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### **D1.6 – Guidance document for an enhanced HRIV concept for drinking water**

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## Executive Summary

In Germany, substances in drinking water that are not subject to a limit value according to the Drinking Water Ordinance are assessed using two different concepts, which differ depending on the available toxicological data. If there is sufficient data (e.g. chronic study data according to standardized test procedures), health-based guide values are calculated. If this data is missing, the assessment gap is closed using the Health-Related Indicator Value (HRIV) concept. This is based on a precautionary approach that uses hierarchical assessment steps to assign corresponding concentration values (e.g. 0.1 µg/l) to various endpoints (e.g. genotoxicity). In the absence of exculpatory data for an endpoint, the HRIV is set at the corresponding value until new data is available and exoneration makes a higher concentration value possible. Although the HRIV is not legally binding, it is accepted in practice by all stakeholders in Germany (authorities, water suppliers and industry).

In Germany, there has been a limit value for 20 PFAS in the drinking water regulations since 2023. However, there is no conclusive regulatory assessment concept for the much larger number of PFAS. Due to the large number of PFAS, data from chronic animal experiments cannot be generated for all substances, so an alternative approach, i.e. via the HRIV concept, must be created in order to protect drinking water and consumers.

The question of a corresponding endpoint that could be used as an indicator for the large heterogeneous number of PFAS was open. In PROMISCES, various mechanisms of action of PFAS were examined using a large in vitro test battery. This was supplemented by in silico modeling in order to be able to represent an even larger number of PFAS. The results show that PFAS trigger endocrine mechanisms of action (receptor binding/inhibition). In the cell-based bioassays, these were mainly effects on the binding of a thyroid hormone to the corresponding transport protein (TTR-TR).

Endocrine effects can subsequently cause further adverse effects that can result in serious impairments in humans. Therefore, a sufficiently protective level for drinking water should be set for the effects of PFAS identified in PROMISCES. We therefore propose a further enhancement of the current HRIV concept with an endpoint for thyroid effects, which can be used as an indicator effect for PFAS until further data are available. The corresponding HRIV would be in the concentration range 0.1 µg/l or 0.01 µg/l.

A decision on this must be discussed in Germany in the relevant committees (Drinking Water Commission) and decided on with the Federal Ministry of Health.

One possibility at EU level could be to discuss this endpoint identified in PROMISCES as a basis for a holistic (individual) PFAS assessment. Existing assessments are mostly limited to the endpoint of liver toxicity, for which the data are certainly incomplete. A precautionary approach such as the HRIV concept based on the comprehensive data base from PROMISCES could be a solution.

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## List of Abbreviations:

ADONA	chemical substance; 3H-perfluoro-3-[(3-methoxy-propoxy)propanoic acid], ammonium salt
CALUX	bioassay; Chemical Activated LUCiferase gene eXpression
DIN	German Institute for Standardisation; Deutsches Institut für Normung e.V.
EQS	Environmental quality standard
ER	Estrogen Receptor
EU	European Union
HRIV	Health-Related Indicator Value
ISO	International Organization for Standardization
OECD	Organisation for Economic Co-operation and Development
PFAS	Per- and Polyfluoroalkyl Substances
PFOA	Perfluorooctanoic acid
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SCHEER	Scientific Committee on Health, Environmental and Emerging Risks
T <sub>4</sub>	thyroid hormone; Thyroxine
TR $\beta$	Thyroid hormone receptor beta
TTR	transport protein for T <sub>4</sub> ; Transthyretin
UBA	German Environment Agency; Umweltbundesamt
WHO	World Health Organization

# 1 The Health-Related Indicator Value (HRIV) concept for drinking water in Germany

## 1.1 Introduction

The unmanageably large amount of organic and inorganic substances in production and use means that a significant proportion can be detected in almost all environmental compartments. In addition, there are persistent and mobile metabolites and transformation products, which can further increase the number of substances. The existing European and national legal regulations (in particular REACH) do not completely prevent non-evaluated substances from entering the environment, including substances that are only slowly broken down, are easily soluble in water and are not or hardly at all withheld by sediment passage and/or drinking water treatment. In addition, modern analysis methods allow an increasing number of substances and substance classes to be recorded with high sensitivity in the range of nanograms and picograms per liter.

