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2-Mercaptobenzothiazole in urine of children and adolescents in Germany – Human biomonitoring results of the German Environmental Survey 2014–2017 (GerES V)

Aline Murawski[a,*], Maria I.H. Schmied-Tobies[a], Gerda Schwedler[a], Enrico Rucic[a], Wolfgang Gries[b,1], Christoph Schmidtkunz[b], Katja Küpper[b], Gabriele Leng[b], André Conrad[a], Marike Kolossa-Gehring[a]

[a] German Environment Agency (UBA), Berlin, Germany
[b] Currenta GmbH & Co. OHG, Institute of Biomonitoring, Leverkusen, Germany

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ABSTRACT

2-Mercaptobenzothiazole (2-MBT) is widely used as a vulcanisation accelerator and is contained in many products made from natural rubber, e.g. car tires. Additionally, it is used as a fungicide in paint or fibre. Systemically human exposure to 2-MBT can occur via dermal and oral uptake or inhalation. Locally, 2-MBT can cause skin sensitisation. The International Agency for Research on Cancer (IARC) classified 2-MBT as probably carcinogenic to humans.

516 urine samples of 3- to 17-year-old children and adolescents living in Germany were analysed for the concentration of 2-MBT in the population representative German Environmental Survey for Children and Adolescents 2014–2017 (GerES V). 2-MBT was quantified above the limit of quantification (LOQ) of 1.0 µg/L in 50% of the 516 samples analysed. The geometric mean of urinary 2-MBT concentration was 1.018 µg/L and 0.892 µg/$g_{creatinine}$, the arithmetic mean was 1.576 µg/L (1.351 µg/g_{crea}). The median concentration was below the LOQ.

Analyses of subgroups revealed higher 2-MBT concentrations in children aged 3–5 years compared to 14- to 17-year-old adolescents. All urinary 2-MBT concentrations were well below the health-based guidance value HBM-I for children of 4.5 µg/L. Therefore, current exposure levels are – according to current knowledge – not of concern. For the first time, reference values can be derived for 2-MBT for children and adolescents in Germany. This will facilitate to recognise changing exposure levels in this population group in Germany and identification of unusually high exposures.

1. Introduction

2-Mercatobenzothiazole (2-MBT) is a vulcanisation accelerator and widely used in the rubber production, particularly in natural rubber. Thus, it is contained in many commodities such as car tires, cables, rubber gloves, seals, shoes, but also toys and swimwear (Emmett et al., 1994; Gries et al., 2015). It also is sporadically found in baby soothers made from natural rubber (Bekanntmachung des Umweltbundesamtes, 2015). 2-MBT is also a decomposition product of biocides used for paper and leather products (Gries et al., 2015). Furthermore, its salts are used as a fungicide in latex, oil paints, and textile fibres (EPA, 1994). Humans can be exposed to 2-MBT by dermal, and oral or inhalative uptake. Due to the usage in car tires, tire tread wear and hence particulate matter as an ubiquitous source of exposure especially in traffic intense areas (Avagyan et al., 2014). The authors tested the hypothesis, if 2-MBT can serve as an indicator for traffic intensity.

Abbreviations: 2-MBT, 2-Mercaptobenzothiazole; AM, arithmetic mean; CI GM, 95% confidence interval for the geometric mean; CLP, Classification, Labelling and Packaging; crea, creatinine; DFG, German Research Foundation; GerES V, German Environmental Survey for Children and Adolescents; GM, geometric mean; HBM, human biomonitoring; HBM-I, human biomonitoring value I; KiGGS Wave 2, German Health Interview and Examination Survey for Children and Adolescents; MAX, maximum value; N, sample size; P, percentiles; RKI, Robert Koch Institute; SES, socio-economic status

* Corresponding author.
E-mail address: aline.murawski@uba.de (A. Murawski).
[1] Present address: Currenta GmbH & Co. OHG, Environmental Analytics, Krefeld-Uerdingen, Germany.

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Table 1
Urinary concentrations of 2-MBT (µg/L) in a subpopulation of GerES V participants. LOQ = 1 µg/L.

	N	N < LOQ	% ≥ LOQ	P10	P50	P90	P95	P98	MAX	AM	GM	CI GM
Total	516 (100%)	260	50	< LOQ	< LOQ	3.14	4.81	8.53	43.5	1.576	1.018	–
Sex												
male	266 (52%)	133	50	< LOQ	< LOQ	3.56	5.06	8.50	38.0	1.666	1.075	–
female	250 (48%)	126	49	< LOQ	< LOQ	2.42	4.39	10.3	43.5	1.482	< LOQ	–
Age group*												
3–5 years	99 (19%)	39	61	< LOQ	1.28	4.60	5.45	11.5	14.1	1.864	1.231	1.035–1.463
6–10 years	166 (32%)	83	50	< LOQ	< LOQ	3.56	6.06	10.3	38.0	1.693	1.040	–
11–13 years	103 (20%)	66	35	< LOQ	< LOQ	1.96	3.06	5.68	43.5	1.143	< LOQ	–
14–17 years	149 (29%)	71	52	< LOQ	1.00	3.34	5.47	8.53	17.1	1.555	1.044	–
Community size (inhabitants)												
< 50,000	168 (33%)	89	47	< LOQ	< LOQ	3.34	4.81	7.43	43.5	1.503	< LOQ	–
50,000 - < 100,000	31 (6%)	17	45	< LOQ	< LOQ	2.27	4.13	–	5.96	1.109	< LOQ	–
≥ 100,000	317 (61%)	154	52	< LOQ	1.06	3.19	5.80	10.3	38.0	1.661	1.054	–
Socio-economic status												
low	103 (20%)	56	46	< LOQ	< LOQ	2.06	4.33	5.07	11.7	1.180	< LOQ	–
medium	309 (60%)	153	50	< LOQ	1.02	3.34	4.84	8.53	43.5	1.602	1.046	–
high	82 (16%)	41	50	< LOQ	< LOQ	3.10	4.46	7.81	38.0	1.615	1.034	–
Region of residence												
former West Germany (including West Berlin)	434 (84%)	222	49	< LOQ	< LOQ	3.13	4.81	8.53	38.0	1.515	< LOQ	–
former East Germany (including East Berlin)	82 (16%)	37	55	< LOQ	1.10	4.08	7.81	14.1	43.5	1.902	1.126	–
Migration background												
no migration background	358 (69%)	179	50	< LOQ	< LOQ	3.13	4.81	7.83	43.5	1.579	1.030	–
one-sided migration background	59 (11%)	27	54	< LOQ	1.12	3.56	5.28	14.1	14.1	1.719	1.090	–
two-sided migration background	83 (16%)	45	45	< LOQ	< LOQ	2.02	3.27	3.34	8.46	1.100	< LOQ	–
Traffic intensity at home												
very/extremely busy through road	69 (13%)	34	51	< LOQ	1.07	5.07	8.48	14.1	14.1	1.944	1.113	–
busy minor road	75 (15%)	44	42	< LOQ	< LOQ	2.22	3.56	4.88	5.09	1.095	< LOQ	–
moderately busy minor road	127 (25%)	58	55	< LOQ	1.12	2.66	4.28	5.22	43.5	1.456	1.031	–
very little traffic (residential street, lane, restricted traffic zone)	208 (40%)	105	49	< LOQ	< LOQ	3.34	4.81	8.47	38.0	1.601	1.035	–

The sample and subsample sizes were calculated as the sum of case weights. Due to rounding to nearest whole numbers, the sum of stratified sample sizes does not always exactly match the total sample size. Further discrepancies may be due to missing values in stratification criteria.N = sample size; P10, P50, P90, P95, P98 = percentiles; MAX = maximum value; AM = arithmetic mean; GM = geometric mean; % of values ≥ LOQ (limit of quantification); CI GM = approximate 95% confidence interval for the GM; – = not calculated due to too few measurements above LOQ (CI GM not given, if lower limit < LOQ).
Significance test: χ^2 test of independence (number of measurements below and above LOQ): *p ≤ 0.01.

Another potentially relevant source of exposure to 2-MBT are synthetic turf fields (Ginsberg et al., 2011). Pors and Fuhlendorff (2003) analysed the migration properties of 2-MBT in different natural rubber products and found substantial migration from elastic bandages (sports equipment) and gloves in contact with sweat. Occupational exposure to 2-MBT is observed for individuals with extended contact to rubber products, e.g. shoemakers and cleaning staff (Gries et al., 2015). 2-MBT can cause skin sensitisation (Bekanntmachung des Umweltbundesamtes, 2015) and is suspected to cause “bikini dermatitis” (Jung et al., 2006), “tennis shoe dermatitis” (Jung et al., 1988), and atypical diaper dermatitis (Onken et al., 2011). 2-MBT is classified and labelled according to EC No 1272/2008 (CLP) as skin sensitising category 1 (H317: may cause an allergic skin reaction) (European Commission, 2008). It is also suspected to increase the risk of bladder cancer, but the hypothesized relation could not clearly be confirmed (Bekanntmachung des Umweltbundesamtes, 2015). The International Agency for Research on Cancer (IARC) classified 2-MBT as “probably carcinogenic to humans” (IARC group 2A) (IARC, 2018). The Human Biomonitoring Commission derived a toxicologically-based HBM-I value of 4.5 mg/(L urine) for children and 7 mg/(L urine) for adults, based on the no observed adverse effect level (NOAEL) of 94 mg/(kg bodyweight) and relative liver weight as the endpoint in a sub-chronic oral study in mice (Bekanntmachung des Umweltbundesamtes, 2015). The German Federal Institute for Risk Assessment (BfR) found that the emission of 2-MBT from consumer products should be minimised as far as possible (BfR, 2008) and the European Chemicals Agency (ECHA) included 2-MBT in the Community Rolling Action Plan (CoRAP) for substance evaluation, calling for including 2-MBT into biomonitoring programs (ECHA, 2014).

2-MBT was identified as a chemical of relevance by a cooperation between the German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) and the German Chemical Industry Association (VCI), in which scientific activities the German Environment Agency (UBA) plays a vital role (Kolossa-Gehring et al., 2017). The cooperation aims at developing new, sensitive, and specific analytical human biomonitoring methods to determine the body burden of substances with potential health relevance or substantial exposure. The development of the analytical biomonitoring method for 2-MBT was successfully completed in 2011 (Apel et al., 2017; Gries et al., 2015; Leng and Gries, 2017).

The German Environmental Survey (GerES) is a representative cross-sectional study on human exposure to environmental chemicals and its sources (Kolossa-Gehring et al., 2012a, 2012b) repeatedly been conducted since the 1980s. The field work of the fifth cycle (GerES V) was conducted from 2014 to 2017 (Schulz et al., 2017) and 2294 children and adolescents living in Germany aged 3–17 years were investigated. Three main instruments of GerES V are human biomonitoring (HBM) in combination with ambient monitoring and collection of information on exposure relevant conditions, habits and behaviours (e.g. dietary habits, use of cosmetics and consumer products, characteristics of the residential environment) via questionnaires (Schulz et al., 2017).

