.
Using the conversion factors it is also possible to determine what the TDI ought to be in the event of exposure only to PFOA&PFNA. EFSA makes no distinction between PFOA and PFNA as regards potency and kinetics (model). Consequently no distinction can be made between these two PFASs.
The maximum permissible PFOA&PFNA exposure (which makes up the PoD in the 1-year-old child) is equal to 17.5/50 ≈ 0.4 ng/kg bw/day, and that is markedly lower than the TDI of 0.63 ng of the sum of the PFAS/kg bw/day. This does not apply to PFOS&PFHxS, where the maximum permissible exposure is 17.5/20 ≈ 0.9 ng/kg bw/day. That is higher than the TDI of 0.63 ng of the sum of the PFAS/kg bw/day.
The line in Figure A2.1 indicates all PFOA&PFNA PFOS&PFHxS combinations for the exposure of the mother which fulfil the PoD = 17.5 ng/mL in serum of 1-year-old infants in accordance with the above conversion factors.
It should be noted that these calculations could be carried out more accurately if the PBK models for mother and child were used to calculate the exposure of the mother to the serum concentration of the child (or vice versa).

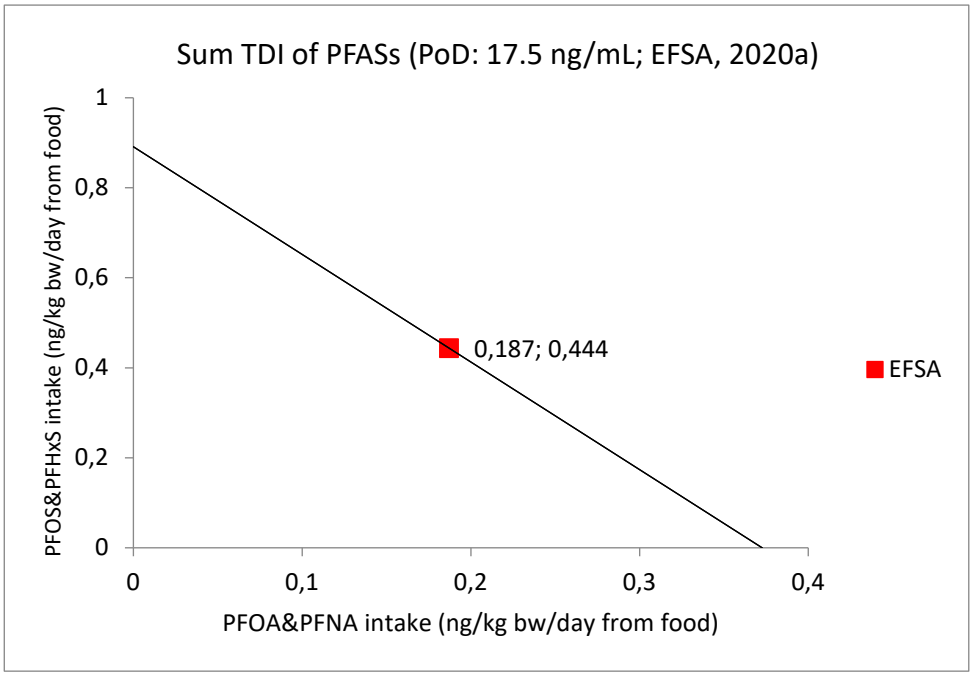


Figure A2.1 Line under which a mixture does not make up the PoD. The EFSA equipotency point corresponds with 8.78 ng/mL of serum for PFOA&PFNA and 8.72 ng/mL of serum for PFOS&PFHxS.

Appendix B: Description and comparison of the different methods for applying the EFSA TWI

The term EFSA PFASs is used below to refer to the four PFASs assessed by EFSA, namely PFOA, PFNA, PFOS and PFHxS. The possible applications for the (EFSA) sum-TWI (4.4 ng/kg bw/week) are described below. The possibilities are summarised in the event that one, two, or three of the four PFASs selected by EFSA have to be assessed, or PFASs other than the EFSA PFASs (options e and f) as well.
Given that EFSA does not make any distinction between PFOA and PFNA as regards potency and kinetics, nor between PFOS and PFHxS, these are jointly described as PFOA&PFNA and PFOS&PFHxS, unless they are explicitly referred to separately.

The table below summarises the extent to which the described methods a to f fulfil the points to consider described in the main text, namely that:

1) the method is applicable to individual PFASs, the EFSA PFASs and for more PFASs than the four considered by EFSA;
2) PFASs mixtures can be assessed in different compositions;
3) differences in potency between PFASs are taken into account,
4) the method is conceptually simple and usable in practice.
   As regards the practical applicability, an assessment is made as to whether the method is unambiguous. In other words, whether there is a single approach which is applicable to individual PFASs and different PFASs mixtures and to different concentrations (e.g. in water and food) and exposures. In addition, the method is considered impractical when resulting in multiple limit values, risk limits, or standards which may be dependent on the mixture composition.