International institutions such as the World Health Organization (WHO) or national authorities such as the German Umweltbundesamt (UBA) have so far only been able to fully toxicologically assess a comparatively small proportion of the substances released into the environment or water. This is due to the large amount of time and money involved in toxicity studies. To date, the process for the complete toxicological examination and evaluation of a substance usually requires a long-term animal test over at least two years and a genotoxicity study. Ideally, such studies lead to a guideline value, which, among other things, can be used when assessing drinking water quality. If these toxicologically derived values are included in (German) laws or regulations, they are usually set as limit values or requirements.

It is inevitable that substances are repeatedly found in drinking water - usually in low concentrations, for a limited time and only in some places - for which there are no limit values or guide values, but which require a timely toxicological assessment. Therefore, an assessment concept is required with which a statement can be made, even if toxicological data is incomplete or even missing, as to whether the concentration of a substance found in drinking water poses a risk to health, or whether this can be excluded or assumed to be very unlikely.

Improved *in vitro* and *in silico* methodologies for toxicological assessment of environmental media/drinking water were carried out in PROMISCES (see [Deliverable 1.5](#)) to close the assessment gap for PFAS. Based on this work – the development of *in silico* and *in vitro* toxicity profiles of the selected PFAS – the aim of this deliverable will be to select the most relevant toxicological endpoint for PFAS to include this substance class into the current HRIV concept.

The expected goal of this deliverable is to propose a “new” HRIV category for PFAS as a substance group into the existing concept, based on the scientific approach of task 1.3 in PROMISCES. It is not the goal to set specific HRIV for individual PFAS (see beyond).

## 1.2 The current HRIV concept

An HRIV closes the time, data and legal gap between the analytical detection of a substance in drinking water above 0.1 µg/l and the existence of a guideline or limit value. A concentration falling below the HRIV provides sufficient human toxicological safety; exceeding the HRIV does not inevitably lead to a health impact or risk due to the strong precautionary nature.

The basis of the Health-Related Indicator Value (HRIV) concept as it is used in Germany is the general precautionary value of 0.1 µg/l (HRIV<sub>1</sub>). This is based on studies by Dieter (2014), according to which a total of 140 internationally derived drinking water limit and guide values were always higher for 50 toxic and fully evaluated (reference) substances. To date, the only exceptions to this are a few extremely genotoxic substances relevant to drinking water with human-relevant metabolism, for which a lower HRIV<sub>0</sub> of 0.01 µg/l has been set. Certain structural properties of the substances to be examined also provide an indication of possible genotoxicity. Examples of these substances are aromatic amines or nitroso compounds. A more detailed discussion of these so-called structural alerts can be found in the guide “Hazard-based risk management for anthropogenic trace substances to secure drinking water supplies (Tox Box)” (Grummt et al. 2020).

The HRIV is set so low that, even in a subsequent complete human toxicological assessment, it is sufficiently certain that no damage to human health is to be expected if the substance in question is consumed daily over a lifetime through drinking water. This precautionary aspect ensures that increasing completion of the toxicological data usually leads to the same or a higher value, but not a lower value. The less extensive the human toxicological database, the lower the HRIV.

**The German Umweltbundesamt (UBA) only determines an HRIV for a specific substance upon application or request by authorities or water suppliers and only if the substance is detected in German drinking waters with concentrations > 0.1 µg/l.**

First, data on genotoxicity is researched and evaluated, as laid out in figure 1. If studies demonstrate a genotoxic effect or if there are insufficient data to safely rule out genotoxicity, the HRIV is set at 0.1 µg/l (HRIV<sub>1</sub>). If there is also a human-relevant metabolism that activates the substance in the body with a strong genotoxic effect, the HRIV is reduced to 0.01 µg/l (HRIV<sub>0</sub>).