In the present paper, we describe and evaluate 2-MBT concentrations in urine samples of children and adolescents, collected in GerES V, also for different population subgroups. It is the first HBM study worldwide to give data on current 2-MBT exposure in a representative sample of the general population.

Table 2
Urinary concentrations of 2-MBT (µg/$g_{creatinine}$) in a subpopulation of GerES V participants.

	N	P10	P50	P90	P95	P98	MAX	AM	GM	CI GM
Total	516 (100%)	*0.30*[1]	*0.85*[1]	2.66	3.52	5.42	42.7	1.351	0.892	*0.829–0.959*[1]
Sex										
male	266 (52%)	*0.28*[1]	*0.85*[1]	2.70	3.52	5.42	42.7	1.365	0.912	*0.822–1.012*[1]
female	250 (48%)	*0.31*[1]	*0.86*[1]	2.47	3.36	6.10	21.6	1.336	*0.870*[1]	*0.785–0.965*[1]
Age group										
3–5 years	99 (19%)	*0.60*[1]	1.74	3.54	5.42	15.9	21.6	2.291	1.672	1.441–1.940
6–10 years	166 (32%)	*0.37*[1]	*1.01*[1]	2.93	5.25	6.57	42.7	1.538	1.040	*0.920–1.177*[1]
11–13 years	103 (20%)	*0.28*[1]	*0.55*[1]	1.54	2.45	3.30	21.1	0.823	*0.601*[1]	*0.524–0.690*[1]
14–17 years	149 (29%)	*0.27*[1]	0.62	1.70	2.57	3.15	9.40	0.883	0.649	*0.575–0.733*[1]
Community size (inhabitants)										
< 50,000	168 (33%)	*0.30*[1]	*0.68*[1]	2.59	3.13	5.42	21.1	1.246	*0.825*[1]	*0.724–0.940*[1]
50,000 - < 100,000	31 (6%)	*0.32*[1]	*0.78*[1]	1.78	2.88	–	3.39	0.954	*0.788*[1]	*0.624–0.995*[1]
≥ 100,000	317 (61%)	*0.31*[1]	0.92	2.70	4.26	5.72	42.7	1.445	0.940	*0.856–1.033*[1]
Socio-economic status										
low	103 (20%)	*0.33*[1]	*0.63*[1]	2.68	3.15	3.95	8.30	1.069	*0.773*[1]	*0.666–0.899*[1]
medium	309 (60%)	*0.28*[1]	0.95	2.58	3.26	5.42	21.6	1.373	0.908	*0.825–1.000*[1]
high	82 (16%)	*0.37*[1]	*0.74*[1]	2.16	5.23	8.77	42.7	1.411	0.883	*0.742–1.051*[1]
Region of residence										
former West Germany (including West Berlin)	434 (84%)	*0.30*[1]	*0.84*[1]	2.60	3.43	5.36	42.7	1.267	*0.867*[1]	*0.802–0.938*[1]
former East Germany (including East Berlin)	82 (16%)	*0.39*[1]	0.95	3.07	7.86	21.5	21.6	1.794	1.033	*0.847–1.260*[1]
Migration background										
no migration background	358 (69%)	*0.30*[1]	*0.80*[1]	2.56	3.23	5.47	42.7	1.270	0.851	*0.780–0.928*[1]
one-sided migration background	59 (11%)	*0.32*[1]	1.14	3.92	4.70	21.6	21.6	2.009	1.201	*0.948–1.522*[1]
two-sided migration background	83 (16%)	*0.28*[1]	*0.76*[1]	1.84	2.17	2.97	11.0	0.985	*0.782*[1]	*0.673–0.910*[1]
Traffic intensity at home										
very/extremely busy through road	69 (13%)	*0.33*[1]	1.16	2.68	4.51	21.6	21.6	1.688	0.967	*0.775–1.207*[1]
busy minor road	75 (15%)	*0.30*[1]	*0.78*[1]	1.98	4.49	4.70	4.70	1.078	*0.795*[1]	*0.670–0.943*[1]
moderately busy minor road	127 (25%)	*0.27*[1]	0.86	2.35	3.12	4.87	21.1	1.201	0.844	*0.730–0.976*[1]
very little traffic (residential street, lane, restricted traffic zone)	208 (40%)	*0.33*[1]	*0.80*[1]	2.49	3.27	6.51	42.7	1.338	0.884	*0.790–0.990*[1]

The sample and subsample sizes were calculated as the sum of case weights. Due to rounding to nearest whole numbers, the sum of stratified sample sizes does not always exactly match the total sample size. Further discrepancies may be due to missing values in stratification criteria.N = sample size; P10, P50, P90, P95, P98 = percentiles; MAX = maximum value; AM = arithmetic mean; GM = geometric mean; % of values ≥ LOQ (limit of quantification); CI GM = approximate 95% confidence interval for the GM; – = not calculated due to too few measurements above LOQ (CI GM not given, if lower limit < LOQ).[1] = corresponding volume-based value is < LOQ.

2. Material and methods

2.1. Study population and sample collection

GerES V was conducted in close cooperation with the German Health Interview and Examination Survey for Children and Adolescents (KiGGS Wave 2) of the Robert Koch Institute (RKI) (Mauz et al., 2017). For KiGGS Wave 2 a population-representative sample of children and adolescents was drawn by first selecting 167 sampling locations in Germany, reflecting the grade of urbanisation and geographic distribution of the population, and secondly selecting children and adolescents randomly from the respective inhabitant registries (Mauz et al., 2017). Out of the KiGGS Wave 2 participants, a stratified random subsample of the 3- to 17-year-olds was invited to also participate in GerES V (Schulz et al., 2017).

A key part of sample collection in GerES V was a visit at the participants' home. During the home visits, the interviewer received, inter alia, samples of first void urine and conducted an interview with the participants' parents/guardians and the participants themselves (if aged 11 and older).

The fieldwork preparation for GerES V was started in 2014 and sample collection took place from January 2015 to June 2017. It was conducted by Kantar Health on behalf of UBA.

For assessing the internal exposure to environmental contaminants, the complete amount of first void urine was collected by the participants themselves or with help from their parents/guardians. First void urine was defined as correctly sampled if the last urination was at least 4 h before sampling and the child did not wear diapers. The urine samples were taken either in polypropylene receptacles or in wide-necked polyethylene containers, depending on sex and age group of the participant. The samples were kept cold, aliquoted in polypropylene vials, frozen (−20 °C) the same day, and kept frozen until analysis. Samples were analysed in randomised order to avoid observer bias. 2-MBT was analysed in 516 first void urine samples stratified randomly selected from the GerES V sample of 2294 participants.

The Ethics Committee of the Berlin Chamber of Physicians (Eth-14/14) and the Federal Officer for Data Protection and Freedom of Information (III-425/009#0018) had approved the project. All parents/legal guardians and all adolescents themselves provided written informed consent.

2.2. Chemical analysis

Creatinine content of the urine samples was photometrically determined by a validated standard assay based on the Jaffé method (Blaszkiewicz and Liesenhoff-Henze, 2010). Creatinine was analysed by the Analytisch-Biologisches Forschungslabor (ABF GmbH), Munich (Germany).

2-Mercaptobenzothiazole (2-MBT, CAS number 149-30-4) was analysed according to Gries et al. (2015) with a limit of quantification (LOQ) of 1.0 µg/L. In brief, urine samples were hydrolysed enzymatically. After centrifugation, the supernatant was analysed by high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) in positive electrospray ionization mode. 2-MBT was determined using isotope-dilution quantification. The analytical method was validated according to guidelines of the German Research Foundation (DFG) (Bader et al., 2012). Determination of 2-MBT was performed by the Institute of Biomonitoring of Currenta GmbH & Co. OHG, Leverkusen (Germany).

Internal quality control was ensured by analysis of quality control (QC) samples with known concentration. Additionally, blinded control samples were randomly included in all analytical cycles. Furthermore,

field blanks (ultrapure water) were also used for quality control. 2-MBT was not detected in any of the field blanks and the internal quality control samples were always measured within the ± 3σ range.

External quality control was ensured for creatinine by successful participation in regular biannual ring trials of the German External Quality Assessment Scheme (G-EQUAS) from 2014 to 2017. No external quality control schemes are available for 2-MBT. However, the analytical method was verified by an independent laboratory according to the guidelines of the DFG working group on Analyses in Biological Material (Bader et al., 2012).

2.3. Statistical analysis

To compensate for minor deviations of the realised sample from characteristics of the population in Germany aged 3–17 years, case weights were provided by the RKI (Hoffmann et al., 2018) for the statistical data evaluation. All results presented here are based on weighted calculations. Sample sizes are given as the rounded sum of respective case weights.

For all measured urinary concentrations, the following basic statistical characteristics are presented here: sample size (N), number of samples below LOQ (N < LOQ), sample fraction equal to or exceeding LOQ (% ≥ LOQ), selected percentiles (P), maximum value (MAX), geometric mean (GM), arithmetic mean (AM), and 95% confidence interval for the geometric mean (CI GM). Weighted sample sizes and fractions are rounded to the nearest whole number. P and MAX values are presented with three significant places, calculated values (AM, GM, and CI GM) are presented with one additional significant place. Volume-based (in µg/L) as well as creatinine-adjusted (in $\mu g/g_{crea}$) concentrations are presented for 2-MBT. Urinary concentrations below LOQ were assigned a value of LOQ/2 for calculation purposes.

Statistical characteristics are described for the total sample, for standard stratification variables (sex, age group, community size, socio-economic status, region of residence (former East or West Germany), migration background), as well as for self-reported traffic intensity in the vicinity of the home, which is suspected to have an effect on 2-MBT exposure. The socio-economic status (SES) was derived in KiGGS Wave 2 based on parent's income, education, and profession (Lampert et al., 2018). Migration background was based on the country of birth of the participant and her or his parents, and on the parents' nationality. One-sided migration background was defined as having one parent not born in Germany or without German citizenship. Two-sided migration background included children and adolescents who themselves migrated to Germany and have at least one parent who was not born in Germany. Children and adolescents belong also to this group, when both parents were born in a country other than Germany or when they are non-German nationals (Frank et al., 2018).

Each of the stratification variables was tested for significance of differences in the fraction of (volume-based) values below and above LOQ between different groups by applying a χ^2 test of independence. All statistical analyses were performed with SPSS statistical package (versions 20 and 25).

3. Results and discussion

3.1. Urinary concentrations

Characteristics of the subsample of 516 urine samples analysed for 2-MBT are given in Table 1. The subsample is in good agreement with the total GerES sample of 2294 participants in terms of population structure regarding sex, age group, SES, region of residence, and migration background. Minor deviations from the total sample only occur for community size, but are of no further relevance here. Volume-based urinary 2-MBT concentrations are presented in Table 1 and creatinine-adjusted concentrations in Table 2.

