WCSR Advice 2022-18

SCIENTIFIC COMMITTEE REACH (WCSR)

ADVICE ON THE PAPER OF SKOCZYNSKA ET AL. (2021) ON THE ANALYSIS OF PAH IN RUBBER : DEVELOPMENT OF AN ANALYTICAL METHOD AND DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS AND HETEROCYCLIC AROMATIC COMPOUNDS IN RUBBER MATRICES.

WCSR Advice 2022-18

MEMBERS OF THE SCIENTIFIC COMMITTEE

The members are :

Willy Baeyens; Lieve Geerts; Lode Godderis; Phillippe Maesen; Birgit Mertens; Guy Schroyen; Guido Vanermen; Nicolas Van Larebeke; Frank Van Belleghem; Griet Jacobs

CONFLICT OF INTEREST

No member has declared any conflict of interest.

RAPPORTEUR(S)

The Scientific Committee REACH thanks the rapporteur Griet Jacobs.

ADOPTION OF THE ADVICE

The Scientific Committee REACH advice was adopted by consensus on 20/10/2022.

LEGAL FRAMEWORK OF THE ADVICE

Cooperation agreement of 17 October 2011 between the Federal State, the Flemish Region, the Walloon Region and the Brussels Capital Region concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

Ministerial decree of 8 July 2014 appointing the members of the Scientific Committee REACH established under Article 3, § 3 of the Cooperation Agreement of 17 October 2011 between the Federal State, the Flemish Region, the Walloon Region and the Brussels Capital Region concerning the Registration, Evaluation, Authorisation and restriction of Chemicals (REACH)

Ministerial decree of 2 June 2016 on dismissal and appointment of members of the Scientific Committee REACH

Ministerial decree of 5 October 2016 on appointment of members of the Scientific Committee REACH

Ministerial decree of 28 July 2022 on appointment of members of the Scientific Committee REACH.

DISCLAIMER

The Scientific Committee REACH reserves, at any time, the right to change this advice when new information and data become available after the publication of this version.

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1 Concern

Review of paper: Skoczynska et al. (2021): Analysis of recycled rubber: Development of an analytical method and determination of polycyclic aromatic hydrocarbons and heterocyclic aromatic compounds in rubber matrices.

Recently a REACH restriction process was voted at EU level targeting 8 listed PAHs (Benzo(a)pyrene, (BaP), Benzo(e)pyrene (BeP), Benzo(a)anthracene (BaA) , chrysene (CHR), Benzo(b)fluoranthene (BbFA), Benzo(j)fluoranthene (BjFA), Benzo(k)fluoranthene (BkFA) and Dibenzo(a,h)anthracene (DBAhA)) in rubber granules or mulches used in infill material in synthetic turf pitches (for sport fields application) (Annex XVII entry 50 PARA 9-14 Legal ref = Conditions of restriction (Regulation number (EU) 2021/1199 of 20 July 2021)). While a REACH restriction on these 8 PAHs was already in place for the consumer articles (Annex XVII entry 50 PARA_5-8), it did not capture the “mixture” (vs. “article”) at use by consumers and therefore a dedicate assessment was launched for safe level PAH concentration for the use of rubber granulates (which - for the most part of it – originate from used tyres).

2 Analysis of available information

3 standard methods were accepted (WCSR Advice 2017-13A1) for the determination of 16 EPA PAH in plastics and rubbers (CEN/TS 16181; NEN 7331 and AfPS GS 2014:01 PAK).However, it should be noted that the 3 standard methods did not contain all 8 listed PAHs of the REACH restriction. The following compounds were missing for 2 of the 3 standards; Benzo(e)pyreen (BeP) and Benzo(j)fluoranthene (BjFA).

The method of Skoczynska et al. 2021 is in this review compared with the 3 accepted standard methods. The 3 standard methods measured only the 16 EPA-PAHs, while the paper of Skoczynska et al. 2021 identified and quantified 46 (16 EPA PAHs included) sample specific compounds, including several heterocyclic PAHs and aromatic amines. **The publication of Skoczynska et al. 2021 used a good strategy (combination of screening and target analysis) to identify the possible compounds present in the samples** and this analytical set-up can be used for further research. Only a limited of different plastics were analyzed (granulates, tiles and care tires). When analyzing more relevant matrices more compounds will be found.

This assessment will help BE CA REACH for its coordination of the BE position and negotiation at the EU level under REACH regulation (Restriction), as ECHA (RAC) is expected to deliver more assessment opinions on 2 Commission's mandates on this subject and the Commission will (probably) present the corresponding Restriction proposal(s) for vote:

1) A mandate 'review clause' (reassessment) for the existing restriction on (8 listed) PAHs into consumer articles (Annex XVII entry 50 PARA 5-8). (Ref: text = '8. By 27 December 2017, the Commission shall review the limit values in paragraphs 5 and 6 in the light of new scientific information, including migration of PAHs from the articles referred to therein, and information on alternative raw materials and, if appropriate, modify these paragraphs accordingly.')
2) A mandate on a (separate) 'review clause' for the above (new) restriction (described into the introductory section) notably for the reassessment of the new restriction targeting risk for 'playground' usage of the rubber granulates infill – as a number of Member States (including BE) did not agree with that 'allowed use' being proposed by the Commission and questioned some aspects of the risk assessment by the RAC.

The article describes the development of an analytical method that allows identification and quantification of a wider range of organic compounds extractable from the complex rubber matrix. The developed analytical method involves sonication extraction, followed by solid phase extraction (SPE) fractionation that enables simple and efficient separation of analytes with broad polarity scale. The application of SPE fractionation resolves coelution problems and simplifies the chromatograms. This analytical approach allowed to identify and quantify 46 sample specific compounds, including several heterocyclic PAHs like 2-methylthiobenzothiazole, benzonapthothiophenes, benzonaphthofuranes and aromatic amines like diphenylamine and N-phenyl-2-naphthylamine.

In the article an analytical set-up is developed including:

- target analysis of 21 heterocyclic aromatic compounds (including polyaromatic nitrogen- sulfur- and oxygenheterocycles: PANHs, PASHs and PAOHs), 2 aromatic amines (diphenylamine (DPA) and N-phenyl-2-naphthylamine (PNA)). and 3 methyl PAHs (1-methylpyrene (1MPYR), 2-methylphenanthrene (2MPHN), 5-methylchrysene (5MCHY)). Additionally, several methyl-PAHs (structural isomers of 1MPYR, 2MPHN and 5MCHY and a suite of dimethyl- (and/or ethyl-) dibenzothiophenes (structural isomers of 4,6-Dimethyldibenzothiophene

(46DMDBT)). Moreover, 16-EPA PAHs together with Triphenylene (TPH) and perylene (PER) were identified by target analysis.

