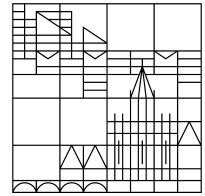


“WHO and EFSA Risk evaluations”

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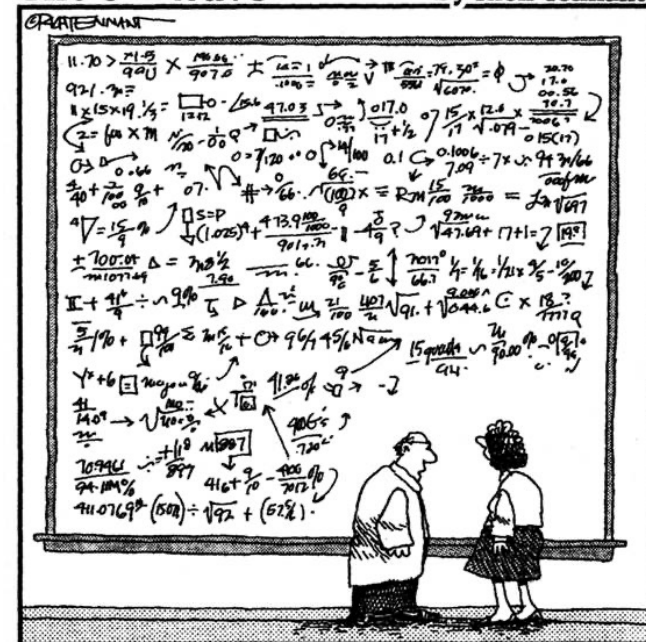
TDI and tolerable drinking-water levels derived by Germany, EFSA, ECHA and WHO

Daniel Dietrich & Helmut Greim

Disclaimer: Helmut never saw the final version, so the opinions expressed are not necessarily all supported by Helmut Greim

The 5th Wave

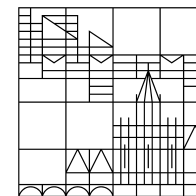
By Rich Tennant



"WHAT EXACTLY ARE WE SAYING HERE?"

Evaluations of PFAS in Europe

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Generally, no regulation of chemicals in individual EU member state:

Chemical dossiers are submitted

- to the European Chemicals Agency (ECHA) to classify, authorize or restrict chemicals: Authorizations and restrictions usually include identification of ADI unless EFSA already developed such data.
- to the European Food Safety Agency (EFSA) to regulate chemicals in food: supported by the competent authorities of member states evaluates risk of food contaminants and proposes ADI values. The documents are prepared by committees of experts from academia and representatives of competent authorities of member states.

European activities on PFAS

UBA 2016: German Drinking-Water Commission: drinking-water standard 0.1 µg/L

EFSA: PFAS in food: tolerable daily intake (TDI): 4.4 ng/kg bw per week (0.63 ng/kg per day) 2020

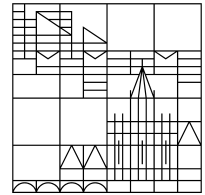
ECHA: Restriction proposal for undecafluorohexanoic acid (PFHxA) oral DNEL 0.03 mg/kg bwt.(2021)

2023: 5 member states propose restriction of about 10 000 per- and polyfluoroalkyl substances (PFASs). ***Basic assumption of a non-threshold toxicity!***

TDI 4.4 ng/kg bw per week referred to EFSA

German Drinking Water Commission (2016)*

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Health Based Values (HBVs) based on TDI $0.1 \mu\text{g/kg bw}$, 70 kg human adult, drinking water intake 2 L/day, 10% allocation factor for presence of similar compounds: $0.1 \mu\text{g/L}$

TDI: NOAEL of 0.1 mg/kg bw for reproductive toxicity of PFOA in rats.