If the substance to be evaluated shows an estrogen-like effect in the human cell-based estrogen receptor (ER)-alpha reporter gene assay, the substance is purchased with an HRIV<sub>0</sub> of 0.01 µg/l on the 17beta-estradiol equivalent concentrations.

**The examination for endocrine effects will gradually be expanded to include further endpoints as soon as standardized test procedures (e.g. according to DIN) are available.**

If genotoxic and endocrine effects can be ruled out, the next step is to look for data on further severe toxic effects as immunotoxicity, reproduction toxicity or neurotoxicity. If at least one of these can be proven with sufficient documentation or cannot be reliably excluded, the HRIV is set at 0.3 µg/l (HRIV<sub>2</sub>). The final two assessment levels include subchronic (HRIV<sub>3</sub> = 1.0 µg/l) and chronic (HRIV<sub>4</sub> = 3.0 µg/l) toxicity.

As soon as sufficient toxicological data is available (chronic study data), the HRIV will be replaced by a health-related guide value (h-r guide value). If an h-r guide value can be derived, UBA is removing the previous HRIV for the substance from the HRIV list.

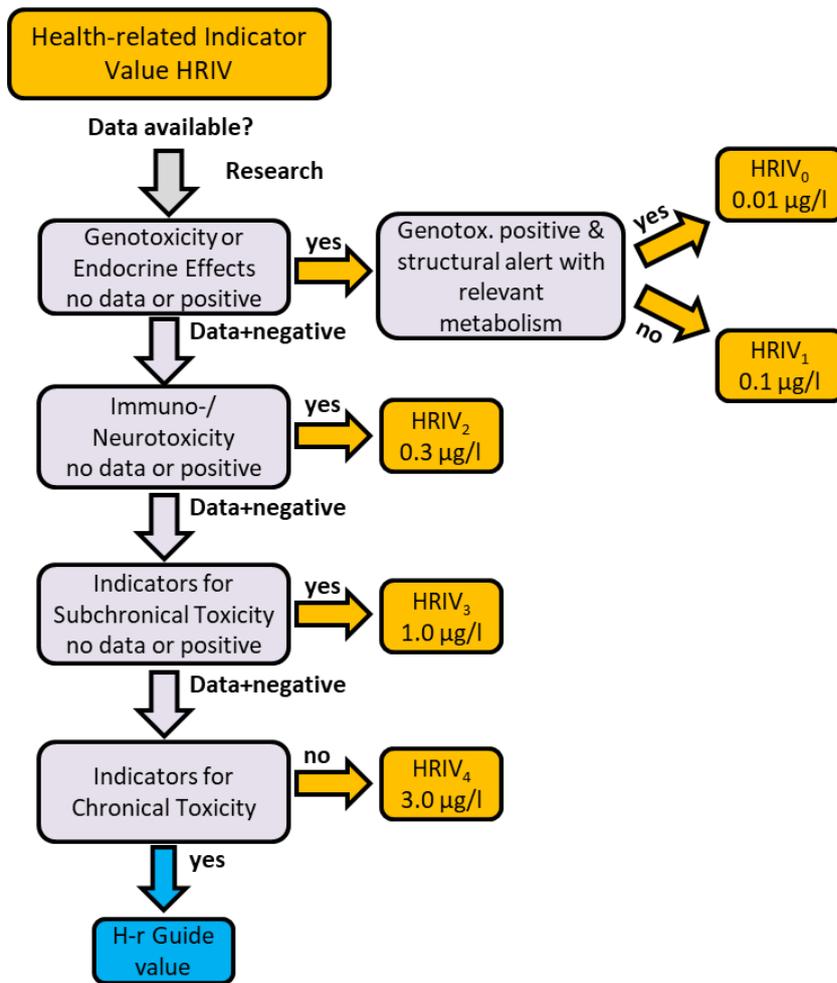


Figure 1: Current HRIV concept in Germany

## 2 Enhancement of the HRIV concept for PFAS

With regard to PFAS, the in vitro bioassays and in silico modeling carried out in the PROMISCES project indicate primarily an endocrine mechanistic effect (PROMISCES D1.5, 2024), which may not necessarily be clinically expressed, but is of concern for precautionary reasons. Endocrine disruptors can trigger a variety of effects in humans, some of them serious, through different mechanisms of action (neurotoxicity, genotoxicity, ...). (figure 2). The HRIV should therefore be set as sufficiently protective.