- suspect screening of raw extracts to tentatively identify primary organic compounds present, which are not included in the standard target analysis of recycled rubber The screening is based on the list of the suspected compounds, including inter alia vulcanization accelerators, antioxidants and other compounds used in rubber production. This list was set using previous studies (European chemicals Agency (ECHA) 2017, Perkins et al., 2019; US Environmental Protection Agency (US EPA); Centres for Disease Control and Prevention(CDC), 2019)
- addition of newly identified compounds to the target list and improvement of the analytical method.

The developed analytical method involves:

- extraction by sonication
- solid phase extraction (SPE) that enables simple and efficient separation (fractionation) of analytes with broad polarity scale.
- Screening of the raw extracts

3 Discussion

3 standard methods were accepted (WCSR Advice 2017-13A) for the determination of PAH in plastics and rubbers (CEN/TS 16181; NEN 7331 and AfPS GS 2014:01 PAK). The method of Skoczynska et al. 2021 is in this review compared with the 3 accepted standard methods. The 3 standard methods measured only the 16 EPA-PAHs, while the paper of Skoczynska et al. 2021 identified and quantified 46 (16 EPA PAHs included) sample specific compounds (Table 1), including several heterocyclic PAHs and aromatic amines (table 1)

However, some important remarks on the used analytical method by Skoczynska et al. 2021 should be addressed;

1) The sample intake (100 mg) is too small for a sample size of 3 mm. For plastic materials we recommend a particle size reduction to <1 mm in order to guarantee sufficient extraction yields.
2) The used extraction solvents (DCM/EtAc) are different when compared to the 3 standard methods (PE, DMSO, hexane, aceton and toluene). The extraction is based on the method developed by Menichini et al. (2011), but is slightly changed by replacing hexane with more polar EtAc. The recoveries of the extractions with DCM/hexane and DCM/EtAc were similar for all studied compounds. However no detailed information is given about the obtained recoveries (extraction efficiency).
3) The extraction is done with ultrasonication. However, nothing is mentioned about the conditions (used time and temperature). The conditions are a critical step in the sample preparation and should be clearly specified in the method!
4) Additional sample clean-up with reversed phase SPE is performed. Further clean-up is necessary due to coelution of unknown compounds with the target compounds. Most of the heterocyclic PAHs, like PASHs, eluted together with PAHs in the first fraction but this procedure allowed fractionation of PANHs: (acridine and carbazole). In the standard methods, clean-up with SPE is optional.
5) The standard method used the internal standard method with isotope labelled PAH standards as internal standards. The method of Skoczynska et al. 2021 used external calibration as quantification though the samples are spiked with d-labelled standards. Nothing is mentioned on how this is done, a lot of compounds are quantified but nothing is specified if this is done against a corresponding reference standard.
6) The first diagnostic ion is for all the methods the same. The second diagnostic ion used in the method of Skoczynska et al. 2021, differs from the one used in the standard methods (Table 3), the reason for this is not specified in the paper. Also the relative abundance of the diagnostic ions is not mentioned in the paper. The relative abundance gives an extra certainty for identification of the PAHs compounds.
7) LOD is very low in comparison with the 3 standard methods. The LOD was calculated from the concentration of the lowest standard as the concentration giving a signal-to-noise of three (S/N=3). In practice, the LOD (based on reel samples) will be much higher and probably in the range of the 3 standard methods!

The analytical method of Skoczynska et al. 2021 looks promising for analysis of PAHs and other relevant compounds, however some important remarks are specified in the above points. Due to these remarks and the limited detailed information about the results of the method optimization, **the analytical method cannot be accepted as an alternative to the 3 standard methods**. In a first step, the missing details should be clarified and in a second step a broad validation should be done before acceptance of the method.

However, **the publication of Skyczynska et al. 2021 used a good strategy (combination of screening and target analysis) to identify the possible compounds present in the** samples and this analytical set-up can be used for further research. This analytical approach allowed to identify and quantify 46 sample specific compounds. A complete list of compounds is listed in Table 1. The PAHs profiles showed **valuable information about the different origin of the samples**. Different patterns were noticed between samples originating from Spanish and from the Netherlands (Figure 1).

Not only the 8 REACH PAHs and the 16 EPA-PAHs are analyzed in the publication of Skyczynska et al. 2021, also the methyl-PAHs, are measured because of their ubiquity and toxicity, the latter sometimes higher than that of the parent PAHs. The concentrations of the three methyl PAHs isomers 5MCHY (possibly carcinogenic to humans; one of the PAHs included by US EPA in the Toxic Release Program (Environmental Protecti, 2018)), 1MPYR and 2MPHN were determined. **The results presented in this study show that the three groups of methylated PAHs have a substantial contribution to the sum of all quantified PAHs (20-40% of all measured PAHs).** Acridine and carbazole, suspected carcinogens, could be identified in most samples. Also two aromatic amines were identified and quantified: diphenylamine (DPA) and N-phenyl-2-naphthylamine (PNA) (a suspected carcinogen according to ECHA, which can be metabolized to the carcinogenic b-naphthylamine). Also N-1,3-dimethylbutyl-N0-phenyl-p-phenyldiamine (6PPD) was identified by NIST, but was not confirmed by a standard.

The authors state finally that the obtained results stress **the need for expanding the list of target compounds analysed in crumb rubber** and the need for longitudinal studies on weathering processes taking place in CR. Proper toxicological risk assessment of recycled rubber used in consumer products cannot be based on a limited set of target analytes alone, while ignoring many other hazardous compounds present in CR. The limited screening approach, applied in this study, shows that comprehensive non-target screening study is needed in order to better characterize toxic compounds associated with rubber matrix and ELT.

4 Conclusion

The publication of Skoczynska et al. 2021 showed that not only the 8 REACH PAHs (annex 17 entry 50) are present at significant levels in the tested samples. Also other compounds including 21 heterocyclic aromatic compounds, **two aromatic amines and several methylated PAHs** together with the **16 EPA PAHs and TPH, BeP, BjF and PER** were identified and quantified in CR samples originating from football pitches, rubber tiles and car tires.