Extrapolation to humans: extrapolation factors (EF) of 10 each for inter- and intraspecies differences, additional SF of 10 for uncertainties (much longer elimination half-life of PFOA in humans than in rats): TDI = $0.1 \mu\text{g/kg bw}$

Drinking-water standard:

TDI $0.1 \mu\text{g/kg bw}$ per 70 kg, drinking 2 L = $3.5 \mu\text{g/L}$,

10% of total PFOA intake via drinking water = **$0.35 \mu\text{g/L}$**

In consideration of the simultaneous presence of similar compounds (PFOA, PFNA, PFHxS and PFOS) (dose addition model) :

Drinking-Water Standard for total PFAS: $0.1 \mu\text{g/L}$

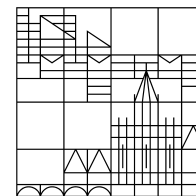
* UBA Drinking-Water Commission (Wilhelm et al 2010, Int J Hygiene Environ Health 213, 224-232)

ECHA (2021)

Restriction proposal for undecafluorohexanoic acid (PFHxA)

Oral DNEL 0.03 mg/kg bwt.

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DNEL-derivation for general population, long-term, systemic effects, all routes, based on:

- Rat, subacute: decreased total T3 and T4, LOAEL 62 mg/kg, AF 1800 (100 x subacute-chronic 6 x LOAEL-NOAEL 3) **DNEL 0.03 mg/kg** (NTP 2018)⁺
- Rat, subchronic: lower body weight NOAEL 10 mg/kg AF 300 (100 x subchronic-chronic 3*, **DNEL 0.03 mg/kg** (Chengelis et al 2009b)
- Rat, chronic: kidney papillary necrosis, NOAEL 30, AF 100, **DNEL 0.3 mg/kg** (Klaunig et al 2015a)

⁺ Due to difference in pathophysiology human relevance of thyroid effects in rats questionable.

* According to ECHA Guidance: subchronic-chronic 2.

ECHA in conjunction with EU- CSS (*Chemicals Strategy for Sustainability*): Proposal for Summary RA and thus limit values (2023)*

RA based on EFSA (2020) TWI of 4.4 ng/kg bw week

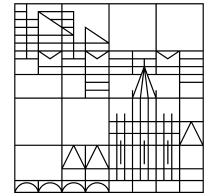
**ECHA ANNEX XV RESTRICTION REPORT version 2, 22.3.2023*

European Food Safety Authority (EFSA) - I

Risk to human health related to the presence of perfluoroalkyl substances in food (2020)

<https://doi.org/10.2903/j.efsa.2020.6223>

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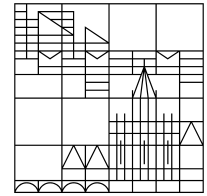
- Epidemiological studies provide clear evidence for ***an association*** between exposure to PFOS, PFOA and PFNA and increased serum levels of cholesterol.
- Epidemiological studies provide evidence for ***an association*** between exposure to PFASs and increased serum levels of the liver enzyme alanine transferase (ALT).
- Studies on PFOS and PFOA published since 2018 confirm previous conclusions from the PFOS and PFOA Opinion (EFSA CONTAM Panel, 2018) that 'there may well be a causal association between PFOS and PFOA and birth weight'. **HOWEVER:** The literature is highly contradictory (meaning pos. and neg. findings on par); No Mode of action of weight by PFASs has been established; no credible indication as to how important a loss of 10g weight / 1 ng/mL increased maternal plasma PFOA with regard to the future development of the offspring is!
- Epidemiological studies published since the publication of the previous EFSA CONTAM Panel Opinion on PFOS and PFOA provide further support for the conclusion that PFOS and PFOA ***are associated*** with reduced antibody response to vaccination, observed in several studies (albeit the most recent study by Abraham et al 2020 appears to dispute the latter, see next slides). Some of the studies suggest that serum levels of PFOS and PFOA are associated with increased propensity for infection. **HOWEVER:** *No Mode of action of immunotoxicity by PFASs has been established!*

European Food Safety Authority (EFSA) - II

Risk to human health related to the presence of perfluoroalkyl substances in food (2020)

<https://doi.org/10.2903/j.efsa.2020.6223>

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Based on studies in animals and humans, effects on the immune system were considered the most critical:

- Children showed an inverse association between serum levels of PFOA, but also the sum of PFOA, PFNA, PFHxS and PFOS, and antibody titers against *haemophilus influenzae* type b (Hib), diphtheria and tetanus in serum sampled from 1-year-old children, predominantly breastfed. NOAECs determined fitting a “knee-function” of 12.2, 16.9, and 16.2 µg/L of PFOA were determined (Abraham et al 2020*).
- A lowest BMDL10 of 17.5 ng/mL at the age of 1 year was derived by EFSA for the sum of PFOA, PFNA, PFHxS and PFOS.
- Using PBPK modelling, a serum level of 17.5 ng/mL in children was estimated to correspond to long-term maternal exposure of 0.63 ng/kg bw per day. Since accumulation over time is important, a **tolerable intake (TWI) of 4.4 ng/kg bw per week** was established. This TWI also protects against other potential adverse effects observed in humans. ***The CONTAM Panel concluded that parts of the European population exceed this TWI, which is of concern.***

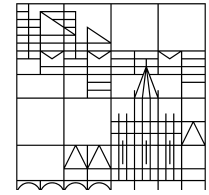
*Abraham et al (2020). Internal exposure to perfluoroalkyl substances (PFASs) and biological marker in 101 healthy 1-year-old children: associations between levels of perfluorooctanoic acid (PFOA) and vaccine response. Arch Toxicol 94, 2131–2147.

Critical Publication for EFSA Evaluation -III

Abraham et al Archives of Toxicology (2020) 94:2131–2147;

<https://doi.org/10.1007/s00204-020-02715-4>

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“Internal exposure to perfluoroalkyl substances (PFASs) and biological markers in 101 healthy 1-year-old children: associations between levels of perfluorooctanoic acid (PFOA) and vaccine response”

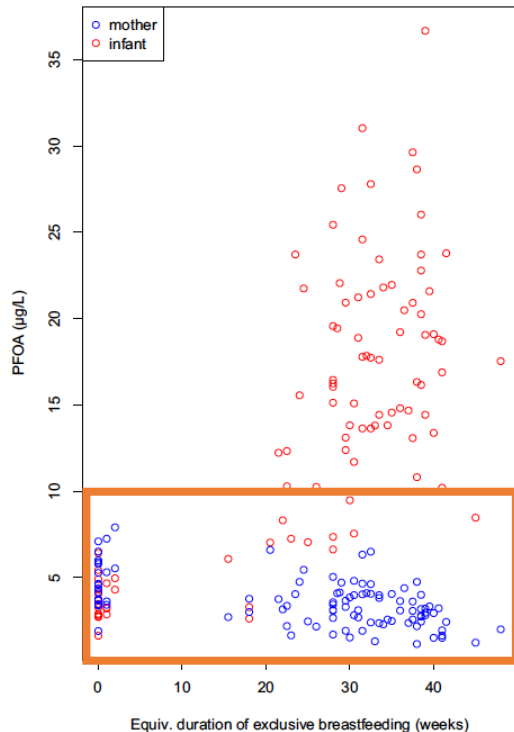


Fig.1 Scatter plot of plasma levels of PFOA in children and their mothers in relation to the equivalent duration of exclusive breastfeeding ($n=101$)

21 Formula-fed; 80 Breast-fed infants:

- Formula-Fed: breast-fed for max 2 weeks
- Breast-Fed for >4 months

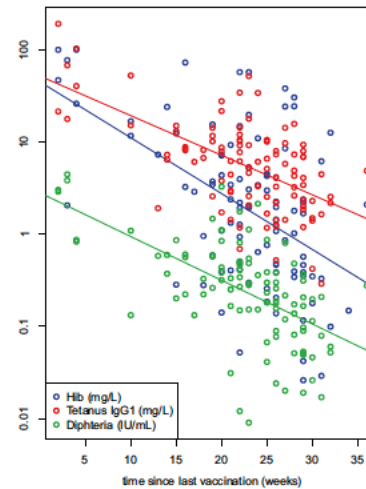


Fig.2 Scatter plot of levels of vaccine antibodies as measured in plasma for Hib ($n=98$), tetanus IgG1 ($n=100$) and diphtheria ($n=100$), in relation to the time since the last vaccination in children with at least two vaccinations

Pairwise assoc. Statistics: Spearmann-Corr.
Threshold $r=0.2$
corresponding to $p<0.05$ as relevant association cut-off.