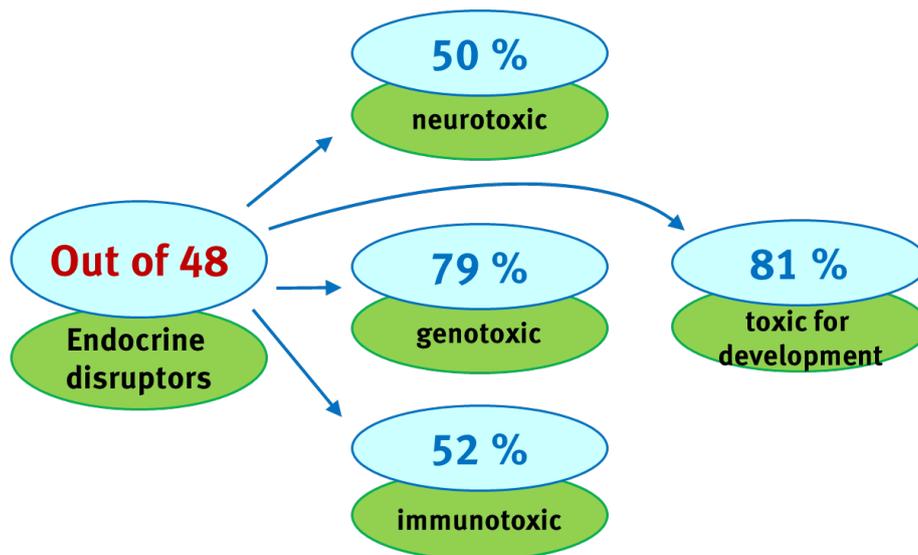


Figure 2: Possible adverse outcomes of endocrine disruption (adapted from Choi et al. 2004)

If an HRIV is exceeded by a factor of 10, but with a cap of 10 µg/l for HRIV<sub>4</sub>, it can be considered as improbable that damage to health is to be feared due to the health-related precautionary character of the HRIV<sub>1-4</sub>. An exception is HRIV<sub>0</sub>, which stands for mutagenic substances without an effect threshold. If this is exceeded, measures should be taken to ensure compliance again (Dieter 2014). In all other cases an exceedance within this framework should therefore primarily be seen as a compelling reason to expand knowledge about the entry pathways into or occurrence in drinking water and the toxicological profile of the substance, as well as for an analysis of possible minimization measures.

We were able to demonstrate that sensitive effect-based in vitro toxicity tests with human cells can play an important role to assess the in vitro toxicity of the selected 45 PFAS and some industrial PFAS products e.g., ADONA, GenX (table 1). This data shows that the most promising bioassay for the sum of known and unknown PFAS is the TTR-TRβ CALUX, which is based on their common property to bind to specific thyroid hormone transport proteins and thereby interfering with the thyroid-hormone system with possible adverse health consequences. Details can be found in PROMISCES [deliverable D1.5](#).

Table 1: Tested PFAS with endocrine effects (ER: estrogen receptor; AR: androgen receptor; TTR-TR $\beta$ : Thyroxin-Transthyretin-thyroidal receptor): red: effect; yellow: equivocal; green: no effect.