From the publication of Skoczynska et al. 2021 it was clear that the 8 PAHs concentrations determined in crumb rubber tiles purchased in Dutch and Spanish shops **exceed (for all tested materials!) the EU limits for consumer articles (1 mg/kg or 1µg/g) marketed** for use by the public (entry 50, paragraphs 5 - 8 of annex XVII to regulation No 1907/2006). Only, for one material (Spanish mats) (1861 mg/kg), the limit of 20 mg/kg for the **8 listed PAHs was exceeded (**the new restriction targeting specifically the rubber granulates infills (entry 50, paragraphs 9 - 14 of annex XVII to regulation No 1907/2006) (Table2). **When looking at the sum of the 16 EPA-PAHs all concentrations found in the crumb rubber tiles exceed the EU limits for consumer articles (1 mg/kg) and the limit of the granulates infills (20 mg/kg)!**

- It should be pointed out, that legal limits relatively to the PAHs in rubber are still expected to be reviewed based on a (future) advice by the RAC of ECHA. For mixtures the maximum allowable concentration limits for mixtures are set at 1000 mg/kg for PAHs classified as Cancerogenic or Mutagen (except if a specific stronger limit has been decided under the CLP regulation: 0.01% for BaP and DBahA). Nonetheless the recent voted restriction (2020) (entry 50, paragraphs 9 and 10 of annex XVII to regulation No 1907/2006) lowers the total concentration limit of eight PAHs to 20 mg/kg for *granules and mulches to be used as infill material in synthetic turf pitches or in loose form on playground or in sport applications*.

- Other maximum tolerance limits apply to shock absorbing mats, used e.g. on children playgrounds, in nurseries or sport schools (entry 50, paragraphs 5 - 8 of annex XVII to regulation No 1907/2006). They fall under the category of rubber (or plastic) consumer 'articles' (vs. 'mixtures' – *see above*) products and therefore, the applied maximum concentration limit is 1 mg/kg for each of eight carcinogenic PAHs.

Not only the 8 REACH PAHs are present in the crumb rubber tiles, also the presence of the 16 EPA-PAHs and 3 methylated PAHs isomers cannot be ignored. 5MCHY is classified by ECHA as suspected of causing cancer and 1MPYR and 2MPHN are likely to meet criteria for CMR (carcinogenic, mutagenic and reprotoxic) category 1A or 1B (Part 3 of Annex I of Regulation (EC) No 1272/2008). It is therefore advised to add more compounds (at least the 16 EPA-PAHs and the 3 methylated PAHs isomers) to the REACH restriction (annex 17 entry 50). Substances that are related to PAHs and measured at such quantity, considering the non-threshold character of the hazard, confirmed for at least 16 PAHs and the 3 Methylated PAHs isomers, should not be permitted and the regulatory limits under REACH REG (EU 1907/2006) is unacceptable (certainly for the 'Rubber Granulates' : entry 50 para 9-14) as they do not provide an adequate level of protection to workers and the general public.

5 Recommendations relative to the analytical method

The **analytical method of Skoczynska et al. 2021 looks promising** for analysis of PAHs and other relevant compounds (listed in table 1) in rubber material (originating from football pitches, rubber tiles and car tires) however some important remarks are specified in this document (point 1 – 7, listed above). At the moment (due to this remarks), **the analytical method cannot be used as an alternative to the 3 standard methods** (point 1 – 7, listed above). The missing details should be clarified and a complete validation should be done before acceptance of the considered published method as the basis for tests to identify (un)compliance to the obligations under REACH Annex XVII entry 50.

3 standard methods were accepted (WCSR Advice 2017-13A) for the determination of 16 EPA PAHs in plastics and rubbers (CEN/TS 16181; NEN 7331 and AfPS GS 2014:01 PAK). **The following compounds were missing for 2 of the 3 standards**; Benzo(e)pyreen (BeP) and Benzo(j)fluoranthene (BjFA). These compounds should be (as a

minimum) included in the 2 of the 3 standard methods. Based on the findings of the screening method of Skoczynska et al. 2021, the target method should be expanded with the compounds found in the samples (**two aromatic amines and several methylated PAHs**, **TPH, BeP, BjF and PER**). The standard methods can be used as a base and should be updated regularly on experimental evidence (obtained by screening) or on literature searches.

Currently, a new publication of Armada et al. 2022[2] is published. In this publication a global evaluation is performed on the chemical hazard of recycled tire crumb rubber employed on worldwide synthetic turf football pitches. The presence of **hazardous substances** in the recycled crumb rubber samples collected around the world was confirmed. Three crumb rubber **samples exceeded the limit of 20 µg/g for the sum of the 8 REACH PAHs**. In total 17 PAHs were measured with targeted analysis. The combination of screening and target analysis that was developed by Skoczynska et al. 2021 was not applied. The compounds found from the screening (Skoczynska et al. 2021) were added to the target method. It can be a strategy to do on a regularly basis a screening and update (e.g. yearly) the target list of the target method (if commercial reference standards are available) and validate the compounds that were added.

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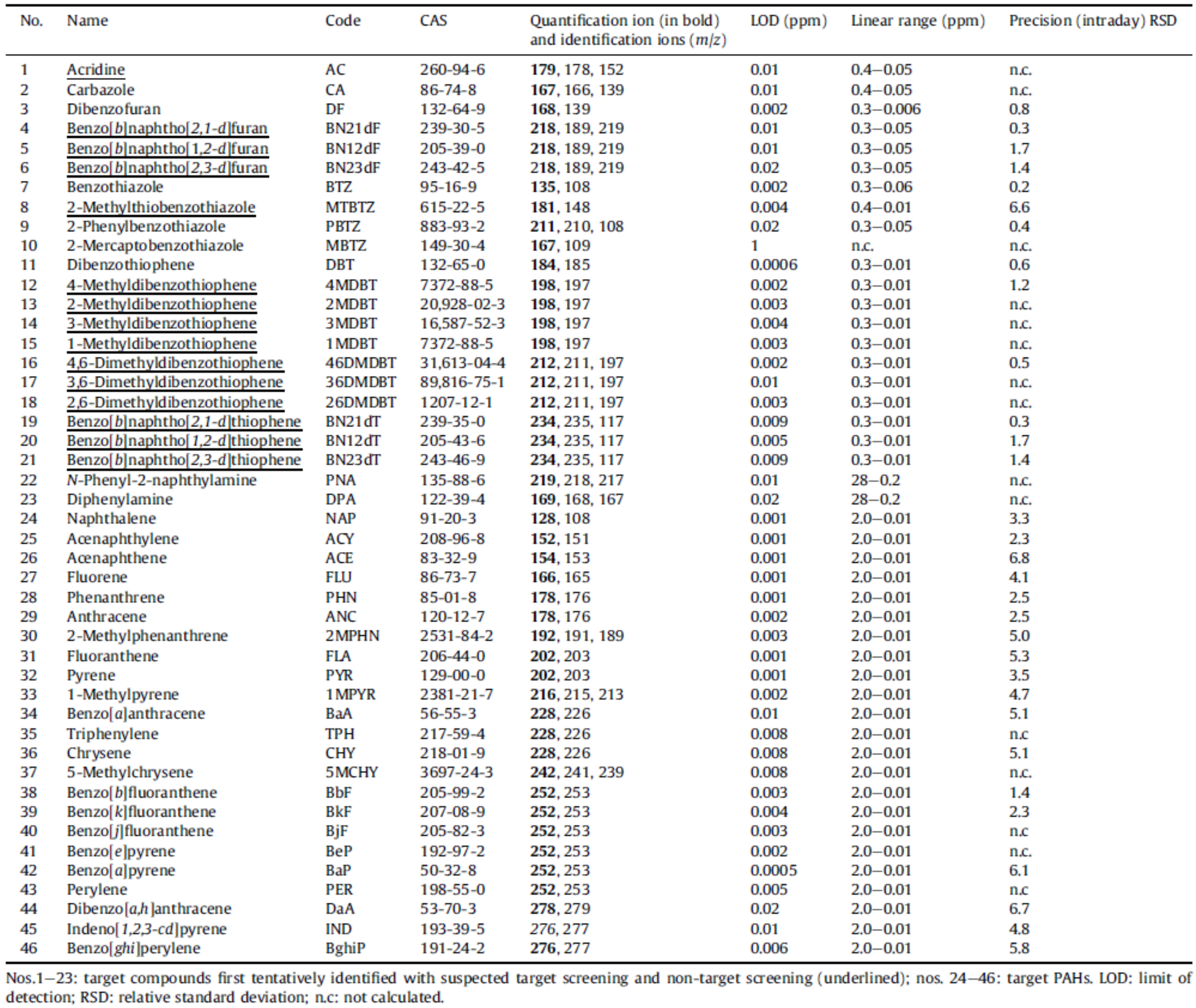
[1] *2017-13 : ADVICE ON MEASURING METHODS FOR CERTAIN RESTRICTED SUBSTANCES OF THE ANNEX XVII OF REACH - PART A : Nonylphenol and nonylphenol ethoxylates in textile, Phthalates in soft PVC, PAH in plastics*