Problem: Pairwise comparison of >111 parameters with $n=101$

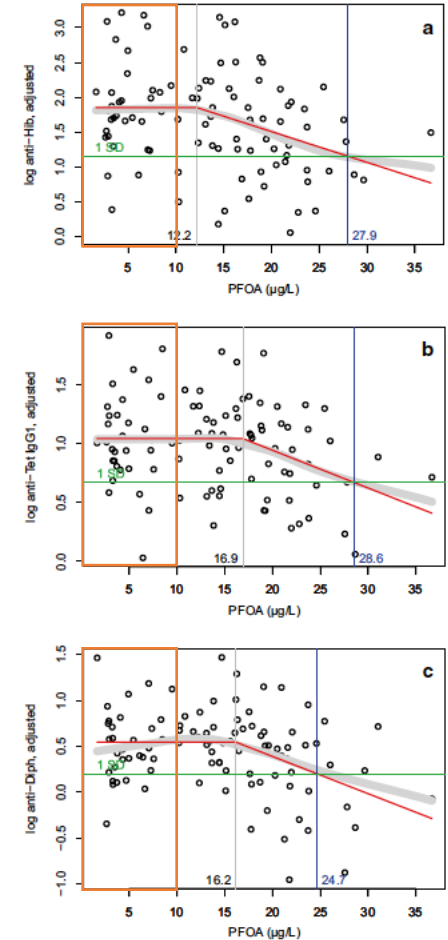
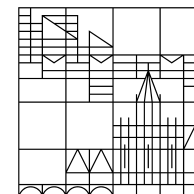


Fig.3 a–c Scatter plot of levels of vaccine antibodies adjusted for the number of vaccinations (in case of tetanus only) and for the time since the last vaccination for Hib (a $n=98$), tetanus IgG1 (b $n=100$) and diphtheria (c $n=100$), in relation to the levels of PFOA. Broad gray band: moving average. Red line: Fitted ‘knee’ function. Horizontal green line: mean minus one standard deviation of the antibody levels below the PFOA ‘knee’ level. Vertical gray line: PFOA level of the ‘knee’. Vertical blue line: PFOA level of the ‘knee’ function with antibody levels averaged lowered by one standard deviation

Critical Publication for EFSA Evaluation -IV

Abraham et al Archives of Toxicology (2020) 94:2131–2147; Universität Konstanz

<https://doi.org/10.1007/s00204-020-02715-4>



“Internal exposure to perfluoroalkyl substances (PFASs) and biological markers in 101 healthy 1-year-old children: associations between levels of perfluorooctanoic acid (PFOA) and vaccine response”

Cytokine production after stimulation	PFOA	PFOS
IFN γ (PHA, PBMCs after 2d)	0.07	-0.03
IL-5 (PHA, PBMCs after 2d)	-0.04	-0.10
IL-10 (PHA, PBMCs after 2d)	-0.22	-0.04
IFN γ (tet. tox. after 3d, n=55)	-0.33	-0.10
IFN γ (diph. tox. after 3d, n=55)	-0.24	-0.21

Lymphocyte proliferation after stimulation with	PFOA	PFOS
PHA (3 days)	-0.08	-0.06
ConA (3 days)	-0.05	0.00
PWM (3 days)	0.09	0.13
Candida (5 days)	-0.17	-0.07
SEB (5 days)	-0.14	-0.07
Tetanus (5 days)	-0.08	0.03
Diphtheria (5 days)	-0.12	-0.02

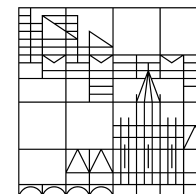
Excerpts from Abraham et al 2020, Table 4