Quantifiable amounts of 2-MBT were found in 50% of the samples. The geometric mean of 2-MBT concentrations in urine of 3- to 17-year-old children and adolescents was 1.018 µg/L (0.892 $\mu g/g_{crea}$), the arithmetic mean was 1.576 µg/L (1.351 $\mu g/g_{crea}$), and the median (P50) was below LOQ. The maximum urinary 2-MBT concentration found in a GerES V sample was 43.5 µg/L (42.7 $\mu g/g_{crea}$).

No significant differences between boys and girls in 2-MBT exposure were found. Urine concentrations above LOQ were detected in 50% of males and 49% of females. The exposure levels were found to be significantly decreasing ($p \leq 0.01$) by age. This was true for the number of samples above LOQ as well as for average exposure levels. While 2-MBT was quantified in 61% of the 3-5-year-old children, it was found in 50% and 52% of the age group of 6–10 years and 14–17 years, respectively, and in only 35% of the 11-13-year-old adolescents. Accordingly, the mean (GM) 2-MBT concentration in 3-5-year-old children was 1.2 µg/L, whereas it was near or below the LOQ of 1.0 µg/L in all other age groups. The higher exposure of young children might possibly result from chewing on soothers and toys, which, if made from natural rubber, may contain 2-MBT, as described above. The observed age gradient was even more pronounced in the creatinine-adjusted concentrations, because age-related differences in diuresis and renal function result in much higher creatinine concentrations in adolescents' urine than in those of young children (Barr et al., 2005). For SES and migration background, no significant differences between subgroups were found. Slightly higher urinary concentrations of 2-MBT were found in participants living in large communities with ≥100,000 inhabitants compared to those living in smaller communities and for study participants living in former East Germany compared to those living in former West Germany. However, these differences were small and not statistically significant.

The hypothesis that 2-MBT could serve as a marker for traffic intensity in the close vicinity of the home could not be confirmed. No association between 2-MBT concentrations in urine and traffic intensity could be found. This may indicate that exposure in the close vicinity of the home is an unsuitable proxy for overall exposure, or particulate matter resulting from tire tread wear is spread over greater distances and thus affecting not only the immediate proximity.

Up to now, no other data on 2-MBT urinary concentrations are available, neither for children and adolescents nor for adults representing the general population. GerES V is the first population representative survey to have 2-MBT included in its biomonitoring program.

Future surveys might additionally investigate further specific sources of potential exposure to 2-MBT, such as exposure to natural rubber products, to give a more detailed picture on relevant sources of urinary 2-MBT.

3.2. Comparison with health-based guidance values

The human biomonitoring value HBM-I of 4.5 mg/L is by two orders of magnitude higher than the maximum concentration found (43.5 µg/L). Hence, the observed internal exposures of German children and adolescents to 2-MBT are, according to the current state of knowledge, of no concern regarding systemic health impacts. However, the HBM I-value does not account for local effects and sensitisation.

4. Conclusions and outlook

The results shown here are representative of children and adolescents in Germany aged 3–17 years and reveal their exposure to 2-MBT. This study presents the worldwide first data for exposure of the general 3-17-year-old population to 2-MBT on a national level. Despite its ubiquitous usage, 2-MBT was detected in only about every second study participant living in Germany. Exposure levels were well below the existing health-based guidance value for systemic exposure. According to current knowledge they do not pose a risk to human health from internal exposure. The skin sensitising properties were not included in

the derivation of the HBM-I value and therefore, a conclusion of the health relevance concerning sensitisation cannot be drawn. For two reasons 2-MBT should be included in future HBM studies to further monitor potential changes in exposure levels: 1. because the youngest children show the most frequent and highest exposure levels like for other chemicals (Schwedler et al., 2019, 2020), and 2. because the internal exposure of 50% of the study population to a sensitising agent should be followed up. The GerES V data will also be the basis for statistically derived reference values on 2-MBT.

Declaration of competing interest

Lanxess Deutschland GmbH, a producer of 2-MBT, holds 40% of the Currenta GmbH & Co. OHG shares and is an important customer of the Institute of Biomonitoring in the field of occupational medicine. However, regarding the work reported in the present paper, Lanxess was never involved in any aspect of the study, including study design, the collection, analysis, and interpretation of data, the writing of the report, and the decision to submit the paper for publication. Currenta's Institute of Biomonitoring is accredited to DIN EN ISO/IEC 17025 and is thus subject to the requirements for impartiality defined in this standard and to internal and external audits. Risks to impartiality are identified, documented and regularly reviewed, and appropriate measures to mitigate these risks are implemented in our Institute.

All authors affiliated to UBA declare no conflict of interest related to this work.

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A biomonitoring study assessing the exposure of young German adults to butylated hydroxytoluene (BHT)

Christoph Schmidtkunz[a,*], Katja Küpper[a], Till Weber[b], Gabriele Leng[a], Marike Kolossa-Gehring[b]

[a] Currenta GmbH & Co. OHG, Institute of Biomonitoring, 51368 Leverkusen, Germany
[b] German Environment Agency (UBA), Corrensplatz 1, 14195 Berlin, Germany

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ABSTRACT

The antioxidant 2,6-di-*tert*-butyl-4-methylphenol (butylated hydroxytoluene, BHT) is used ubiquitously in food, cosmetics, pharmaceuticals, fuels, plastics, rubbers and many other products. Therefore, exposure of the general population to this substance is likely.

We analyzed the BHT metabolite 3,5-di-*tert*-butyl-4-hydroxybenzoic acid ("BHT acid") in 24-h urine samples from the German Environmental Specimen Bank with the aim of gaining a better understanding of the internal burden of BHT in young nonspecifically exposed adults. The study population consisted of students between 20 and 29 years of age at the time of sampling, all from Halle/Saale in Central Germany. In total, 329 samples collected in the years 2000, 2004, 2008, 2012, 2015, and 2018 were measured by ultra high performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS).

BHT acid was detected above the limit of quantification (0.2 µg/L) in 98% of the samples. The median of the measured concentrations was 1.06 µg/L and 1.24 µg/g creatinine respectively, the median of the daily excretion was 1.76 µg/24 h and – additionally normalized for body weight – 26.8 ng/24 h × kg bw respectively. The corresponding 90th percentiles were 3.28 µg/L, 3.91 µg/g creatinine, 5.05 µg/24 h, and 81.9 ng/24 h × kg bw. Medians of creatinine-corrected values were slightly higher in women than in men, while the opposite situation was observed for the volume concentrations and the 24-h excretion values (not corrected for body weight). Values simultaneously normalized both for 24-h excretion and body weight did not exhibit any significant differences between males and females, probably indicating a virtually identical magnitude of exposure for both genders. The background exposure of the investigated population was found to be largely constant since the year 2000, with only weak temporal trends at most.

Daily intakes were estimated from excretion values and found to be largely below the acceptable daily intake (ADI) of BHT at 0.25 mg/kg bw: our worst-case estimate is a daily BHT intake of approximately 0.1 mg/kg bw at the 95th percentile level. However, these intake assessments rely on very limited quantitative data regarding human metabolism of BHT.

1. Introduction

Butylated hydroxytoluene (BHT, 2,6-di-*tert*-butyl-*p*-cresol, IUPAC name: 2,6-di-*tert*-butyl-4-methylphenol) is widely used as an antioxidant thanks to its properties as a radical scavenger. An acid-catalyzed process for BHT synthesis starting from *p*-cresol and isobutylene was patented in 1947 (Stillson, 1947), while BHT as such had already been described a couple of years earlier (Pardee and Weinrich, 1944; Stevens, 1943; Weinrich, 1943). Since then BHT has found numerous application areas. They are very varied and include food, animal feed, cosmetics, packaging materials, pharmaceuticals, gasolines, mineral oils, polymers, paints, and inks (OECD, 2002). Many of these uses may lead to exposure of the general population with this antioxidant. Furthermore, BHT and its degradation products have also been detected in environmental samples such as rain, river water, ground water, waste water, sediments, and soil (Fries and Püttmann, 2002; Jungclaus et al., 1978; Muszkat et al., 1993; Rodil et al., 2010; Yasuhara et al., 1981).

The toxicology of BHT has been extensively investigated over the past decades, and an ADI value of 0.25 mg/kg bw was derived by the European Food Safety Authority, based on a NOAEL of 25 mg/kg bw per day and an uncertainty factor of 100 (EFSA, 2012). This assessment mainly covers hepatic enzyme induction and resulting thyroid hyperactivity as well as reproductive effects as most sensitive endpoints observed in the studies by Olsen et al. (1986) and Price (1994). The

* Corresponding author.
E-mail addresses: christoph.schmidtkunz@currenta.de (C. Schmidtkunz), katja.kuepper@currenta.de (K. Küpper), gabriele.leng@currenta.de (G. Leng).

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Abbreviations

ADI	acceptable daily intake
BHT	butylated hydroxytoluene (2,6-di-*tert*-butyl-4-methylphenol, 2,6-di-*tert*-butyl-*p*-cresol)
BHT acid	3,5-di-*tert*-butyl-4-hydroxybenzoic acid
bw	body weight
ESB	Environmental Specimen Bank
LOQ	limit of quantification
NOAEL	no observed adverse effect level
QC	quality control
RfD	reference dose
SPE	solid phase extraction
UHPLC-MS/MS	ultra high performance liquid chromatography – tandem mass spectrometry

United States Environmental Protection Agency has derived a chronic provisional RfD at a very similar level: 0.3 mg/kg bw per day, taking decreased body weight as the critical endpoint (EPA, 2013).