Substance	Synonyme	Receptor-binding		
		ER $\alpha$ CALUX	anti- AR CALUX	TTR- TR $\beta$ CALUX
Scotchgard Pre-2002 Formulation (Tech mix)	tech-1			
Scotchgard Post-2002 Formulation (Tech mix)	tech-2			
perfluorobutanoic acid	PFBA			
perfluoro-4-methoxybutanoic acid	PFMOBA			
2-perfluoro-propoxypropanoic acid	PFPrOPrA			
3-perfluoro-methoxypropanoic acid	PFMOPrA			
perfluoropentanoic acid	PFPeA			
perfluorohexanoic acid	PFHxA			
perfluoroheptanoic acid	PFHpA			
7H-perfluoro heptanoic acid	HPFHpA			
perfluorooctanoic acid	PFOA			
perfluoro(3,7-dimethyloctanoic acid)	P37DMOA			
perfluorononanoic acid	PFNA			
perfluorodecanoic acid	PFDCa			
Perfluoroundecanoic acid	PFUnDA			
2H,2H,3H,3H-Heptadecafluoroundecanoic Acid	4H-PFUnDA			
Perfluorododecanoic acid	PFDoDA			
perfluorobutanesulfonic acid	PFBS			
Perfluoropentane sulfonic acid	PFPeS			
Perfluorohexane sulfonic acid	PFHxS			
Perfluoroheptane sulfonic acid	PFHpS			
Perfluorooctane sulfonic acid	PFOS			
3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctane-1-sulfonic acid	6:2 FTSA H4-PFOS			
perfluorononane sulfonic acid	PFNS			
perfluorodecane sulfonic acid	PFDS			
2:2 fluorotelomer alcohol	2:2 FTOH			
1H,1H,2H,2H-Perfluorohexan-1-ol	4:2 FTOH			
1H,1H,2H,2H-Perfluorooctan-1-ol	6:2 FTOH			
1H,1H,2H,2H-Perfluoro-1-decanol	8:2 FTOH			
1H,1H,2H,2H-Perfluorododecan-1-ol	10:2 FTOH			
2H-Perfluoro-2-decenoic Acid	8:2 FTUCA			
1H,1H,2H,2H-Perfluorohexanesulphonic acid	4:2 FTSA H4-PFHxS			
N,N-Dimethyl-3-((perfluorohexyl)ethylsulfonyl)aminopropanamine N-oxide	6:2 FTSAM (DPOSA)			
Bis[2-(perfluorohexyl)ethyl]phosphate)	6:2 diPAP			
4,8-dioxa-3H-perfluorononanoic acid	ADONA			
Ammonium perfluoro(2-methyl-3-oxahexanoate)	HFPO-DA GenX			

Substance	Synonyme	Receptor-binding		
		ER $\alpha$ CALUX	anti- AR CALUX	TTR- TR $\beta$ CALUX
2-[Dimethyl-[3-(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctylsulfonylamino)propyl]azaniumyl]acetate	6:2 FTAB CDPOS Capstone B			
Perfluorobutylsulphonamide	PFBSA			
N-Methyl perfluorobutane sulfonamide	MeFBSA			
Perfluorbutanesulfonylamide(N-methyl)acetate	N-MeFBSAA			
Perfluorooctane sulfonamide	PFOSA			
N-Ethyl perfluorooctane sulfonamide	EtFOSA			
N-Methyl perfluorooctane sulfonamide	MeFOSA			
Perfluorooctane sulfonamidoacetic acid	FOSAA			
N-Methyl perfluorooctane sulfonamidoacetic acid	MeFOSAA			
N-Ethyl perfluorooctane sulfonamidoacetic acid	EtFOSAA			

Based on the results of PROMISCES, it is proposed to expand the HRIV concept at the level of the endpoint endocrine effects (HRIV<sub>0</sub>), in addition to the effect on the estrogen receptor, to include the inhibitory effect of PFAS on the transport protein transthyretin (TTR) of the thyroid hormone thyroxine (T<sub>4</sub>). In the context of the discussions at EU level, to introduce sum parameters for PFAS in the ongoing revision of the Environmental Quality Standards (EQS) Directive, which could be 0.0044 µg/l for 24 PFAS (SCHEER 2022) and 0.0044 µg/l in the Groundwater Directive for the sum of four PFAS (EU 2022), a precautionary embedding of individual PFAS in an HRIV<sub>0</sub> (0.01 µg/l) would be a possible implementation option to be discussed (figure 3).