[2] *Armada, D., Llompart, M., Celeiro, M., Garcia-Castro, P., Ratola, N., Dagnac, T., & de Boer, J. (2022). Global evaluation of the chemical hazard of recycled tire crumb rubber employed on worldwide synthetic turf football pitches. Science of The Total Environment, 812, 152542.* https://doi.org/10.1016/J.SCITOTENV.2021.152542

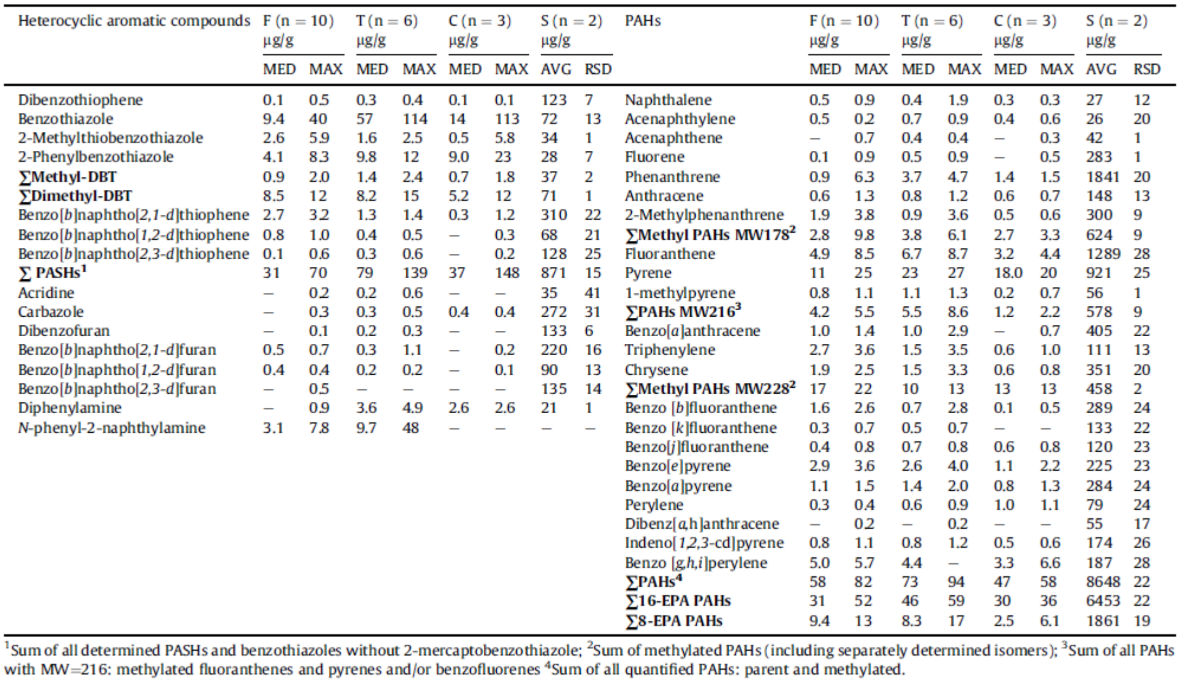
Table 1 shows the list of compounds determined in this study. Compounds 1 to 23 were found using a suspect and non-target screening. After tentatively identification, reference compounds were used for verification. Compounds 24-46 are target PAHs (both from the list of 8 as well as the list of 16 PAHs with extra PAHs outside these lists)