The metabolism of BHT is complex and subject to variability between different animal species. Several metabolic pathways have been described, e.g. via oxidation of the 4-methyl group or the *tert*-butyl side chains. Quinoid metabolites are known as well (DFG, 2004). For humans, it is thought that initial metabolism of BHT proceeds rapidly, but that tissue retention or enterohepatic circulation of BHT metabolites significantly slows down the rate of excretion (Daniel et al., 1967; Wiebe et al., 1978; Witschi et al., 1989). Indeed, approximately 50% of a single oral dose of ^{14}C-BHT were detected in human urine within 24 h after administration, and in total only about 63–67% of the dose after 11 days (Daniel et al., 1967). 3,5-di-*tert*-butyl-4-hydroxybenzoic acid ("BHT acid") has been reported as a characteristic urinary metabolite in humans, arising from stepwise oxidation of the 4-methyl moiety via the corresponding alcohol and aldehyde (Daniel et al., 1968; Holder et al., 1970; Wiebe et al., 1978). BHT acid can be excreted as the free acid or, more importantly, as its ester glucuronide conjugate. Quantitative human metabolism data about the relative amounts of individual metabolites after administration of a BHT dose is scarce and quite variable. For example, reported percentages for urinary excretion of BHT acid, both free and conjugated, range from 0.3% (Wiebe et al., 1978) to 3% (Daniel et al., 1968) and 5.5% (Verhagen et al., 1989) of the oral dose. Metabolites that are more abundant than BHT acid have been reported by various researchers (Daniel et al., 1968; Wiebe et al., 1978). However – in spite of their abundance – they do not seem to be an ideal choice for human biomonitoring: in part because there has been considerable disagreement about the adequacy of their structural elucidation (Holder et al., 1970; Wiebe et al., 1978), but also because quantitative metabolism data for these putative metabolites (Daniel et al., 1968; Wiebe et al., 1978) is even more limited than the data for BHT acid. Specifically, Daniel et al. (1968) reported the ester glucuronide of 4-carboxy-2-(1-carboxy-1-methylethyl)-6-(1-formyl-1-methylethyl) phenol, possibly as a hydrate, in the urine of two volunteers, at an estimated 35% of the administered BHT dose. But Holder et al. (1970) were unable to reproduce these results when they investigated a group of 8 volunteers who had been given BHT. Thus Holder et al. challenged the previous assignment, additionally citing insufficient structure elucidation in the paper by Daniel et al. Later, Wiebe et al. (1978) reported new evidence for the structure first suggested by Daniel et al., albeit in a cyclic hemiacetal form (5-carboxy-7-(1-carboxy-1-methylethyl)-3,3-dimethyl-2-hydroxy-2,3-dihydrobenzofuran). Wiebe and coworkers report that the excretion of this metabolite via urine accounted for approximately 21% (within 72 h) of an oral BHT dose in one volunteer. However, to our knowledge, this metabolite was not investigated by any other scientists since then (Verhagen et al., 1989; Wang and Kannan, 2019). BHT acid, on the other hand, is the one metabolite which most or even all of the relevant literature consistently mentions (Daniel et al., 1968; Holder et al., 1970; Verhagen et al., 1989; Wang and Kannan, 2019; Wiebe et al., 1978).

Hence the analytical determination of urinary BHT acid as a diagnostic marker for low-level exposure of humans to BHT was first reported by Göen et al. (2006a, 2006b). In the context of a cooperation between the German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) and the Verband der Chemischen Industrie e.V. (German Chemical Industry Association – VCI), Gries and colleagues developed and validated a high-throughput analysis method for the determination of BHT acid in human urine, based on UHPLC-MS/MS with online SPE enrichment (Gries et al., 2020; Kolossa-Gehring et al., 2017; Leng and Gries, 2017). We now report the application of this method to the analysis of 329 urine specimens (24-h voids) from the German Environmental Specimen Bank (ESB), collected between the years 2000 and 2018 from students of Martin Luther University in Halle/Saale, Germany. Based on the results we shall attempt to draw conclusions about the exposure level of the study population, aiming to validate previous estimates based on dietary intake figures (EFSA, 2012; Nieva-Echevarría et al., 2015).

2. Material and methods

2.1. Study population

The 24-h urine samples used in this study were collected from male and female students aged 20–29 years studying at Martin Luther University in Halle/Saale, Germany. Students were volunteering participants of the Environmental Specimen Bank (ESB). The study protocol of the ESB has been reviewed by the ethics committee of the Medical Association Westfalen-Lippe, the Medical Faculty of the University of Münster and (since 2012) by the ethical committee of the Medical

Table 1
Characterization of the study population.

year	Sex (m/f)	age [years] median (range)	weight [kg] median (range)	24 h urine volume [mL] median (range)	creatinine [g/L] median (range)
2000	50 (26/24)	21.5 (20–28)	67.5 (50–100)	1640 (540–2620)	1.33 (0.22–4.26)
2004	50 (25/25)	23 (20–29)	68 (50–95)	1733 (601–2789)	0.78 (0.34–1.75)
2008	52 (27/25)	25 (20–29)	65.5 (53–94)	1797.5 (473–3141)	0.96 (0.27–2.82)
2012	58 (28/30)	24 (20–29)	66 (47–110)	1743.5 (586–3799)	0.77 (0.15–2.49)
2015	59 (29/30)	24 (20–28)	70 (50–110)	1971 (645–3056)	0.61 (0.22–2.06)
2018	60 (30/30)	25 (20–29)	67 (49–110)	2023 (605–2993)	0.65 (0.29–2.51)
In total	329	24 (20–29)	67 (47–110)	1826 (473–3799)	0.84 (0.15–4.26)
male	165	25 (20–29)	75 (53–110)	1846 (473–3141)	0.94 (0.30–4.26)
female	164	24 (20–29)	60 (47–110)	1805 (528–3799)	0.64 (0.15–3.24)

Table 2
Concentration of BHT acid in 24-h urine, expressed in µg/L.

Gender	*N*	*N* (%) < LOQ	P.10	P.50	P.90	Max
All	329	7 (2.1%)	0.346	1.06	3.28	18.1
Male	165	3 (1.8%)	0.385	1.22	3.60	18.1
Female	164	4 (2.4%)	0.315	0.945	3.05	16.8

N, number of samples.
LOQ = 0.2 µg/L.
P.10/P.50/P.90, 10th, 50th (median) and 90th percentiles.
Max, highest value observed.

Association of the Saarland. All participants gave written informed consent. Creatinine concentrations were measured using the Jaffe-reaction (Lermen et al., 2019). Sampling took place in the years 2000, 2004, 2008, 2012, 2015 and 2018; since then samples were stored under cryo-conditions (Lermen et al., 2014). The samples were randomized by ESB before shipping to the analytical institute on dry ice. Information on the study population is shown in Table 1.

2.2. Analytical method

The analytical determination of BHT acid in urine samples took place at Currenta's Institute of Biomonitoring and involved enzymatic hydrolysis to liberate the analyte from glucuronide conjugates, followed by online SPE coupled to UHPLC-MS/MS. Calibration was performed using a ^{13}C-labelled internal standard. The limit of quantification (LOQ) thus achieved was 0.2 µg/L, expressed as the urinary concentration of BHT acid. Ubiquitous reagent or instrument blanks were taken into account for determination of this LOQ, which therefore is not a purely instrumental LOQ based, e.g., on a signal-to-noise ratio only (Gries et al., 2020). The key aspects of method development, experimental parameters and validation results have been reported previously in brief (Leng and Gries, 2017) and will be more comprehensively published elsewhere (Gries et al., 2020). Experimental details of the procedure are described in the Supplementary Material (including Figs. S1–S5 and Tables S1–S2).

2.3. Calculations and statistical analysis

Creatinine-normalized metabolite concentrations (c^c_{BHTA} in µg/g creatinine, equation (1)), daily excretion of BHT acid (E_{BHTA} in µg/24 h, equation (2)), and daily excretion corrected for body weight (E^{bw}_{BHTA} in ng/24 h × kg bw, equation (3)) were calculated applying the following formulas:

$$c^c_{BHTA} = \frac{c_{BHTA}}{c_{Crea}} \quad (1)$$

$$E_{BHTA} = c_{BHTA} \times V_{24h} \quad (2)$$

$$E^{bw}_{BHTA} = 1000\frac{ng}{\mu g} \times \frac{c_{BHTA} \times V_{24h}}{m} = 1000\frac{ng}{\mu g} \times \frac{E_{BHTA}}{m} \quad (3)$$

with c_{BHTA} as the urinary concentration of BHT acid (in µg/L), c_{Crea} as the urinary concentration of creatinine (in g/L), V_{24h} as the 24 h urine volume (in L), and m as the body weight of the volunteer (in kg). Urinary concentrations of BHT acid below the LOQ of 0.2 µg/L were reported as LOQ/2 (i.e., 0.1 µg/L) for the purpose of these calculations and all statistics.

All statistical measures were calculated using Microsoft Excel 2010.

2.4. Evaluation of time trends

Temporal trends of urinary BHT acid parameters (c_{BHTA}, c^c_{BHTA}, E_{BHTA}, and E^{bw}_{BHTA}) were evaluated based on the median values of the respective parameters. Trends were tested with two approaches (linear regression analysis, Neumann trend test). Details can be found in the Supplementary Material.

2.5. Daily intake estimation

Daily intakes of BHT (DI_{BHT} in µg/24 h × kg bw) were estimated from the daily excretion corrected for body weight, E^{bw}_{BHTA} (see above), the molar masses of BHT acid and BHT, $M_{BHTA} = 250.3\frac{g}{mol}$ and $M_{BHT} = 220.3\frac{g}{mol}$, and the urinary excretion factor relative to the administered dose, f_{BHTA}, via equation (4):

$$DI_{BHT} = \frac{E^{bw}_{BHTA} \times M_{BHT}}{f_{BHTA} \times M_{BHTA}} \times 0.001\frac{\mu g}{ng} \quad (4)$$

Different values of the excretion factor f_{BHTA} were actually used; details are reported in the Results and discussion section.

3. Results and discussion

3.1. Urinary concentration of BHT acid

BHT acid was quantifiable in the vast majority of the samples, being detected above 0.2 µg/L (LOQ) in 322 of the 329 samples (detection rate: 98%). Table 2 summarizes key statistical figures of the dataset. All in all, male participants were found to exhibit slightly higher (but not significantly) urinary concentrations of BHT acid than female participants.

In general, the urinary concentrations of BHT acid found in the present study are quite similar to previously published data from other, mostly smaller populations (Table 3). In this context we should add that our Institute also performs occupational biomonitoring of BHT acid. The metabolite levels in industrial workers handling BHT do not seem to differ much from values found in the general population, with the notable exception of a few isolated, extreme values (Table 3). These

Table 3
Overview of biomonitoring studies dealing with urinary BHT acid.

Country	Collection year(s)	*N*	Urine type	Median [µg/L] (range)	Reference
Germany	2000–2018	329	24-h	1.06 (< 0.2–18.1; P.90 = 3.28)	This study
Germany	2005[a]	22	Spot[a]	0.912 (< 0.132–12.7)	Göen et al. (2006a)
Germany	2005[a]	16	Spot[a]	0.118 (< 0.044–3.86)	Göen et al. (2006b)
Germany	2015	80	Spot	0.66 (< 0.2–7.55; P.90 = 1.89)	Leng and Gries (2017)
China	2010–2012	53	Spot	0.26 (< 0.1–4.98)	Wang and Kannan (2019)
India	2010–2012	36	Spot	2.24 (< 0.1–24.0)	Wang and Kannan (2019)
Japan	2010–2012	24	Spot	3.86 (< 0.1–24.4)	Wang and Kannan (2019)
Saudi Arabia	2010–2012	9	Spot	0.44 (0.11–5.64)	Wang and Kannan (2019)
United States	2010–2012	23	Spot	1.78 (< 0.1–46.0)	Wang and Kannan (2019)
Germany	2018–2019	622[b]	Spot	1.20 (< 0.2–142; P.90 = 4.93)	Küpper, Gries, Schmidtkunz and Leng, unpublished data (Currenta GmbH & Co. OHG)

[a] T. Göen, personal communication
[b] Routine biomonitoring of workers who are occupationally exposed to BHT (all other studies involve samples from non-specifically exposed populations). Of the 622 samples, 15 were above 18.0 µg/L (5 of them > 50.0 µg/L) and 40 were below 0.2 µg/L.

obviously hint to a substantial workplace exposure for these individual persons.