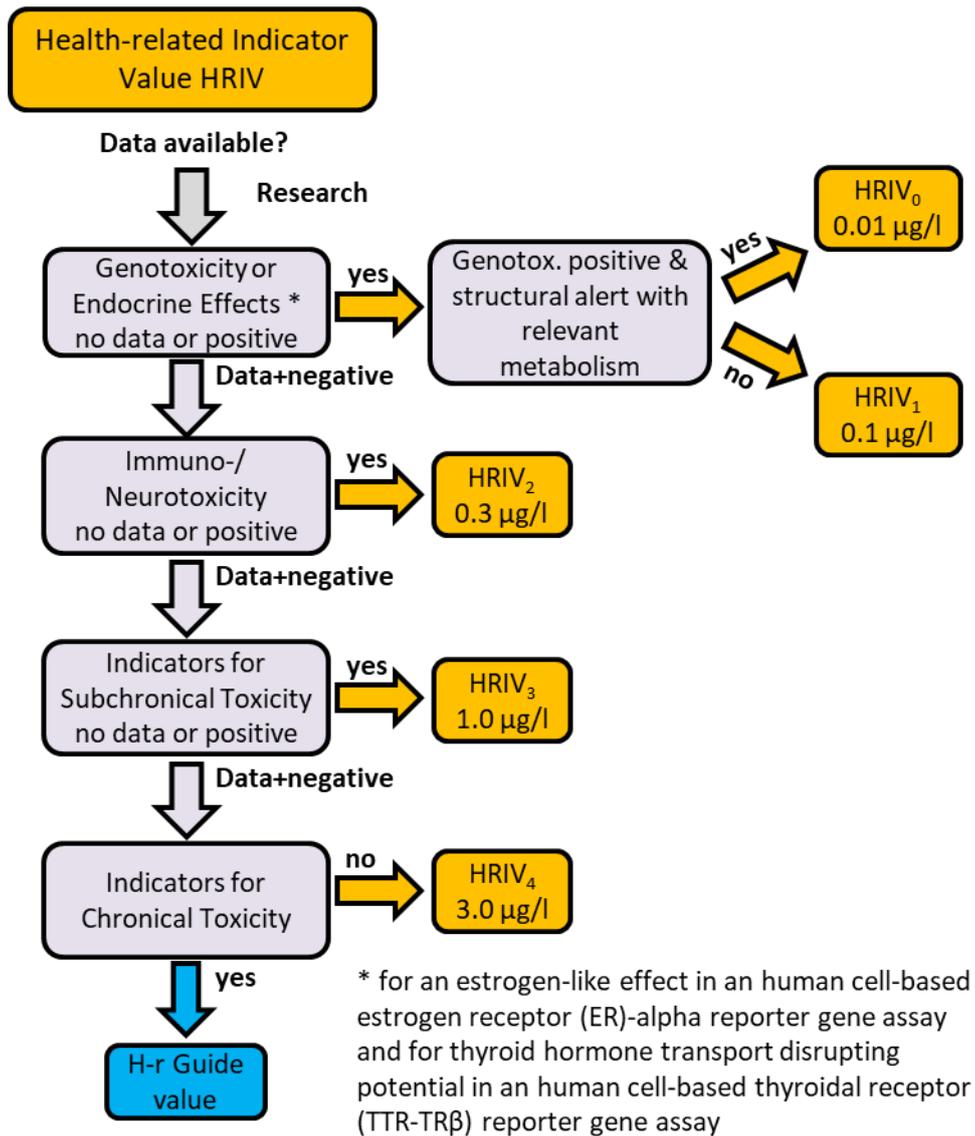


Figure 3: Proposal for an enhanced HRIV concept in Germany for individual PFAS

### 3 Take home message

This project and the data collected are an important **first step** in achieving the possibility to prospectively and uniformly assess individual PFAS in drinking water at a regulatory level in a quick, animal-testing free and efficient procedure.