No.	Name	Code	CAS	Quantification ion (in bold) and identification ions (*m*/*z*)	LOD (ppm)	Linear range (ppm)	Precision (intraday) RSD
1	Acridine	AC	260-94-6	**179**, 178, 152	0.01	0.4–0.05	n.c.
2	Carbazole	CA	86-74-8	**167**, 166, 139	0.01	0.4–0.05	n.c.
3	Dibenzofuran	DF	132-64-9	**168**, 139	0.002	0.3–0.006	0.8
4	Benzo[*b*]naphtho[*2,1-d*]furan	BN21dF	239-30-5	**218**, 189, 219	0.01	0.3–0.05	0.3
5	Benzo[*b*]naphtho[*1,2-d*]furan	BN12dF	205-39-0	**218**, 189, 219	0.01	0.3–0.05	1.7
6	Benzo[*b*]naphtho[*2,3-d*]furan	BN23dF	243-42-5	**218**, 189, 219	0.02	0.3–0.05	1.4
7	Benzothiazole	BTZ	95-16-9	**135**, 108	0.002	0.3–0.06	0.2
8	2-Methylthiobenzothiazole	MTBTZ	615-22-5	**181**, 148	0.004	0.4–0.01	6.6
9	2-Phenylbenzothiazole	PBTZ	883-93-2	**211**, 210, 108	0.02	0.3–0.05	0.4
10	2-Mercaptobenzothiazole	MBTZ	149-30-4	**167**, 109	1	n.c.	n.c.
11	Dibenzothiophene	DBT	132-65-0	**184**, 185	0.0006	0.3–0.01	0.6
12	4-Methyldibenzothiophene	4MDBT	7372-88-5	**198**, 197	0.002	0.3–0.01	1.2
13	2-Methyldibenzothiophene	2MDBT	20,928-02-3	**198**, 197	0.003	0.3–0.01	n.c.
14	3-Methyldibenzothiophene	3MDBT	16,587-52-3	**198**, 197	0.004	0.3–0.01	n.c.
15	1-Methyldibenzothiophene	1MDBT	7372-88-5	**198**, 197	0.003	0.3–0.01	n.c.
16	4,6-Dimethyldibenzothiophene	46DMDBT	31,613-04-4	**212**, 211, 197	0.002	0.3–0.01	0.5
17	3,6-Dimethyldibenzothiophene	36DMDBT	89,816-75-1	**212**, 211, 197	0.01	0.3–0.01	n.c.
18	2,6-Dimethyldibenzothiophene	26DMDBT	1207-12-1	**212**, 211, 197	0.003	0.3–0.01	n.c.
19	Benzo[*b*]naphtho[*2,1-d*]thiophene	BN21dT	239-35-0	**234**, 235, 117	0.009	0.3–0.01	0.3
20	Benzo[*b*]naphtho[*1,2-d*]thiophene	BN12dT	205-43-6	**234**, 235, 117	0.005	0.3–0.01	1.7
21	Benzo[*b*]naphtho[*2,3-d*]thiophene	BN23dT	243-46-9	**234**, 235, 117	0.009	0.3–0.01	1.4
22	*N*-Phenyl-2-naphthylamine	PNA	135-88-6	**219**, 218, 217	0.01	28–0.2	n.c.
23	Diphenylamine	DPA	122-39-4	**169**, 168, 167	0.02	28–0.2	n.c.
24	Naphthalene	NAP	91-20-3	**128**, 108	0.001	2.0–0.01	3.3
25	Acenaphthylene	ACY	208-96-8	**152**, 151	0.001	2.0–0.01	2.3
26	Acenaphthene	ACE	83-32-9	**154**, 153	0.001	2.0–0.01	6.8
27	Fluorene	FLU	86-73-7	**166**, 165	0.001	2.0–0.01	4.1
28	Phenanthrene	PHN	85-01-8	**178**, 176	0.001	2.0–0.01	2.5
29	Anthracene	ANC	120-12-7	**178**, 176	0.002	2.0–0.01	2.5
30	2-Methylphenanthrene	2MPHN	2531-84-2	**192**, 191, 189	0.003	2.0–0.01	5.0
31	Fluoranthene	FLA	206-44-0	**202**, 203	0.001	2.0–0.01	5.3
32	Pyrene	PYR	129-00-0	**202**, 203	0.001	2.0–0.01	3.5
33	1-Methylpyrene	1MPYR	2381-21-7	**216**, 215, 213	0.002	2.0–0.01	4.7
34	Benzo[*a*]anthracene	BaA	56-55-3	**228**, 226	0.01	2.0–0.01	5.1
35	Triphenylene	TPH	217-59-4	**228**, 226	0.008	2.0–0.01	n.c
36	Chrysene	CHY	218-01-9	**228**, 226	0.008	2.0–0.01	5.1
37	5-Methylchrysene	5MCHY	3697-24-3	**242**, 241, 239	0.008	2.0–0.01	n.c.
38	Benzo[*b*]fluoranthene	BbF	205-99-2	**252**, 253	0.003	2.0–0.01	1.4
39	Benzo[*k*]fluoranthene	BkF	207-08-9	**252**, 253	0.004	2.0–0.01	2.3
40	Benzo[*j*]fluoranthene	BjF	205-82-3	**252**, 253	0.003	2.0–0.01	n.c
41	Benzo[*e*]pyrene	BeP	192-97-2	**252**, 253	0.002	2.0–0.01	n.c.
42	Benzo[*a*]pyrene	BaP	50-32-8	**252**, 253	0.0005	2.0–0.01	6.1
43	Perylene	PER	198-55-0	**252**, 253	0.005	2.0–0.01	n.c
44	Dibenzo[*a,h*]anthracene	DaA	53-70-3	**278**, 279	0.02	2.0–0.01	6.7
45	Indeno[*1,2,3-cd*]pyrene	IND	193-39-5	*276*, 277	0.01	2.0–0.01	4.8
46	Benzo[*ghi*]perylene	BghiP	191-24-2	**276**, 277	0.006	2.0–0.01	5.8

Nos.1–23: target compounds first tentatively identified with suspected target screening and non-target screening (underlined); nos. 24–46: target PAHs. LOD: limit of detection; RSD: relative standard deviation; n.c: not calculated.

TABLE 2 SHOWS THE CONCENTRATIONS OF THE DIFFERENT COMPOUNDS IN DIFFERENT SUBSETS OF SAMPLES



Heterocyclic aromatic compounds	F (n = 10) µg/g		T (n = 6) µg/g		C (n = 3) µg/g		S (n = 2) µg/g	
	MED	MAX	MED	MAX	MED	MAX	AVG	RSD
Dibenzothiophene	0.1	0.5	0.3	0.4	0.1	0.1	123	7
Benzothiazole	9.4	40	57	114	14	113	72	13
2-Methylthiobenzothiazole	2.6	5.9	1.6	2.5	0.5	5.8	34	1
2-Phenylbenzothiazole	4.1	8.3	9.8	12	9.0	23	28	7
∑Methyl-DBT	0.9	2.0	1.4	2.4	0.7	1.8	37	2
∑Dimethyl-DBT	8.5	12	8.2	15	5.2	12	71	1
Benzo[*b*]naphtho[*2,1-d*]thiophene	2.7	3.2	1.3	1.4	0.3	1.2	310	22
Benzo[*b*]naphtho[*1,2-d*]thiophene	0.8	1.0	0.4	0.5	–	0.3	68	21
Benzo[*b*]naphtho[2,3-*d*]thiophene	0.1	0.6	0.3	0.6	–	0.2	128	25
∑ PASHs[1]	31	70	79	139	37	148	871	15
Acridine	–	0.2	0.2	0.6	–	–	35	41
Carbazole	–	0.3	0.3	0.5	0.4	0.4	272	31
Dibenzofuran	–	0.1	0.2	0.3	–	–	133	6
Benzo[*b*]naphtho[*2,1-d*]furan	0.5	0.7	0.3	1.1	–	0.2	220	16
Benzo[*b*]naphtho[*1,2-d*]furan	0.4	0.4	0.2	0.2	–	0.1	90	13
Benzo[*b*]naphtho[2,3-*d*]furan	–	0.5	–	–	–	–	135	14
Diphenylamine	–	0.9	3.6	4.9	2.6	2.6	21	1
N-phenyl-2-naphthylamine	3.1	7.8	9.7	48	–	–	–	–