3.2. Creatinine-adjusted BHT acid concentration

While uncorrected BHT acid concentrations in the ESB samples of our present study were higher in men than in women, the opposite situation was observed when the concentrations were normalized to creatinine: the medians were 1.24 µg/g creatinine overall, 1.15 µg/g creatinine for men, and 1.35 µg/g creatinine for women.

It is well-established that urinary creatinine is considerably higher in adult men than in adult women (Barr et al., 2005), and this is most probably also the reason for our observation regarding creatinine-corrected concentrations of BHT acid. As a matter of fact, the median urinary creatinine levels in our study population were 0.84 g/L overall, and 0.94 g/L or 0.64 g/L for men or women, respectively. Additionally, the medians of the daily creatinine excretion – calculated using a modified equation (2) – depended on the gender of the participants in a quite similar fashion, confirming our hypothesis: 1.38 g/24 h overall, 1.68 g/24 h in men and 1.17 g/24 h in women.

3.3. Daily excretion values of BHT acid

Daily excretion of BHT acid was significantly higher in men than in women: the medians were 2.11 and 1.58 µg/24 h, respectively (overall: 1.76 µg/24 h). Two factors contributed to this result, first the higher urinary concentration of BHT acid in men, and second a somewhat higher 24-h urine volume for men than for women (medians: 1.85 vs. 1.81 L/24 h, 90th percentiles: 2.89 vs. 2.69 L/24 h).

However, significant differences between male and female participants were not detectable any more when daily excretion of BHT acid was normalized to body weight – neither at the median nor at the 10th or the 90th percentiles (Table 4). This seems to indicate an essentially identical magnitude of exposure for both genders in our study population. Appreciable differences were only noticed above the 90th percentile, for example at the 95th percentile. The 95th percentile is obviously beyond a threshold to highly exposed subjects and hence not representative for the great majority of the study population, but these levels may, of course, be very important for the individual persons.

3.4. Comparison of normalization methods for urinary BHT acid

Differences (or the substantial absence thereof) between results for men vs. women, depending on the type of normalization – urinary concentration, creatinine-normalized urinary concentration, daily excretion, and daily excretion normalized to body weight –, are visualized in Fig. 1. A more detailed insight into the result distributions for all parameters is available in the Supplementary Material, Figs. S6–S9 (showing data for all participants/only men/only women).

The effects of different normalization approaches on the interpretation of human biomonitoring data were recently assessed by Lermen et al. (2019), with a case study focused on urinary calcium (Ca^{2+}) levels. Time trends and sex specific differences in urinary Ca^{2+} were found to vary significantly depending on the type of normalization. While our present results for BHT acid are not directly comparable with the situation for Ca^{2+}, they do nevertheless confirm the advice by Lermen et al. (2019) to implement tailored normalization methods when interpreting urinary biomonitoring data. Lermen and coauthors evaluated the trends of urinary creatinine (UC) and total urine volume (UVtot) in ESB samples over time and found a strong dependency of UC on UVtot. The increase in UVtot was linked to a rise in liquid uptake of the participants in the same time period. Thus the decrease in UC is most likely a dilution effect. As our study population (Table 1) is for the most part a subsample of the population investigated by Lermen et al., the similar trends observed in both studies make a lot of sense.

Daily excretion of BHT acid normalized to body weight is, in our opinion, clearly the most meaningful parameter (see also "Daily intakes of BHT" below). Yet we should acknowledge that such data will often not be readily available in biomonitoring studies: it requires collecting 24-h urine which is not always practical, depending on study design and other limitations (Barr et al., 2005). Thus it may be acceptable to use other normalization methodologies as well. This is why we chose to report and discuss a variety of normalized parameters so that comparability with other studies is assured.

3.5. Evolution of BHT acid levels over time

In order to assess potential temporal trends of the exposure level, BHT acid results were further broken down into the years of sample collection, i.e. the years 2000, 2004, 2008, 2012, 2015, and 2018. Regardless of the parameter under scrutiny, whether concentration or daily excretion values, either normalized or not, a clear trend could not be established. At most, fairly weak increases or decreases of median levels were observed between different years, but significant ($p < 0.05$) trends were not detectable by statistical evaluation (see the Supplementary Material, Table S3 for detailed results). An exemplary diagram displaying the variation in time of daily excretion normalized to body weight is shown in Fig. 2.

The fact that temporal trends are weak at most has two major implications. Firstly, we may assume that the exposure of our study population has remained largely unchanged since at least the year 2000. This is not too surprising, as BHT is a substance that has been in widespread use for decades. While BHT has faced some criticism due to its toxicological profile, evidence for highly critical properties like endocrine disruption or carcinogenicity is still rather limited at present (EFSA, 2012). Hence BHT uses have not been restricted or voluntarily been reduced in recent years, which is consistent with our findings of a constant exposure level. Nonetheless we should note that BHT is currently (status "ongoing" as of February 22, 2019 according to ECHA, 2019) being evaluated in the European Union under the Community rolling action plan (CoRAP), so that more thorough insights into the human toxicity of BHT might be gained in the years to come (ANSES, 2016). The second implication of the absence of an unambiguous time trend for BHT acid excretion is that we may reasonably draw on the full data set covering all sampling years for further investigations into the extent of exposure to BHT (see the following subsection "Daily intakes of BHT"), instead of focusing only on the most recent specimens.

3.6. Daily intakes of BHT

3.6.1. Scenarios

Estimating intake levels of BHT was one of the main goals of our human biomonitoring study. The excretion values, E^{bw}_{BHTA}, reported above (see also Table 4) can be taken as the basis for such an estimation: the 10th percentile would represent a low level of exposure, the median an average level, the 90th or the 95th percentile a boundary to high exposure, and the maximum value an example of extremely high exposure. With our total sample number of 329, the 90th percentile is without any doubt a more robust estimate than the 95th percentile; nonetheless we chose to report the 95th percentile as well for better comparability with previous studies. Daily intakes may in principle be

Table 4
Daily excretion of BHT acid (normalized to body weight), expressed in ng/24 h × kg bw.

Sex	P.10	P.50	P.90	P.95	Max
All	10.4	26.8	81.9	118	740
Male	10.7	25.2	84.2	118	365
Female	9.28	27.0	80.7	132	740

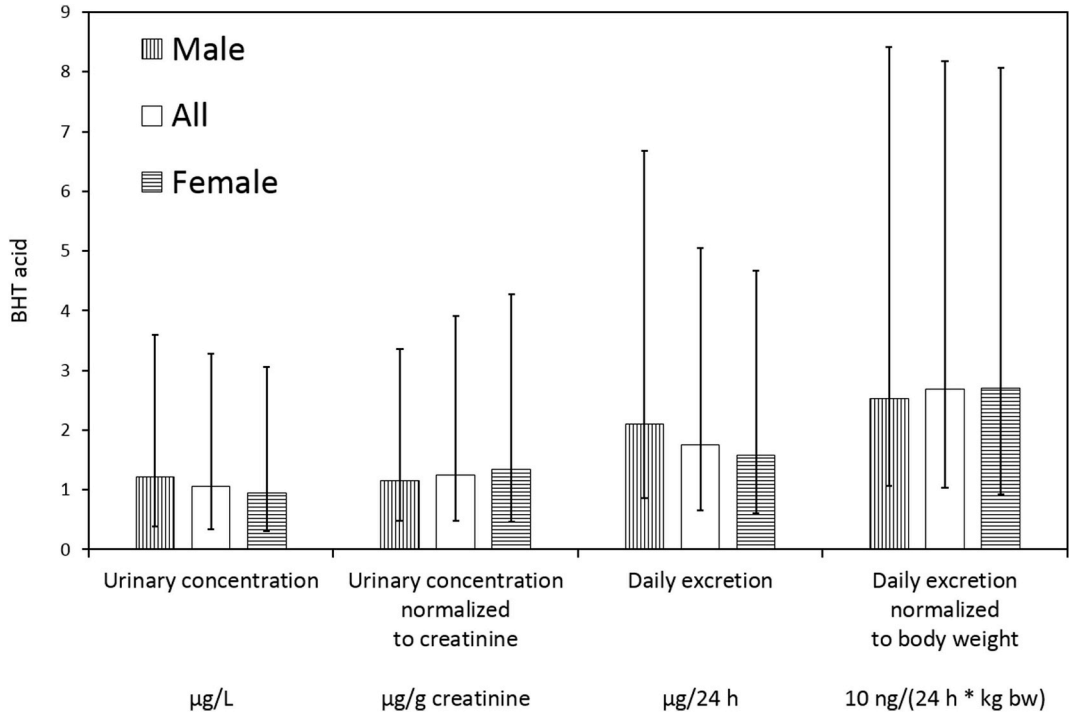

Fig. 1. Different parameters for BHT acid. Columns represent the medians, while error bars show the range between the 10th and the 90th percentiles. Note the different units (for the y axis) corresponding to each parameter – in particular, note that the daily excretion normalized to body weight is expressed in 10 ng/24 h × kg bw so as to permit graphical representation in one common diagram along with the other parameters.

calculated using equation (4) with our values of E^{bw}_{BHTA}, but the urinary excretion factor f_{BHTA} constitutes a major element of uncertainty in this case. In fact, as mentioned in the Introduction, urinary excretion factors reported in the literature for BHT acid cover quite a vast range (Daniel et al., 1968; Verhagen et al., 1989; Wiebe et al., 1978). Additionally, the kinetics of BHT metabolism are yet incompletely understood, especially considering that some evidence exists for a delayed excretion (several days) of a significant fraction of BHT intakes (Daniel et al., 1967). On the other hand, for BHT acid (including conjugates) as a particular metabolite, it has been reported that the majority is excreted via urine on the first day after ingestion of an oral BHT dose, while only a very small amount was found on the second day, and none on later