Lymphocyte subpopulations	PFOA	PFOS
T cells (CD3+, % lymph.)	0.08	0.04
CD4 cells (CD3+, % lymph.)	0.11	0.02
CD8 cells (CD3+, % lymph.)	-0.01	0.02
CD4/CD8 ratio	0.05	-0.00
B cells (CD19+, % lymph.)	-0.07	-0.04
T/B ratio	0.08	0.04
NK cells (CD3-CD56+, % lymph.)	0.03	-0.01
TCR $\gamma\delta$ + $\alpha\beta$ - (% CD2+ lymph.)	0.09	0.04
TCR $\gamma\delta$ - $\alpha\beta$ + (% CD2+ lymph.)	-0.12	-0.07
CD45RO+CD45RA- cells (% CD4+ lymph.)	0.05	-0.06
CD27- (% CD4+ lymph.)	0.10	0.16
CD25+ (% CD4+ lymph.)	-0.11	-0.16
HLA+ (% CD4+ lymph.)	-0.05	-0.06
CD29 high CD49d high (% CD4+ lymph.)	-0.16	-0.16
CD45RO+CD45RA- cells (% CD8+ lymph.)	0.23	0.13
CD27- (% CD8+ lymph.)	0.27*	0.25*
CD25+ (% CD8+ lymph.)	-0.10	-0.13
HLA+ (% CD8 high lymph.)	0.12	0.03
CD56+CD57- (% CD8 high lymph.)	0.07	0.13
CD56-CD57+ (% CD8 high lymph.)	0.15	0.12
CD11a high (% CD8+ lymph.)	0.19	0.14
CD21+ (% CD19+ lymph.)	-0.11	0.06

Conclusions: The study revealed **no influence of PFOA and PFOS on infections during the first year of life and on levels of cholesterol.** *“Our results confirmed the negative associations of PFAS levels and parameters of immune response observed in other epidemiological studies, with high consistency as well as comparable NOAECs and effects sizes for the three vaccine antibodies investigated, but for PFOA only. No significant/relevant associations with PFOA levels were observed for other components of the immune system, apart from a positive correlation of PFOA levels and CD45RO+ CD45RA– cells as well as CD27– cells as percentage of CD8+ T cells (but not of CD4+ T cells).”*

Many questions remain!

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- EFSA ***assumes equal or similar activity of the 4 PFAS evaluated***, despite that in the Abraham 2020 study no effect was observed for PFOS. POD is a BMDL10 of 17.5 ng/ml serum for the sum of PFOS, PFOA, PFNA and PFHxS, which was converted to a value of 0.63 ng per kg body weight per day using the Loccisano et al. (2011) PBPK model.
- No Mode of Action and biological relevance for none of the “critical endpoints”:
 - Increased cholesterol (rodent)
 - Increased liver weight (rodent)
 - Decreased body weight (infants)
 - Reduced antibody response to infectious agents (diphtheria, tetanus, influenza)
- More “associative than strong” evidence of reduced birth weight
- Contrary data to the reduced antibody response (reduction of antibody titre) and increased number of infections in infants and children. Questions regarding the design and outcome of the study: Is an association or causality required?
- Is the reduced antibody response (reduction of antibody titre) sufficiently demonstrated to imply a challenged or afunctional immune system to define this as an adverse/unwanted effect.

“Approaches to mixture risk assessment of PFASs in the European population based on human hazard and biomonitoring data” Bil et al 2023

International Journal of Hygiene and Environmental Health 247 (2023) 114071; <https://doi.org/10.1016/j.ijheh.2022.114071>

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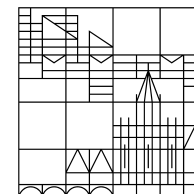


Table 2

Overview of the different mixture risk assessment approaches, the number of PFASs included in these assessments, and the different data that were used in these approaches.

	Hazard index	Sum value	Relative potency factor
Exposure assessment			
PFASs included	PFNA, PFDA, PFUnDA, PFDoDA, PFOS ^a PFOA, PFDA, PFHxS ^b PFOA, PFNA, PFHpS, PFOS ^c PFNA, PFDA, PFDoDA, PFUnDA ^d	PFOA, PFNA, PFHxS, PFOS	PFHxA,* PFOA, PFNA, PFDoDA, PFBS, PFHxS, PFOS
Type of data	Summary data, P50 and P95 per substance	Individual data, P50 mixture, P95 mixture	Individual data, P50 mixture, P95 mixture
Hazard assessment			
Type of effect studied in the mixture risk assessment	Birth weight reduction Immunotoxicity	Immunotoxicity	Immunotoxicity
Type of data used to account for differences in toxic potency	Effect levels (ELs) based on immunotoxicity and birth weight reductions in epidemiological studies (Table 3)	None	Internal relative potency factors (RPFs) obtained from rodent liver toxicity data (Appendix C) to convert PFAS serum concentrations to PFOA Equivalents (PEQs)
HBM GV used	None	EFSA sum value for teenagers and their (future) children	EFSA sum value for teenagers and their (future) children

^a Using the ELs based on Kielsen et al. (2016).