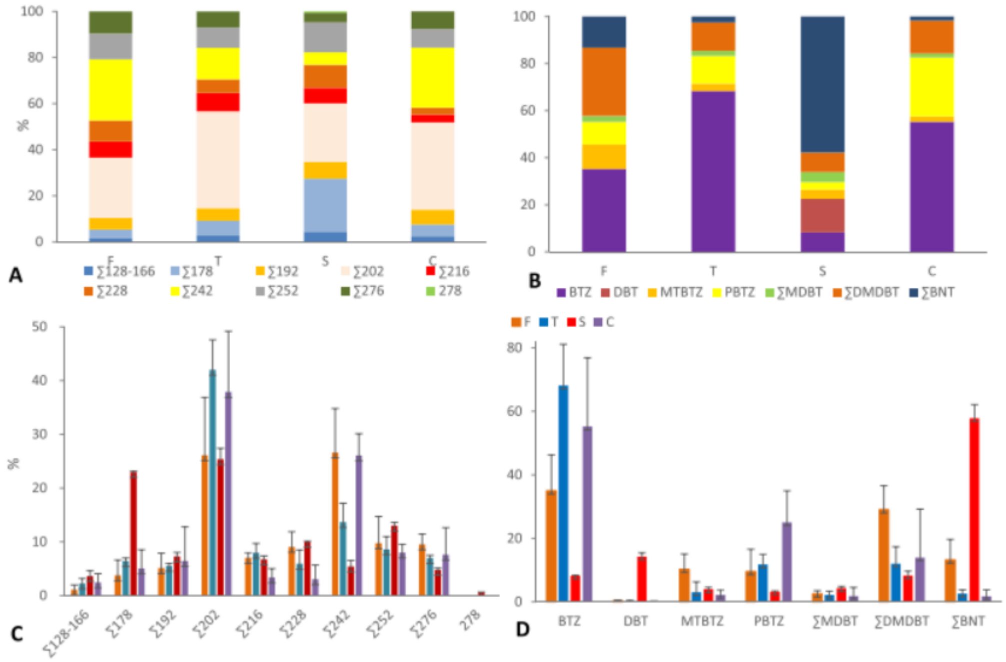
PAHs	F (n = 10) µg/g		T (n = 6) µg/g		C (n = 3) µg/g		S (n = 2) µg/g	
	MED	MAX	MED	MAX	MED	MAX	AVG	RSD
Naphthalene	0.5	0.9	0.4	1.9	0.3	0.3	27	12
Acenaphthylene	0.5	0.2	0.7	0.9	0.4	0.6	26	20
Acenaphthene	–	0.7	0.4	0.4	–	0.3	42	1
Fluorene	0.1	0.9	0.5	0.9	–	0.5	283	1
Phenanthrene	0.9	6.3	3.7	4.7	1.4	1.5	1841	20
Anthracene	0.6	1.3	0.8	1.2	0.6	0.7	148	13
2-Methylphenanthrene	1.9	3.8	0.9	3.6	0.5	0.6	300	9
∑Methyl PAHs MW178[2]	2.8	9.8	3.8	6.1	2.7	3.3	624	9
Fluoranthene	4.9	8.5	6.7	8.7	3.2	4.4	1289	28
Pyrene	11	25	23	27	18.0	20	921	25
1-methylpyrene	0.8	1.1	1.1	1.3	0.2	0.7	56	1
∑PAHs MW216[3]	4.2	5.5	5.5	8.6	1.2	2.2	578	9
Benzo[*a*]anthracene	1.0	1.4	1.0	2.9	–	0.7	405	22
Triphenylene	2.7	3.6	1.5	3.5	0.6	1.0	111	13
Chrysene	1.9	2.5	1.5	3.3	0.6	0.8	351	20
∑Methyl PAHs MW228[2]	17	22	10	13	13	13	458	2
Benzo [*b*]fluoranthene	1.6	2.6	0.7	2.8	0.1	0.5	289	24
Benzo [*k*]fluoranthene	0.3	0.7	0.5	0.7	–	–	133	22
Benzo[*j*]fluoranthene	0.4	0.8	0.7	0.8	0.6	0.8	120	23
Benzo[*e*]pyrene	2.9	3.6	2.6	4.0	1.1	2.2	225	23
Benzo[*a*]pyrene	1.1	1.5	1.4	2.0	0.8	1.3	284	24
Perylene	0.3	0.4	0.6	0.9	1.0	1.1	79	24
Dibenz[*a*,h]anthracene	–	0.2	–	0.2	–	–	55	17
Indeno[*1,2,3*-cd]pyrene	0.8	1.1	0.8	1.2	0.5	0.6	174	26
Benzo [*g,h,i*]perylene	5.0	5.7	4.4	–	3.3	6.6	187	28
∑PAHs[4]	58	82	73	94	47	58	8648	22
∑16-EPA PAHs	31	52	46	59	30	36	6453	22
∑8-EPA PAHs	9.4	13	8.3	17	2.5	6.1	1861	19

[1] Sum of all determined PASHs and benzothiazoles without 2-mercaptobenzothiazole; [2] Sum of methylated PAHs (including separately determined isomers); [3] Sum of all PAHs with MW=216: methylated fluoranthenes and pyrenes and/or benzofluorenes [4] Sum of all quantified PAHs: parent and methylated.

TABLE 3: *GC-MS DIAGNOSTIC IONS FOR PAH*

	Skoczynska et al. 2021		Standard methods (NEN 7331 - PAH part , CEN/TS 16181, AfPS GS 2014:01 PAK)		
m/z	diagnostic ion 1	diagnostic ion 2	diagnostic ion 1	diagnostic ion 2	diagnostic ion 3
Naphthalane	128	108	128	102	
Acenaphthene	154	153	154	153	76
Acenaphthylene	152	151	152	150	76
Fluorene	166	165	166	165	139
Anthracene	178	176	178	152	76
Phenanthrene	178	176	178	152	76
Fluoranthene	202	203	202	200	100
pyrene	202	203	202	200	101
Benzo(a)anthracene	228	226	228	226	114
Chrysene	228	226	228	226	113
Benzo(b)fluoranthene	252	253	252	250	126
benzo(k)fluroanthene	252	253	252	250	126
Benzo(a)pyrene	252	253	252	250	113
Indeno(1,2,3-cd)pyrene	276	277	276	138	274
Dibenz(a,h)anthracene	278	279	278	139	276
Benzo(ghi)perylene	276	277	276	138	274

Figure 1 Profiles of PAHs (A), and PASHs and benzothiazoles (B) observed in turf rubber granulate (F), tiles (T), Spanish tile (S), car tires (C).