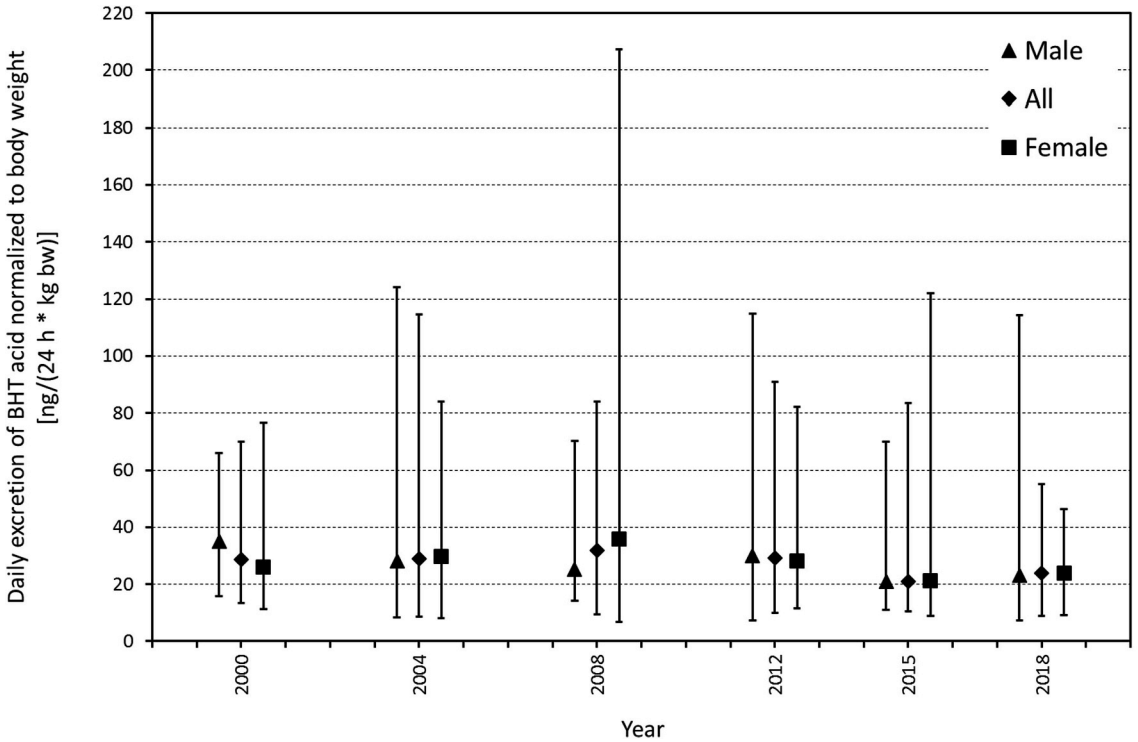

Fig. 2. Daily excretion of BHT acid normalized to body weight for the years 2000, 2004, 2008, 2012, 2015, and 2018. Markers depict medians, and error bars show the 10th and 90th percentiles.

days (2.6% ± 2.2% on day 1, 0.0% ± 0.1% on day 2; Verhagen et al., 1989). To account for the high uncertainty concerning the urinary excretion factor, we have calculated BHT daily intakes for a variety of scenarios, with f_{BHTA} between 0.1% and 5.5%.

3.6.2. BHT intake estimates based on human biomonitoring

The data are presented in Table 5. Depending on which excretion factor we choose, the estimates for a low level of BHT daily intake vary between roughly 0.2 and 9 μg/24 h × kg bw. For the median level, they range from approximately 0.4 to 24 μg/24 h × kg bw. The 95th percentile as a measure for high exposure is between 2 and 100 μg/24 h × kg bw, while the highest value would be between 12 and 650 μg/24 h × kg bw. Obviously these estimates suffer from considerable uncertainty but given the current lack of in-depth quantitative understanding of BHT metabolism in man, more precise assessments do not seem realistic at present.

In spite of these limitations it seems sensible to compare our daily intake results with similar findings reported very recently by Wang and Kannan (2019) in a study which we have already cited above in our discussion of urinary BHT acid concentrations. Indeed the methodologies for intake calculations are essentially identical between their study and ours, aside from the facts that the sample preparation and analytical measurements were carried out according to different protocols, that Wang and Kannan only had spot urine at their disposal, and that the investigated populations were not comparable in terms of age ranges and gender ratios. Table 5 includes the figures reported by Wang and Kannan for a comparison with our results. Notwithstanding all uncertainties associated with the derivation of the daily intakes, we might conclude that exposure to BHT varies between different regions of the world but perhaps not to a very large extent. As a matter of fact, the various intake estimates do not span more than about one order of magnitude (within a given scenario for the urinary excretion factor f_{BHTA}). This is true for the medians, but also for the maximum values.

3.6.3. Biomonitoring, a validation tool for BHT intake estimates based on food surveys

We may, furthermore, compare our intake estimates with published estimates based on food consumption statistics and reported use levels of BHT in various food types. Taking into account exposure from BHT as a food additive only, it was estimated that daily BHT intakes for adults in the European Union are in the range of 0.01–0.03 mg/kg bw (i.e. 10–30 μg/kg bw) on average and 0.03–0.17 mg/kg bw (i.e. 30–170 μg/kg bw) at the 95th percentile, thus remaining below the ADI or the provisional RfD (EFSA, 2012). If migration of BHT from food contact materials is additionally considered, this might account for an increase of the daily intake by 0.05 mg/kg bw (i.e. 50 μg/kg bw; EFSA, 2012). Our human biomonitoring-based data broadly fit in this picture if worst-case urinary excretion factors are used (f_{BHTA} = 0.1%–0.3%), but otherwise our estimates are rather on the low side. Yet this might not be implausible, since EFSA describe their estimates as conservative, based on worst-case scenarios. It must be noted at any rate that published assessments of dietary intake for various populations throughout the world show tremendous variability (see Nieva-Echevarría et al., 2015, for a review), ranging from less than 1 μg/kg bw per day to several hundred μg/kg bw per day.

For the sake of completeness we shall mention that still another situation arises when occupational rather than dietary exposure is considered: Unlike the general population, workers handling BHT in industry are expected to be mainly exposed via inhalation of the substance. Occupational exposure limits are enforced in various countries. For instance Germany applies a MAK (Maximum Workplace Concentration) value of 10 mg/m³ for the inhalable fraction, which would correspond to an intake of 1400 μg/kg bw per day (assuming 70 kg bw, an air volume of 10 m³ inhaled during an 8 h working shift, and 100% absorption of BHT; DFG, 2012). In practice, significantly lower exposure levels (< 2.5 mg/m³) have been viable for BHT production facilities as early as 1985 (OECD, 2002).

To summarize, comparison of different studies dealing with BHT intake estimates based on dietary surveys and BHT levels in food is

Table 5
Biomonitoring-based estimates for BHT daily intake levels.

Country (Reference)	Exposure level	f_{BHTA}				
		0.1%	0.3%	2.6%	3%	5.5%
		DI_{BHT} $\frac{\mu g}{24\,h \times kg\ bw}$				
Germany (This study)	**P.10 (low exposure)**	9.15	3.05	0.352	0.305	0.166
	P.50 (average exposure)	23.6	7.87	0.908	0.787	0.429
	P.90 (high exposure)	72.0	24.0	2.77	2.40	1.31
	P.95 (high exposure)	104	34.8	4.01	3.48	1.90
	Max (very high exposure)	652	217	25.1	21.7	11.8
China (Wang and Kannan, 2019)	**P.50 (average exposure)**	NA	2.07	NA	0.21	NA
	Max (very high exposure)	NA	40.3	NA	4.03	NA
India (Wang and Kannan, 2019)	**P.50 (average exposure)**	NA	18.1	NA	1.81	NA
	Max (very high exposure)	NA	194	NA	19.4	NA
Japan (Wang and Kannan, 2019)	**P.50 (average exposure)**	NA	31.3	NA	3.12	NA
	Max (very high exposure)	NA	198	NA	19.8	NA
Saudi Arabia (Wang and Kannan, 2019)	**P.50 (average exposure)**	NA	3.58	NA	0.36	NA
	Max (very high exposure)	NA	45.7	NA	4.57	NA
United States (Wang and Kannan, 2019)	**P.50 (average exposure)**	NA	14.4	NA	1.44	NA
	Max (very high exposure)	NA	372	NA	37.2	NA

DI_{BHT}, estimated daily intake of BHT, normalized to body weight. This study: based on actual 24-h urine volume and actual body weight of the participants. Wang and Kannan (2019): estimates for adult participants only; based on spot urine, with average values for daily urine volume (1.7 L/d) and body weight (70 kg).
Explanation of urinary excretion factors cited from literature.
0.1% – Urine collected on day 2 after BHT ingestion (0.5 mg/kg bw), mean + 1 standard deviation of 7 male volunteers (the mean itself was reported as 0.0%; Verhagen et al., 1989).
0.3% – Pooled urine collected on days 1–3 after BHT ingestion (200 mg total dose), 1 volunteer (Wiebe et al., 1978).
2.6% – Urine collected on day 1 after BHT ingestion (0.5 mg/kg bw), mean of 7 male volunteers (Verhagen et al., 1989).
3% – Urine collected on day 1 (16 h or 24 h) after BHT ingestion (0.5 mg/kg bw), estimated mean of 2 male volunteers (Daniel et al., 1968).
5.5% – Urine collected after BHT ingestion (0.5 mg/kg bw), maximum value of 7 male volunteers (Verhagen et al., 1989).

often very difficult, since many factors influence the outcome. These factors include the selection of relevant food categories, the determination of BHT levels in food (maximum permitted levels, data from industry, or actual analytical measurements), consumer brand loyalty, or the fate of BHT during food processing such as heating (Nieva-Echevarría et al., 2015). Human biomonitoring as performed in our present study can, in principle, contribute to a much clearer and more refined picture of actual exposure to BHT, without risking either to overlook certain exposure routes, or to overestimate exposure due to dependence on worst-case data. We can describe in a pretty detailed way the level at which our study population has been excreting BHT acid (Table 4; Supplementary Material, Fig. S9) but unfortunately translating these comprehensive results into actual BHT intake estimates is hampered by the lack of reliable, quantitative metabolism data. Therefore, a human kinetic study will be performed soon which might help to enhance the confidence of our intake estimates and to narrow the range of possible values (Table 5). If possible, future studies should also focus on other metabolites besides BHT acid (see Introduction). Indeed, the conclusion which Wiebe and coauthors drew more than 40 years ago probably still holds true to the present day: "Undoubtedly, the metabolism and excretion of BHT in man has not been adequately resolved" (Wiebe et al., 1978).

3.6.4. Toxicological relevance of BHT exposure: a provisional assessment

We may already assume, at least with an acceptable degree of certainty, that the majority of our study population was not exposed to BHT above the EFSA's ADI (0.25 mg/kg bw = 250 µg/kg bw) since our worst-case 95th percentile was at approximately 100 µg/kg bw (Table 5). In some extreme cases the ADI might, however, have been reached or exceeded in the population under investigation. Assuming an excretion factor f_{BHTA} of 0.1% (worst-case, Verhagen et al., 1989), a total of four persons or 1.2% of our study population would most probably have exceeded the ADI, with intake figures of 652, 322, 269, and 256 µg/24 h × kg bw respectively. The single highest value would amount to a daily intake of 2.6 × ADI. Two more persons (0.6%) would be within 70% of the ADI (intake values: 201 and 176 µg/24 h × kg bw), indicating a high but probably not yet critical level of exposure. With a slightly higher f_{BHTA} of 0.3% (Wiebe et al., 1978), only one person (0.3% of the study population) would be within 70% of the ADI and none above the ADI. Nevertheless 70% of ADI for one single substance might be considered as critical if co-exposures to chemicals with similar toxicity are taken into account. In all other scenarios (Table 5) the calculated BHT intake level would reach at most one tenth of the ADI, even for the most highly exposed individuals.