^b Using the ELs based on Grandjean et al. (2012).

^c Using the ELs based on Meng et al. (2018).

^d Using the ELs based on Wang et al. (2016).

^e The Norwegian Environmental Biobank (NEB) II did not contain measurements on PFHxA.

Conclusions of the study:

“The mixture risk assessments show that combined exposure to PFASs is too high and may result in a human health risk in a substantial fraction of the HBM4EU study population, thereby confirming the conclusion drawn in the recent EFSA scientific opinion.”

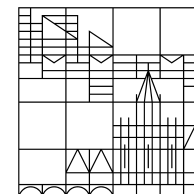
CAVEAT of EFSA opinion and Bil et al 2023 study:

Assumption that all PFAS induce immunotoxicity (reduction in antibody titer) despite that the Abraham et al 2020 study found significant changes only for PFOA but not PFOS.

Thus, is a TEQ and furthermore a HI / Sum Value using the “immunotoxicity endpoint” appropriate?

WHO -I
PFOS and PFOA in drinking-water
Background document for development of *WHO Guidelines*
for Drinking-water Quality
29 September 2022 Version for public review

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Health based values based on ATSDR **Minimal Risk Levels** (2021)

PFOS MRL: 2 ng/kg bw/day (14 ng/kg bw/week)

PFOA MRL: 3 ng/kg bw/day (21 ng/kg bw/week)

Conversion to drinking-water values by applying WHO default parameters :

Adults: PFOS 12 ng/L, PFOA 18 ng/L

Children: PFOS 3 ng/L, PFOA 4 ng/L.

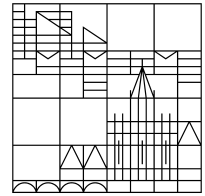
WHO default parameters:

Adults: body weight 60 kg, daily water intake 2 L, allocation factor 20%, subchronic to chronic 2

Children: body weight 5 kg, daily water intake 0.75 L , allocation factor 60%, subchronic to chronic 2

**WHO -II
ATSDR (2021)
Perfluoralkyls Minimal Risk Levels (MRLs)**

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Intermediate (15-364 days) oral MRLs (data for chronic MRLs insufficient)

PFOA:

Critical endpoint; Skeletal effects in mice LOAEL 0.821 $\mu\text{g}/\text{kg}$ bw, AF 300.

MRL: 3×10^{-6} mg/kg

PFOS:

Critical endpoint: Delayed eye opening, decreased pup weights in rats. NOAEL 0.515 $\mu\text{g}/\text{kg}$ bw, AF 300,

MRL: 2×10^{-6} mg/kg

PFHxS:

Critical endpoint: Thyroid follicular epithelial hypertrophy/hyperplasia in rats. NOAEL 4.7 $\mu\text{g}/\text{kg}$ bw, AF 300. (Questionable transferability to humans, see Bartsch et al. Reg Tox Pharm, 2018; <https://doi.org/10.1016/j.yrtph.2018.07.025>)

MRL: 2×10^{-5} mg/kg

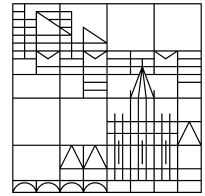
PFNA:

Critical endpoint: Decreased body weight and developmental delays in mice. NOAEL 1 $\mu\text{g}/\text{kg}$ bw, AF 300.

MRL: 3×10^{-6} mg/kg

WHO –III Critical issues

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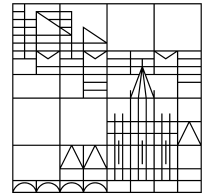


- Primarily relies on ATSDR derived MRLs based on animal studies
- WHO appears critical of both the PBPK data in animals and humans as well as of the half-lives derived thereof
- WHO appears to question the critical health end points used to derive a **Health Based Guidance Value** from various other institutions (incl. EFSA), e.g. **reduced antigen-response** (antigen-titre) due to inconsistencies in the relationship between PFAS exposure and infection propensity in early life and therefore the clinical relevance of these findings.