We have used a complementary approach of *in silico* and *in vitro* testing through parallel testing of a select group of approximately 40 PFAS to identify a toxicological mechanism of action that makes it possible to expand the HRIV assessment concept and thus provide a regulatory tool for the large group of PFAS.

The *in vitro* bioassay analysis, shown in Appendix, applied to 40 PFAS compounds (e.g. all regulated 20 PFAS in drinking water and industrial standards as ADONA and GenX) confirmed that hormone receptors (TR, anti-AR and ER) and especially the inhibition of the thyroid hormone transport protein transthyretin (**TTR**) **are of main importance for testing of single PFAS compounds** and most **promising as an indicator of possible contaminations with PFAS** in general for monitoring complex mixtures.

Therefore, we would recommend focusing further on the research gaps of testing complex mixtures for the sum of known and unknown PFAS via *in vitro* toxicity bioequivalents (e.g., PFOA-equivalents/liter water in case of PFAS).

However, there are still some limitations that need to be overcome before the revised concept is published and implemented. For historical reasons, the authorities and water suppliers often do not have cell culture laboratories where biotests can be carried out, but "only" chemical-analytical laboratories. In addition, there are a few service providers on the market that can offer these cell-based tests. This gap in laboratory infrastructure could cause problems if cell-based assessment methods were to be widely used to test PFAS in drinking water. The lack of international standardization of test methods (e.g. OECD test guidelines) is an additional barrier for accredited laboratories to implement these methods.

In summary, there is still a lack of knowledge, experience and acceptance for short-term implementation. Therefore, the approval of the German ministry as well as the supportive vote of the Drinking Water Commission will be necessary. Furthermore, it would be very useful and helpful to stipulate the test methods used in an international standard (DIN/ISO/OECD/...).

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## 5 Appendix

Table A.1: *In silico* and *in vitro* toxicological endpoints proposed to be used within PROMISCES by BDS, UBA, QSAR Lab and INERIS for selected PFAS and PMTs (see for references: Behnisch et al., 2021; Crowley et al., 2016; Crouch et al., 1993; DIN 2009; Ding et al., 2000; Eruslanov et al., 2010; Griffiths et al., 1985; Holdenrieder et al., 2001; ISO 2005; Kangas et al., 1984; Kolsek et al., 2014; OECD 2016; OECD 2016; Phan et al., 2022; Scelfo et al., 2012; Stefanowicz-Hajduk et al., 2020; Wang et al., 1999; Wojtala et al., 2014).

Number	Cell-based Endpoints	Bioassays methods	QSAR methods
1	Cell death	Cytotox CALUX RTCA, ATP, CDD+, LDH, necrosis PI	x
2	Genotoxicity	P53 CALUX Ames and micronuclei	x
3	Oxidative stress	Nrf2 CALUX ROS detection (DCFHDA, DHE, GHS)	x
4	Estrogenicity (ER)	ER CALUX	x
5	Androgenicity (AR)		x
6	Inhibition androgenicity (anti-AR)	Anti-AR CALUX	x
7	Progesterone activity (PR)		x
8	Inhibition thyroid hormone activity (anti-TR)	Anti-TR $\beta$ CALUX	x
9	Inhibition thyroid hormone transport activity	TTR TR $\beta$ CALUX	x
10	Inhibition of obesity	Anti-PPAR $\alpha$ CALUX	x
11	Inhibition of obesity	Anti-PPAR $\gamma$ CALUX	x
12	Mineralocorticoid receptor (MR)		x
13	Early warning	PXR CALUX	x
14	Glucocorticoid receptor (GR)		x
15	Neurotoxicity	MEA	