4. Conclusions

We have reported a comprehensive evaluation of BHT levels in a non-occupationally exposed population, based on human biomonitoring of 329 urine samples from the German ESB. Our results demonstrate that human biomonitoring is well-suited for assessing the exposure to the antioxidant BHT in investigations involving samples from the general population. With currently available knowledge about human metabolism of BHT our data lead us to assume that, for the majority of the ESB sample, exposure did not exceed the ADI derived by EFSA (2012), maybe with the exception of a few highly exposed individuals. However, quantitative information about BHT metabolism is, alas, very limited to the present date, and therefore our hypothesis would require verification as soon as more meaningful metabolism studies become available.

Declaration of competing interest

Lanxess Deutschland GmbH, a producer of BHT, held 40% of the Currenta GmbH & Co. OHG shares until April 2020 and is an important customer of the Institute of Biomonitoring in the field of occupational medicine. However, regarding the work reported in the present paper, Lanxess was never involved in any aspect of the study, including study design, the collection, analysis, and interpretation of data, the writing of the report, and the decision to submit the paper for publication. Currenta's Institute of Biomonitoring is accredited to DIN EN ISO/IEC 17025 and is thus subject to the requirements for impartiality defined in this standard and to internal and external audits. Risks to impartiality are identified, documented and regularly reviewed, and appropriate measures to mitigate these risks are implemented in our Institute.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2020.113541.

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Comparing the German enabling environment for nationwide Water Safety Plan implementation with international experiences: Are we still thinking big or already scaling up?

Dennis Schmiege[a,b,*], Mariele Evers[a], Verena Zügner[c], Bettina Rickert[c]

[a] Department of Geography, University of Bonn, Meckenheimer Allee 166, 53115, Bonn, Germany
[b] Center for Development Research, University of Bonn, Genscherallee 3, 53113, Bonn, Germany
[c] German Environment Agency (UBA), Schichauweg 58, 12307, Berlin, Germany

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ABSTRACT

Ensuring safe drinking-water is the target of the Water Safety Plan (WSP) approach, which has been successfully applied to a large number of water supply systems around the world. Effective country-wide scaling up of WSP implementation requires an enabling environment at the policy level.

By utilizing a multi-step mixed methods approach, this study summarizes international experience with WSP implementation and scaling-up efforts following the 8 steps of the WSP road map published by WHO and IWA for an enabling environment, shows what steps Germany has in place, and compares this with published international experience to inspire further policy action.

Contrasting the international experience to the German situation revealed several overlaps but also profound differences, which, in turn, offer opportunities for mutual learning. Most experience in Germany and internationally is documented for the earlier steps of the WSP road map. Information particularly on developing a national strategy, securing financial instruments, activities to support continual implementation of WSPs and on review of the overall WSP experiences and sharing lessons learned appears to be scarce, while the importance of training, collaboration and alliances, and the value of a regulatory push are often stressed. In Germany, stakeholder engagement, guidance documents and workshop materials have been of vital importance. Information that could particularly inform further action in Germany mostly relate to considering a national WSP strategy, and how to shape an approach for external quality assurance of WSPs.

1. Introduction

The Water Safety Plan (WSP) approach aims at ensuring safe drinking-water through a thorough system assessment, management and communication measures, as well as monitoring (WHO, 2017). Widespread WSP implementation supports achieving the Sustainable Development Goal (SDG) target 6.1 of providing safe drinking-water for all (UN, 2015). The WSP approach has been successfully applied to a variety of water supply systems globally (WHO and IWA, 2017). This has led to a number of benefits including improved system management, increased awareness among staff, increased knowledge sharing, communication and collaboration, as well as improved water quality (Gunnarsdottir et al., 2012a; Tsitsifli and Tsoukalas, 2019; WHO and IWA, 2017).

Effective scaling up of WSP implementation from demonstration projects to country-wide application needs to be supported by an enabling environment at the policy level. Elements of this include formal rules, which enable and support stakeholder participation, policies, regulations, but also guidelines, tools and resources (Baum and Bartram, 2018).

In 2010, the World Health Organization (WHO) and the International Water Association (IWA) published the WSP road map titled “Think big, Start Small, Scale up. A Road map to Support Country-Level Implementation of Water Safety Plans”. This document supports country-level implementation of WSP and provides information on steps towards introducing, testing, evaluating and then scaling up the approach of governments and regulatory entities that envisage revising or developing new drinking-water quality policies and regulations (WHO and IWA, 2010). The eight road map steps are shown in Fig. 1. These steps do not have to be followed in this specific order and may

* Corresponding author. Department of Geography, University of Bonn, Meckenheimer Allee 166, 53115, Bonn, Germany.
E-mail addresses: d.schmiege@uni-bonn.de (D. Schmiege), mariele.evers@uni-bonn.de (M. Evers), verena.zuegner@uba.de (V. Zügner), bettina.rickert@uba.de (B. Rickert).

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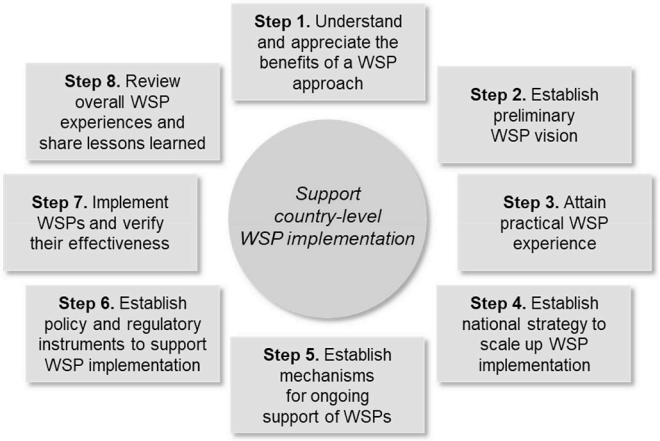

Fig. 1. Eight road map steps to support country-level implementation of Water Safety Plans (WHO and IWA, 2010).

have differing significance in different national settings.

At the European level, the revision of the European Union (EU) Drinking Water Directive (DWD) – the basis for German drinking-water legislation as an EU member state – was proposed by the European Commission in February 2018 (EC, 2018) and political agreement on the revision of the DWD was published in February 2020 (EC, 2020). It includes requirements for a risk-based approach to providing drinking water, including risk assessment and risk management (RA/RM) of catchment areas for the abstraction points of drinking-water, for RA/RM for each water supply system and for RA for the domestic distribution systems. National activities to support this new approach are therefore likely to become necessary for Member States in the near future once the revised DWD enters into force.

This study documents the elements of the WSP road map for which information has been published internationally. This also covers benefits, challenges and good practices that have been emerging from the implementation of these RA/RM approaches globally. It furthermore documents the elements of the WSP road map which are already in place in Germany in order to provide an enabling environment. To inform further implementation and scaling up of WSP in water supplies in Germany as well as in other countries wishing to take respective action, it compares the German experience to the results of the international literature reviewed.

2. Methods

Identifying and understanding the experiences of stakeholders in the German drinking-water sector required a multi-step approach, beginning with a systematic literature review for the German drinking-water sector, followed by qualitative semi-structured expert interviews and a quantitative online survey among water suppliers. Furthermore, national activities with involvement of the Ministry of Health (MoH – in German: BMG) and the German Environment Agency (UBA) for creating an enabling environment for WSP implementation were also summarized for the analysis (see below and Fig. 3).

An additional literature review was conducted to analyse international experiences with the steps of the WSP road map

UBA also hosts a WHO collaborating center (WHO CC) for research on drinking-water hygiene, which has been involved in a number of WSP activities and publications of the WHO, thus contributing to gaining experience with the approach at the national and the international level. Therefore, in addition to the scientific peer-reviewed publications, relevant WHO workshop reports and studies with involvement of UBA's WHO CC were analysed.

2.1. Systematic literature review of experiences with WSPs in the German drinking-water sector

The systematic literature review addressing the question "What experiences do stakeholders in the German drinking-water sector have with WSPs?" covered three areas: advantages, good practices, and obstacles and challenges.

Six databases, i.e. Web of Knowledge, sciencedirect, PubMed, Scopus, Google Scholar and the Environmental Discovery System of UBA, were systematically searched in June and July 2017. English and German search terms used in different combinations were: water safety plan, WSP, *Trinkwassersicherheitskonzept*, water-supply, water-supplier, drinking-water supply, risk, safety, Germany, *Deutschland*, and health. In addition, a hand-search of several journals well-known within the German drinking-water sector was conducted, i.e. German Technical and Scientific Association for Gas and Water (DVGW*) energie | wasserpraxis (EWP), gwf Wasser Abwasser, Hydrologie und Wasserbewirtschaftung, and EUWID Wasser und Abwasser*.

The inclusion criteria encompassed all types of publications dealing with Germany in German or English language published after 2000, explicitly including so-called "grey" literature because not all experiences in the German drinking-water sector are published in peer-reviewed journals. This approach was chosen to minimize the influence of publication bias. A quality appraisal through peer review was not relevant because the main outcome of interest was subjective experience reports. For the final analysis, this systematic review relied on a qualitative narrative synthesis.

2.2. Qualitative semi-structured expert interviews with stakeholders in the German drinking-water sector

Twelve qualitative semi-structured interviews with key stakeholders in the German drinking-water sector were conducted between July and August 2017 to check if experience matched the findings of the systematic literature review but also to gain a deeper insight into experience with WSPs. Twelve open-ended questions were created based on the findings from the systematic literature review and pre-tested, covering the following aspects: advantages of WSP implementation, workload for WSP implementation, challenges, experiences with hazard analysis and risk assessment, transparent communication, data availability and areas of limited responsibility as particular problems, potential inclusion of concern assessment as part of risk assessment following the risk governance framework described by the International Risk Governance Center (IRGC 2017), future legal anchoring, role of local health agencies, useful tools, helpful aspects for future implementation of WSP in Germany, as well as wishes and suggestions for the ministries and their associated authorities.

Expert interviews were conducted with small-scale (2) and large-scale water suppliers (5), local health agencies (2), and experts from other associations or institutions (3), covering five of the 16 German Federal states. One person per institution was interviewed. The interviews were audio-recorded and transcribed using the smooth verbatim transcript model (Mayring, 2014). For analysing the interviews, Mayring's qualitative content analysis was applied which aims at creating rule-based and inter-subjectively understandable categories as basis of a summary (Mayring, 1994). Nevertheless, the purposive sampling procedure and the qualitative content analysis could have introduced potential bias. To address this, the qualitative content analysis was tested for construct validity, stability and reproducibility (Mayring, 2014), all yielding satisfactory results.