Point to consider	**method**					
	a	**b**	**c**	**d**	**e**	**f@**
1) applicable to PFASs other than the four EFSA PFASs?	No	No	No	No	Yes**	Yes**
2) can mixtures of various compositions be assessed?	Yes*	No	Yes*	Yes*	Yes**	No/Yes
3) can different potencies be taken into account?	No	No	Yes, partially#	Yes, partially$	Yes, partially$	No/Yes, partially$
4a) conceptually simple?	Yes	Yes	No	No	Yes	No
4b) usable in practice?	No	Yes	No	No	Yes	No

* only for the four EFSA PFAS
** for the 23 PFASs for which RPFs are available (Appendix C)
# yes for differences in toxicokinetics, incl. milk/serum ratio, but not for differences in toxicodynamics
$ for differences in toxicokinetics and dynamics, but excluding differences caused by the milk/serum ratio.
@ combination of methods b and e

a) Divide TWI by 4:
The TWI for an individual EFSA PFAS is then 4.4*1/4=1.1 ng/kg bw/week (~0.16 ng/kg bw/day). In the event that two or three EFSA PFASs have to be assessed together, the TWI is 4.4*2/4=2.2 ng/kg bw/week (~0.32 ng/kg bw/day) and 4.4*3/4=3.3 ng/kg bw/week (~0.47 ng/kg bw/day) respectively.

- Advantage: conceptually simple.
- Advantage: the TWI/2 (in the event that PFOA and PFNA have to be assessed together, or in the event that PFOS and PFHxS occur together)

and the TWI/4 stay below the limit for PFOA&PFNA and PFOS&PFHxS individually of 0.4 and 0.9 ng/kg bw/day respectively.
- Disadvantage: worst-case, because if there is only a single EFSA PFAS present, it is not allowed to fully make up the PoD. Idem for two or three EFSA PFASs.
- Disadvantage: takes no account of possible potency differences.
- Disadvantage: four risk limits have to be established for instances in which one, two, three and four EFSA PFASs occur.
- Disadvantage: only the four EFSA PFASs can be assessed, meaning other PFASs cannot. To be able to assess other PFASs as well, EFSA's assumption that the four EFSA PFASs are equipotent could also be extended to other PFASs. Given that this assumption is considered improbable (see Appendix A1), it is not explored in any further detail.

b) Filling up the TWI:
If exposure only takes place to one of the four PFASs, that single EFSA PFAS may fill up the TWI. Consequently, the TWI of 4.4 ng/kg bw/week (=0.63 ng/kg bw/day) applies for each EFSA PFAS. Idem for two or three EFSA PFASs, whereby the sum of two or three EFSA PFASs may fill up the TWI.
- Advantage: conceptually simple.
- Advantage: less worst-case compared with a) 'Divide TWI by 4'.
- Advantage: a single TWI is more practical/clearer when it comes to establishing risk limits.
- Disadvantage: as described above the exposure to a single PFOA&PFNA can lead to the PoD being exceeded, while the exposure to just PFOS&PFHxS might be slightly higher before the PoD is made up. In other words, this method takes no account of possible potency differences.
- Disadvantage: only the four EFSA PFASs can be assessed, meaning other PFASs cannot.

c) Filling up the PoD, with due regard for the kinetic differences between PFOA&PFNA and PFOS&PFHxS:
In accordance with Figure A2.1 (Appendix A2) and the description, the TWI for PFOA&PFNA must be slightly lower than the sum TWI of 4.4 ng/kg bw/week, while the TWI for PFOS&PFHxS may be slightly higher in order to make up the PoD. The TWI for PFOA&PFNA is then 2.6 ng/kg bw/week (=0.4 ng/kg bw/day) and for PFOS&PFHxS it is 6.2 ng/kg bw/week (=0.9 ng/kg bw/day). In the case of combinations of PFOA&PFNA and PFOS&PFHxS, the TWIs depend on each other in accordance with the line in Figure A2.1 (Appendix A2).
- Advantage: correctly takes account of the kinetic differences between PFOA&PFNA and PFOS&PFHxS.
- Advantage: Figure A2.1 is clear. If the combination exposure remains below the line, no effects are expected.
- Disadvantage: conceptually more difficult to understand how Figure A2.1 comes about. Requires more explanation.
- Disadvantage: a line (like Figure A2.1) also has to be established for risk limits.

- Disadvantage: possible differences in toxicodynamics are not taken into account.
- Disadvantage: only the four EFSA PFASs can be assessed, meaning other PFASs cannot.

d) Potency dependent on filling up the TWI:

Divide the sum TWI into separate TWIs on the basis of RPF values established by RIVM. The basic principle is that if a single EFSA PFAS is present, this can fill up the TWI, but if there are several, the contribution of each individual EFSA PFAS ought to be lower. The RPFs are 1 (PFOA), 10 (PFNA), 2 (PFOS) and 0.6 (PFHxS). So if, for example, PFNA and PFOS are present, the TWI for PFNA is 4.4*10/(10+2)=3.7 ng/kg bw/week (~0.5 ng/kg bw/day) and 4.4*2/(10+2)=0.73 ng/kg bw/week (~0.10 ng/kg bw/day) for PFOS.