6 References

Apel, P., Angerer, J., Wilhelm, M. & Kolossa-Gehring, M. (2017) New HBM values for emerging substances, inventory of reference and HBM values in force, and working principles of the German Human Biomonitoring Commission. *Int J Hyg Environ Health*, **220**, 152-166.

Beesoon, S., Webster, G.M., Shoeib, M., Harner, T., Benskin, J.P. & Martin, J.W. (2011) Isomer Profiles of Perfluorochemicals in Matched Maternal, Cord, and House Dust Samples: Manufacturing Sources and Transplacental Transfer. *Environmental Health Perspectives*, **119**, 1659-1664.

Blaine, A.C., Rich, C.D., Sedlacko, E.M., Hundal, L.S., Kumar, K., Lau, C., Mills, M.A., Harris, K.M. & Higgins, C.P. (2014a) Perfluoroalkyl acid distribution in various plant compartments of edible crops grown in biosolids-amended soils. *Environ Sci Technol*, **48**, 7858-7865.

Blaine, A.C., Rich, C.D., Sedlacko, E.M., Hyland, K.C., Stushnoff, C., Dickenson, E.R. & Higgins, C.P. (2014b) Perfluoroalkyl acid uptake in lettuce (Lactuca sativa) and strawberry (Fragaria ananassa) irrigated with reclaimed water. *Environ Sci Technol*, **48**, 14361-14368.

Calafat, A.M., Kato, K., Hubbard, K., Jia, T., Botelho, J.C. & Wong, L.Y. (2019) Legacy and alternative per- and polyfluoroalkyl substances in the U.S. general population: Paired serum-urine data from the 2013-2014 National Health and Nutrition Examination Survey. *Environment international*, **131**, 105048.

Chang, S.C., Das, K., Ehresman, D.J., Ellefson, M.E., Gorman, G.S., Hart, J.A., Noker, P.E., Tan, Y.M., Lieder, P.H., Lau, C., Olsen, G.W. & Butenhoff, J.L. (2008) Comparative pharmacokinetics of perfluorobutyrate in rats, mice, monkeys, and humans and relevance to human exposure via drinking water. *Toxicological sciences : an official journal of the Society of Toxicology*, **104**, 40-53.

Chengelis, C.P., Kirkpatrick, J.B., Myers, N.R., Shinohara, M., Stetson, P.L. & Sved, D.W. (2009) Comparison of the toxicokinetic behavior of perfluorohexanoic acid (PFHxA) and nonafluorobutane-1-sulfonic acid (PFBS) in cynomolgus monkeys and rats. *Reproductive toxicology (Elmsford, N.Y.)*, **27**, 400-406.

ECHA (2012) Guidance on information requirements and chemical safety assessment Chapter R.8: Characterisation of dose [concentration]-response for human health. Version: 2.1. November 2012, https://www.echa.europa.eu/documents/10162/13632/information_requirements_r8_en.pdf/e153243a-03f0-44c5-8808-88af66223258

ECHA (2013) Member State Committee support document for identification of Pentadecafluorooctanoic Acid (PFOA) as a substance of very high concern because of its CMR and PBT properties. Adopted on 14 June 2013. https://echa.europa.eu/documents/10162/8059e342-1092-410f-bd85-80118a5526f5, pp. 66.

ECHA (2017a) Guidance on information requirements and chemical safety assessment Chapter R.11: PBT/vPvB assessment. Version 3.0, June 2017, pp. 158.

ECHA (2017b) Member State Committee support document for identification of perfluorohexane-1-sulphonic acid and its salts as substances of very high concern because of their vPvB (Article 57e) properties. Adopted on 15 June 2017. , pp. 161.

ECHA (2019a) Annex XV restriction report. Proposal for a restriction of Undecafluorohexanoic acid (PFHxA), its salts and related substances. 20 December 2019 https://echa.europa.eu/documents/10162/c4e04484-c989-733d-33ed-0f023e2a200e, pp. 95.

ECHA (2019b) Member State Committee support document for identification of perfluorbutane sulfonic acid and its salts as substances of very high concern because of their hazardous properties which cause probable serious effects to human health and the environment which give rise to an equivalent level of concern because of their hazardous properties which cause probable serious effects to human health and the environment which give rise to an equivalent level of concern to those of CMR and PBT/vBvP substances (Article 57F). Adopted on 11 December 2019., pp. 202.

EFSA (2020) Risk to human health related to the presence of perfluoroalkyl substances in food. *EFSA Journal*, **18**.

Fliedner, A., Rudel, H., Dreyer, A., Pirntke, U. & Koschorreck, J. (2020) Chemicals of emerging concern in marine specimens of the German Environmental Specimen Bank. *Environmental Sciences Europe*, **32**.

Glynn, A., Berger, U., Bignert, A., Ullah, S., Aune, M., Lignell, S. & Darnerud, P.O. (2012) Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: serial sampling during pregnancy and nursing, and temporal trends 1996-2010. *Environ Sci Technol*, **46**, 9071-9079.

Groenen, M.A. & Archibald, A.L. & Uenishi, H. & Tuggle, C.K. & Takeuchi, Y. & Rothschild, M.F. & Rogel-Gaillard, C. & Park, C. & Milan, D. & Megens, H.J. & Li, S. & Larkin, D.M. & Kim, H. & Frantz, L.A. & Caccamo, M. & Ahn, H. & Aken, B.L. & Anselmo, A. & Anthon, C. & Auvil, L. & Badaoui, B. & Beattie, C.W. & Bendixen, C. & Berman, D. & Blecha, F. & Blomberg, J. & Bolund, L. & Bosse, M. & Botti, S. & Bujie, Z. & Bystrom, M. & Capitanu, B. & Carvalho-Silva, D. & Chardon, P. & Chen, C. & Cheng, R. & Choi, S.H. & Chow, W. & Clark, R.C. & Clee, C. & Crooijmans, R.P. & Dawson, H.D. & Dehais, P. & De Sapio, F. & Dibbits, B. & Drou, N. & Du, Z.Q. & Eversole, K. & Fadista, J. & Fairley, S. & Faraut, T. & Faulkner, G.J. & Fowler, K.E. & Fredholm, M. & Fritz, E. & Gilbert, J.G. & Giuffra, E. & Gorodkin, J. & Griffin, D.K. & Harrow, J.L. & Hayward, A. & Howe, K. & Hu, Z.L. & Humphray, S.J. & Hunt, T. & Hornshøj, H. & Jeon, J.T. & Jern, P. & Jones, M. & Jurka, J. & Kanamori, H. & Kapetanovic, R. & Kim, J. & Kim, J.H. & Kim, K.W. & Kim, T.H. & Larson, G. & Lee, K. & Lee, K.T. & Leggett, R. & Lewin, H.A. & Li, Y. & Liu, W. & Loveland, J.E. & Lu, Y. & Lunney, J.K. & Ma, J. & Madsen, O. & Mann, K. & Matthews, L. & McLaren, S. & Morozumi, T. & Murtaugh, M.P. & Narayan, J. & Nguyen, D.T. & Ni, P. & Oh, S.J. & Onteru, S. & Panitz, F. & Park, E.W. & Park, H.S. & Pascal, G. & Paudel, Y. & Perez-Enciso, M. & Ramirez-Gonzalez, R. & Reecy, J.M. & Rodriguez-Zas, S. & Rohrer, G.A. & Rund, L. & Sang, Y. & Schachtschneider, K. & Schraiber, J.G. & Schwartz, J. & Scobie, L. & Scott, C. & Searle, S. & Servin, B. & Southey, B.R. & Sperber, G. & Stadler, P. & Sweedler, J.V. & Tafer, H. & Thomsen, B. & Wali, R. & Wang, J. & Wang, J. & White, S. & Xu, X. & Yerle, M. & Zhang, G. & Zhang, J. & Zhang, J. & Zhao, S. & Rogers, J. & Churcher, C. & Schook, L.B. (2012) Analyses of pig genomes provide insight into porcine demography and evolution. *Nature*, **491**, 393-398.