WHO –IV

Provisional Guidance values for drinking water

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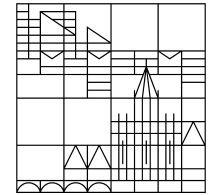


- Provisional Guidance (pGVs) are proposed for individual PFAS
 - **0.1 µg/L for PFOS and PFOA**
- Provisional Guidance (pGVs) are proposed for summary PFAS
 - **0.5 µg/L for summary PFAS**

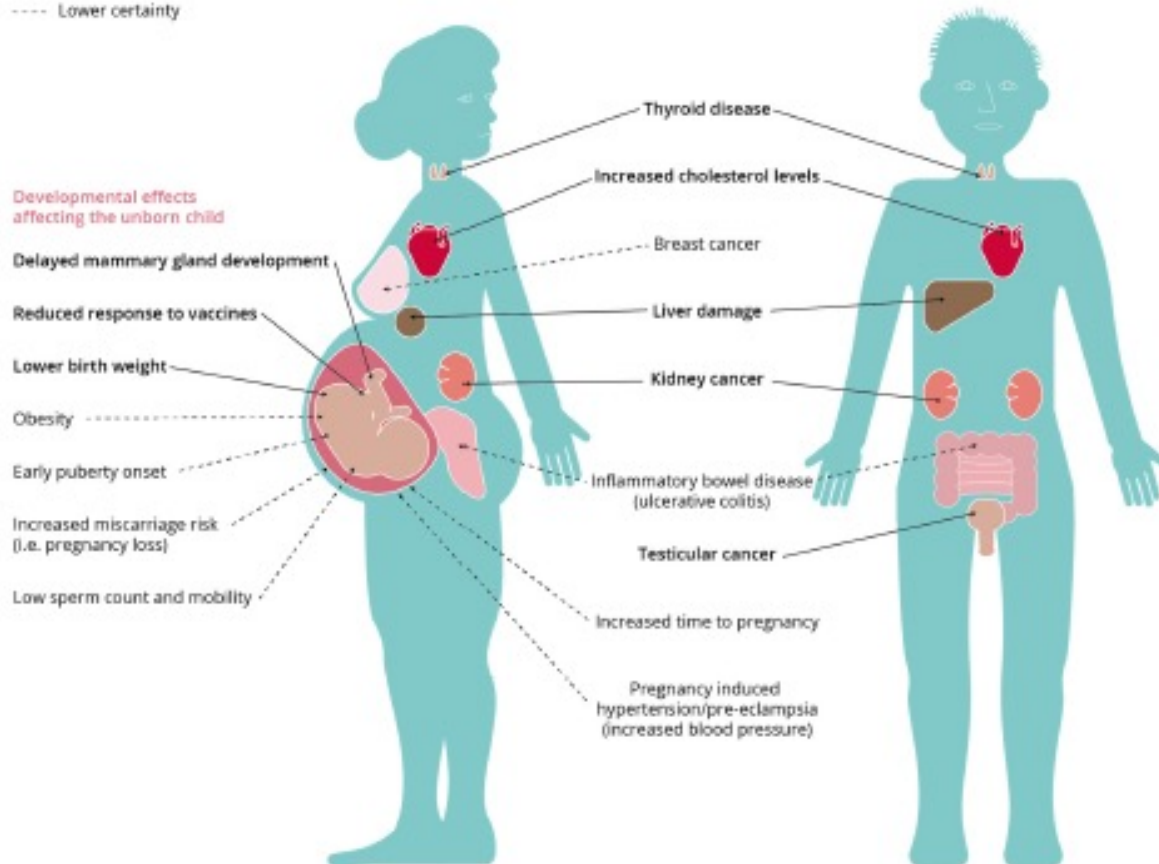
CRITICAL ISSUE: *these pGVs were derived from a pragmatic approach due to the significant uncertainties and absence of consensus with identifying the critical health endpoint to calculate a HBGV!*

Expert Initiatives: Fenton et al 2021; Environmental Toxicology and Chemistry—Volume 40, Number 3—pp. 606–630, 2021. Per- and Polyfluoroalkyl Substance Toxicity and Human Health Review: Current State of Knowledge and Strategies for Informing Future Research

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— High certainty
---- Lower certainty



Health Endpoints are presented as having “High Certainty” that were rejected by WHO and EFSA as having insufficient evidence.