- Advantage: you make a distinction in the potency between the different EFSA PFASs, for example PFOA and PFNA.
- Disadvantage: with four EFSA PFASs there are 15 possible mixtures to come up with (comprising one, two, three or four EFSA PFASs) in an infinite number of compositions and therefore also an infinite number of TWIs. This is impractical when it comes to establishing risk limits.
- Disadvantage: RPFs do not include the possible differences in milk/serum ratio.
- Disadvantage: only the four EFSA PFASs can be assessed, meaning other PFASs cannot.

e) RPF method

Similar to the TEF factors for dioxins. Relative potency factors (RPFs) have been established for 23 PFASs on the basis of differences in liver toxicity (Bil et al. 2021). These RPFs can be used to express concentrations or exposures in the sum of PFOA equivalents, which can then be compared to a risk limit or limit value established for PFOA, e.g. the EFSA TWI of 4.4 ng/kg bw/week (=0.63 ng/kg bw/day).

- Advantage: conceptually simple because similar to TEFs & TEQ for dioxins and only one TDI or risk limit is needed.
- Advantage: takes the different potencies of PFASs into account.
- Advantage: more broadly applicable than the four EFSA PFASs, namely to the 23 PFASs for which RPFs are available. But not for all (the thousands of) PFASs.
- Advantage: possible to apply substance-specific considerations (e.g. BAFs) at a later stage.
- Disadvantage: uncertain whether the assumption applies to all PFASs that RPFs established from liver effects are also applicable to other effects (such as immunotoxicity).
- Disadvantage: RPFs do not include the possible differences in milk/serum ratio.

f) Filling up the TWI + RPF method

If, in addition to the four EFSA PFASs, other PFASs also have to be assessed, a combination of methods can also be used. Method b can be applied to assess the EFSA PFASs, while method e can also be applied in order to assess several PFASs (incl. the EFSA PFAS).

The mixture must then fulfil the (EFSA) sum TWI (for the four EFSA PFASs), but also a PFOA TDI or risk limit after all PFASs (incl. the EFSA PFASs) have been added up using RPFs.

- Advantage: all the advantages of methods b and e, except conceptual simplicity and advantage of one TWI (see below)
- Disadvantage: all disadvantages of methods b and e. However, some disadvantages of one method can be compensated by using the other method.
- Disadvantage: contradictory conclusions from both methods are possible and the combination of methods is difficult to communicate.
- Disadvantage: conceptually more complicated than just method b or e.
- Disadvantage: possibly two different TDIs or risk limits applicable.

Appendix C: Relative potency factors

Table C.1 shows the available RPFs for different PFASs (Bil et al. 2021). For a number of PFASs there was insufficient toxicological information available to establish an RPF. Bil et al. (2021) established an RPF interval on a read-across basis for these PFASs. To simplify the sum of PEQ calculations the table below shows the upper limit of the interval. This maximum value has been chosen from a precautionary point of view and probably completely covers the uncertainty in the RPFs for the PFASs in question. It is also assumed that the RPF of a specific PFAS is applicable to both the linear and the branched isomers of that PFAS.

Table C.1 Relative potency factors of 23 PFASs

PFASs	**PFAS abbreviation**	**CAS number of linear PFASs**	**RPF**
Sulphonic acids			
Perfluorobutanesulfonic acid	PFBS	375-73-5	0.001
Perfluoropentanesulfonic acid *	PFPeS	2706-91-4	0.6
Perfluorohexanesulfonic acid	PFHxS	355-46-4	0.6
Perfluoroheptanesulfonic acid *	PFHpS	375-92-8	2
Perfluorooctanesulfonic acid	PFOS	1763-23-1	2
Perfluorodecanesulfonic acid	PFDS	335-77-3	2
Carboxylic acids			
Perfluorobutanoic acid	PFBA	375-22-4	0.05
Perfluoropentanoic acid *	PFPeA	2706-90-3	0.05
Perfluorohexanoic acid	PFHxA	307-24-4	0.01
Perfluoroheptanoic acid *	PFHpA	375-85-9	1
Perfluorooctanoic acid	PFOA	335-67-1	1
Perfluorononanoic acid	PFNA	375-95-1	10
Perfluorodecanoic acid *	PFDA	335-76-2	10
Perfluoroundecanoic acid	PFUnDA	2058-94-8	4
Perfluorododecanoic acid	PFDoDA	307-06-7	3
Perfluorotridecanoic acid *	PFTrDA	72629-94-8	3
Perfluorotetradecanoic acid	PFTeDA	376-06-7	0.3
Perfluorohexadecanoic acid	PFHxDA	67905-19-5	0.02
Perfluorooctadecanoic acid	PFODA	16517-11-6	0.02
Ether carboxylic acids			
2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid	HFPO-DA (~GenX)	13252-13-6	0.06
Ammonium 4,8-dioxa-3H-perfluorononanoate	ADONA	958445-44-8	0.03
Telomer alcohols			
1H,1H,2H,2H-perfluorooctanol	6:2 FTOH	647-42-7	0.02
1H,1H,2H,2H-perfluorodecanol	8:2FTOH	678-39-7	0.04

* In Bil et al. (2021) the RPF is established as interval on the basis of read-across.