Haug, L.S., Huber, S., Becher, G. & Thomsen, C. (2011) Characterisation of human exposure pathways to perfluorinated compounds--comparing exposure estimates with biomarkers of exposure. *Environment international*, **37**, 687-693.

Hayat K (2012) Difference Between Plasma And Serum. WWW document. https://medimoon.com/2012/07/difference-between-plasma-and-serum/.

Hethey, C., Mielke, H. & Gundert-Remy, U. (2019) Comment on ‘Kim, S.-J., Choi, E.-J., Choi, G.-W., Lee, Y.-B., and Cho, H.-Y. (2019). Exploring sex diferences in human health risk assessment for PFNA and PFDA using a PBPK model, Arch Toxicol 93:311–330’. *Archives of Toxicology*, **93**, 1769-1770.

Kim, S.J., Choi, E.J., Choi, G.W., Lee, Y.B. & Cho, H.Y. (2019) Exploring sex differences in human health risk assessment for PFNA and PFDA using a PBPK model. *Arch Toxicol*, **93**, 311-330.

Lee, H. & Mabury, S.A. (2011) A pilot survey of legacy and current commercial fluorinated chemicals in human sera from United States donors in 2009. *Environ Sci Technol*, **45**, 8067-8074.

Lee, W., An, Y., Yoon, H. & Kweon, H. (2008) Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (Phaseolus radiatus) and wheat (Triticum aestivum): plant agar test for water-insoluble nanoparticles. *Environmental Toxicology and Chemistry*, **27**, 1915-1921.

Li, Y., Fletcher, T., Mucs, D., Scott, K., Lindh, C.H., Tallving, P. & Jakobsson, K. (2018) Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. *Occupational and environmental medicine*, **75**, 46-51.

Numata, J., Kowalczyk, J., Adolphs, J., Ehlers, S., Schafft, H., Fuerst, P., Müller-Graf, C., Lahrssen-Wiederholt, M. & Greiner, M. (2014) Toxicokinetics of seven perfluoroalkyl sulfonic and carboxylic acids in pigs fed a contaminated diet. *Journal of agricultural and food chemistry*, **62**, 6861-6870.

Ohmori, K., Kudo, N., Katayama, K. & Kawashima, Y. (2003) Comparison of the toxicokinetics between perfluorocarboxylic acids with different carbon chain length. *Toxicology*, **184**, 135-140.

Olsen, G.W., Burris, J.M., Ehresman, D.J., Froehlich, J.W., Seacat, A.M., Butenhoff, J.L. & Zobel, L.R. (2007) Half-life of serum elimination of perfluorooctanesulfonate,perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. *Environ Health Perspect*, **115**, 1298-1305.

Olsen, G.W., Lange, C.C., Ellefson, M.E., Mair, D.C., Church, T.R., Goldberg, C.L., Herron, R.M., Medhdizadehkashi, Z., Nobiletti, J.B., Rios, J.A., Reagen, W.K. & Zobel, L.R. (2012) Temporal trends of perfluoroalkyl concentrations in American Red Cross adult blood donors, 2000-2010. *Environ Sci Technol*, **46**, 6330-6338.

Thompson, J., Lorber, M., Toms, L.L., Kato, K., Calafat, A.M. & Mueller, J.F. (2010) Use of simple pharmacokinetic modeling to characterize exposure of Australians to perfluorooctanoic acid and perfluorooctane sulfonic acid. *Environment international*, **36**, 390-397.

Walser, M. (1987) Creatinine excretion as a measure of protein nutrition in adults of varying age. *JPEN. Journal of parenteral and enteral nutrition*, **11**, 73s-78s.

Yeung, L.W., Robinson, S.J., Koschorreck, J. & Mabury, S.A. (2013) Part I. A temporal study of PFCAs and their precursors in human plasma from two German cities 1982-2009. *Environ Sci Technol*, **47**, 3865-3874.

Zhang, Y., Beesoon, S., Zhu, L. & Martin, J.W. (2013) Biomonitoring of perfluoroalkyl acids in human urine and estimates of biological half-life. *Environ Sci Technol*, **47**, 10619-10627.

Zhao, S.Y., Zhu, L.Y., Liu, L., Liu, Z.T. & Zhang, Y.H. (2013) Bioaccumulation of perfluoroalkyl carboxylates (PFCAs) and perfluoroalkane sulfonates (PFSAs) by earthworms (Eisenia fetida) in soil. *Environmental Pollution*, **179**, 45-52.

Annex 1

alternative method									
PFHpA, median	BLOOD	l blood/kg bw	kg bw	serum/blood rate	ng/l serum (Zhang, 2013)	URINE	ng/l urine (Zhang, 2013)	l urine/day	ln 2
		0.077	60	0.57	58		0.82	1.3	0.693147
		4.62	l blood			elimination	1.07	ng/d	
		2.63	l serum						
		152.74	ng						
	T1/2	99.31	days						
	k2	0.0070	d-1						

Annex 2

BMF human					
	Corganisme / C diet				
	Corganisme / C drinking water (main intake source)				
	Corganisme	152,74	ng in 5 l blood	Zhang *et al* . 2013	
		366,57	ng in adult	factor 2.4 (amount whole body/amount in blood)	
		5,24	ng/kg bw	70 kg bw	
	Cdrinking water	97	ng/l	Xu et al., 2020, contaminated	
		194	ng/2l	intake	
		2,77	ng/kg bw	intake	
	BMF	1,89			

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