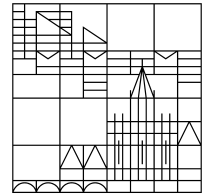
Based on an exchange with one of the coauthors consensus opinions were not always easy to achieve

UK COT panel is undertaking an independent review

FIGURE 1: Effects of per- and polyfluoroalkyl substances on human health. Used with permission from European Environment Agency (2019). Original sources for this figure: National Toxicology Program (2016), CB Science Panel (2012), IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2017), Barry et al. (2013), Fenton et al. (2009), and White et al. (2011b).

CONCLUSIONS

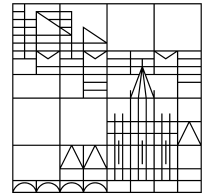
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- Current health based guidance values / TWI appear to largely depend on what information is considered to be relevant and robust
- For both, WHO and EFSA, there is the impression that too little consideration was given to the **biological causality & relevance** as well as **statistical robustness** of the data that was employed as critical endpoints and thus PoD
- For EFSA, in view of supporting evidence from immunological studies in the mouse, the “immunotoxicity” epidemiological studies in humans were key for deriving a TWI. However some experimental design questions and lack of more/better defined data on human “immunotoxicity” (e.g. was/wasn’t there an increased rate of infections // what does a an X% reduction of antigen titer mean) weaken the trust in the most critical health endpoint used for the RA.
- For WHO, the RA heavily relies on the animal studies, where in some endpoints the transferability to humans is questionable. Moreover, although questions were raised as to the different half-lives in rodents and humans, not sufficiently facturing in a longer half-live in humans in the pGV may be cutting it too short.

The way forward -I

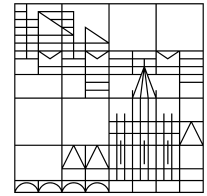
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- **Closure of most important datagaps** via improved experimental designs that specifically address the datagaps in a ***biologically relevant (causality is a must)*** and ***statistically robust*** manner (Aim: consensus that evolving data is sufficiently robust for RA)
- **Improved communication** of certainties und uncertainties in the current RA for example:
 - Avoid ambiguous language in position papers e.g. *‘there may well be a causal association between PFOS and PFOA and birth weight’*
 - why are sum of PFAS used despite adverse effects of a given critical endpoint are available for only a small but important subset of PFAS?
 - How protective are current TDI/TWI’s in light of the apparent general exposure of the broader public to PFAS and in conjunction of above PFAS sum RA? A good example is the current positions paper by the advisory committee of the German Society for Toxicology (Wollin et al. 2023; <https://doi.org/10.1007/s00204-023-03597-y>; published on-line Oct 6, 2023

The way forward -II

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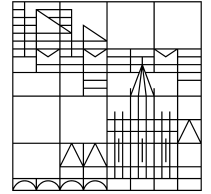


CORRECT AND APPROPRIATE COMMUNICATION:

- Current TWI or TDI are communicated as if they were based on an uncontested dataset of health hazards, which they are obviously not.
- Communicating that health criteria are available but for a very few PFAS but in light of the phys-chem properties of PFAS a precautionary risk management decision was used as guidance is more honest and reduces public anxiety.

TAKING RESPONSIBILITY & THE INITIATIVE:

- All groups/organizations reviewing the current database, despite the best of their intentions „failed“ as lack of sufficient data was bemoaned without defining what criteria and experimental designs would be needed to provide a reasonable basis for a HBGV, individual and summary RA.
- Regulatory authorities need to assume responsibility by defining
 - **criteria** and **fund experiments** that will result in reliable, robust and most importantly biologically relevant causal „exposure-response“ relationships that can be used for the setting of HBGVs
 - **Triaging PFAS into Risk-Benefit** categories in order to manage the overall risk and appropriate remediation procedures.



Thank you for your
attention