2.3. Online survey with water-suppliers in the German drinking-water sector

At the time of the legislative change at the EU level in February 2018, the UBA initialized an online survey addressing water suppliers with experiences in risk management. The main questions covered

aspects around the year of implementation, details of the approach used, advantages and challenges, as well as further remarks. Participation in the survey was on a voluntary basis and completely anonymous and no individual questionnaires were distributed. Water suppliers could complete the questionnaire online on the UBA webpage. It cannot be ruled out that the group of respondents of the quantitative survey partly overlaps with the group of respondents from the qualitative key stakeholder interviews, which could lead to an over-representation of certain answers. The information about the survey was disseminated through the networks of the large German water associations. 26 survey questionnaires of which 24 were from large-scale water suppliers were submitted, 20 of which could be analysed (19 big, one small supply). The overrepresentation of large-scale water suppliers partly limits the validity of findings to this group of supplies.

2.4. Literature review of international experience with WSP road map steps

Documents were searched in three different databases, i.e. Google Scholar, *Scopus* and sciencedirect between July and September 2019. The following search terms were used: "water safety plan" AND one of the following terms: national experience, policy, legislation, national implementation process, enabling environment, scaling up, or national level. The search was limited to peer-reviewed studies published after the year of publication of the WSP road map (2010) in English and German language. In order to identify further publications, the reference lists of those studies were hand-searched yielding additional records, some of them published before 2010.

3. Results

3.1. Study selection

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement was applied to both literature reviews (Moher et al., 2009). For the systematic literature review on experiences with RA/RM systems in the German drinking-water sector, initially, 1193 records were identified through database searching accompanied by 68 records from the hand-search. After removal of duplications, 1037 studies remained. After title- and abstract-screening, 978 studies were excluded based on the inclusion criteria, and 59 studies were assessed for eligibility. Eventually, 40 studies were included in the qualitative synthesis (references of all included studies can be accessed in supplementary material A).

For the literature review on international experiences regarding the WSP road map steps, after removing duplicates, 91 publications were identified. This number was reduced to 44 after title- and abstract-screening. After an evaluation of introductions and conclusions, it was further reduced to 36 studies used for the analysis of the WSP road map (an overview table and references of all included studies as well as the WHO workshop report and publications can be accessed in supplementary material B). Two studies dealt with experiences in the German sector and were excluded from the analysis of the international experience with the WSP road map and added to the German experience.

Out of 34 peer-reviewed studies included in the final synthesis for the international experience regarding the WSP road map steps, the majority touched upon step 1 (22) and step 3 (22) whereas step 7 (8) was covered the least. The distribution of studies for all WSP road map steps is shown in Fig. 2.

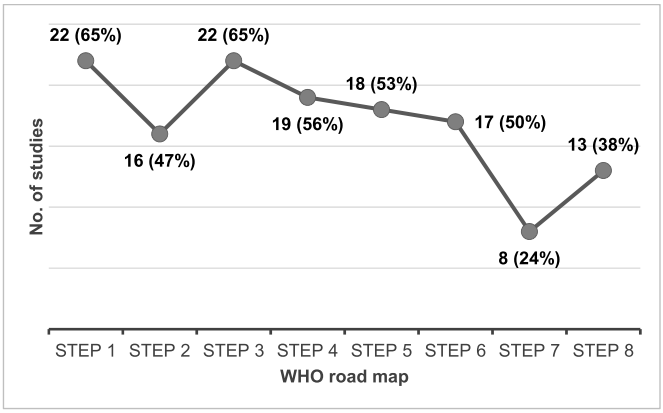

Fig. 2. Number of peer-reviewed studies from the literature review covering each WSP road map step (Note: one study can cover several WSP road map steps).

3.2. International experience with creating an enabling environment documented in the literature

Step 1: Understand and appreciate the benefits of a WSP approach

*Learning about WSPs, creating informal alliances at country level, and agreeing to test and explore the WSP approach are elements of this step (*WHO and IWA, 2010*).*

The interest in WSP can emerge for manifold reasons, including health problems from water-borne diseases (Ezenwaji and Phil-Eze, 2014) or the recognition of issues and limitations of current reactive sampling and analysis regimes (Reid et al., 2014) and that the WSP can help to overcome these challenges. Via different mechanisms, such as awareness raising through partnerships or national conferences (Hubbard et al., 2013; Mahmud et al., 2007), representatives from key stakeholder groups can get familiar with the procedure and benefits of the WSP approach.

WSP entail a number of regulatory, institutional, investment, water quality and operational benefits (WHO EURO, 2014a). For instance, while requiring relatively low development costs (Chang et al., 2013), WSPs help guiding capital improvement planning and investments (Rinehold et al., 2011). In addition, they build upon supply specificity as a key component (Lane et al., 2018). WSPs can also be used as an independent tool to verify existing quality management standards (Viljoen, 2010) and to institutionalize good practices in operation and maintenance of a water supply system (Sutherland and Payden, 2017). Above all, they create and foster a proactive and sound risk governance framework around drinking-water supply systems that helps avoiding complacency (Summerill et al., 2010), with the ultimate goal of more effectively protecting public health. As the main benefits, countries that already have experience with WSP implementation reported improved system management of water supplies; cost savings due to operational efficiencies gained; increased awareness, knowledge and understanding among staff of water supplies; increased promotion and knowledge-sharing of WSP; improved communication and collaboration with other stakeholders; and improved water quality and improved monitoring (WHO and IWA, 2018; WHO and IWA, 2017).

Building upon the benefits, a loose alliance of relevant national stakeholders from key institutions should be formed, which, ideally, evolves into a national steering committee (WHO and IWA, 2010). At an early stage, this loose alliance can also be supported by other partners in terms of advocacy, capacity-building, technical assistance and guidance (Sutherland and Payden, 2017; Rinehold et al., 2011).

Step 2: Establish preliminary WSP vision

*In order to establish a preliminary WSP vision, a national steering committee should be established, and a WSP vision initiated (*WHO and IWA, 2010*).*

The main role of the national steering committee is to establish the WSP vision for the country, as well as to advocate and provide guidance for its implementation. In order to develop the WSP vision, the first step is usually a review of existing standards, regulations, policies, and practices to identify differences between the WSP approach and current national or regional approaches (Baum et al., 2015; Bereskie et al.,

2018; Hubbard et al., 2013; Lane et al., 2018; Ncube and Pawandiwa, 2013). Such an analysis reveals gaps and shows the added value of the WSP approach as a whole or of particular WSP steps (Hubbard et al., 2013). Subsequently, various goals around public health, water supply targets, or operational procedures can be set (Rinehold et al., 2011). Such goals often lead to the implementation of locally appropriate solutions, for example shown by the development of a template with predefined generic risks for a specific region in Canada (Reid et al., 2014). The Tajik Ministry of Health and Social Protection and the State Sanitary Epidemiological Service support WSP uptake to strengthen public health (WHO EURO, 2014). In the Republic of Moldova, a task force to develop and implement WSPs was to be established based on an ordinance (WHO EURO, 2014). In 2016, during a national workshop on small systems, Albania decided to recommend the introduction of WSP requirements, which led to the establishment of a national working group that drafted WSP guidelines and a roadmap for their implementation (WHO EURO, 2017a).

Step 3: Attain practical WSP experience

*Through the implementation and evaluation of WSP demonstration projects in a country, the approach can be tested for nationally prevailing contexts under available resources and it can be evaluated for its longer-term added value and challenges (*WHO and IWA, 2010*).*

WSP demonstration projects have been implemented around the world, including, but not limited to, countries in the Asia-Pacific region (Kumpel et al., 2018; Mahmud et al., 2007; Sutherland and Payden, 2017), Africa (MoWIE, 2015a), Latin America and the Caribbean (Rinehold et al., 2011), South America (Seghezzo et al., 2013), and Europe (Gunnarsdottir et al., 2012b; Roeger and Tavares, 2018; Setty et al., 2017; Six et al., 2015; van den Berg et al., 2019; WHO EURO, 2014a). In a global survey, 30% of the 76 responding countries were at the pilot stage of urban and/or rural WSP implementation (WHO and IWA, 2017). Tajikistan, Kyrgyzstan, the Republic of Moldova, and more than a third of European countries have experience with WSPs at the level of pilot projects, scale-up strategies up to enforceable regulations (WHO EURO, 2014a). Gaining experiences in rural areas through NGOs was complemented by WSP implementation in urban supplies supported by the Kyrgyz public authorities (WHO EURO, 2014a). Common observations among the added values identified were improvement of operational procedures and management as well as improved water quality and thereby reduced disease incidences (Gunnarsdottir et al., 2015; Howard et al., 2005; Mahmud et al., 2007; Ye et al., 2015). Challenges regarding the implementation of WSP demonstration projects were often reported for the catchment area, with key issues being different jurisdictions and a lack of boundary alignment (Keirle and Hayes, 2007).

Step 4: Establish national strategy to scale up WSP implementation

*The development of the national strategy should be supported through promoting multi-stakeholder buy-in (*WHO and IWA, 2010*).*

Good practice in establishing a national strategy is to use international standards as stepping stones (Baum and Bartram, 2018) and to build upon already existing regulatory frameworks as happened, for example, in Iceland (Gunnarsdottir et al., 2015) and Canada (Reid et al., 2014). In Ethiopia, for instance, the Ministry of Water, Irrigation and Energy (MoWIE) expects to utilize existing Water, Sanitation and Hygiene (WASH) mechanisms (MoWIE, 2015a). The implementation of those good practices can be facilitated by the development of a strategic framework for the national WSP implementation and scale-up. This was shown by a study including several demonstration projects in Portugal (Vieira, 2011), and in Ethiopia with its strategic framework on how to ensure climate resilient water safety (CR-WSP) (MoWIE, 2015a). Tackling the challenge of providing safe drinking water to a growing population under climate change conditions, MoWIE published the strategic framework to help raise awareness and understanding of RA/RM approaches with regard to drinking-water (MoWIE, 2015a). Building on existing structures at different levels, the CR-WSP framework represents a good practice for detailing WSP Road Map elements by outlining, *inter alia*.

- the key enabling environment and the use of the existing institutional arrangements within the context of WASH programs,
- the institutional arrangement for WSP,
- timelines,
- roles and responsibilities of institutions at national, regional, local and community levels, capacity building needs at different levels,
- financing of the strategic framework, and
- monitoring and evaluation of the framework (MoWIE, 2015a).

Although not necessarily a formal national WSP strategy, several countries have set related targets under the Protocol on Water and Health of the WHO European Region on scaling up, including Armenia, Hungary, Kyrgyzstan, the Republic of Moldova, Tajikistan (WHO EURO, 2014a), Belgium, Serbia, and Luxembourg (UNECE and WHO EURO, 2019). In Italy, a national WSP roadmap is under development, including the strengthening of legislation on WSP implementation and approval and training programs (WHO EURO, 2018).

Scaling up WSP implementation nationally requires large-scale engagements with water utility operators and other stakeholders. Proactive engagement can lead to acceptance among different stakeholders, which, in turn, often translated into participation that was seen as a first crucial step (Mahmud et al., 2007; Ncube and Pawandiwa, 2013; Rinehold et al., 2011). In Arctic communities, community engagement was key to understanding the perception of the people involved, which helped to identify local needs (Lane et al., 2018). In addition, the provision of training and (technical) support also plays a pivotal role for scaling-up WSP implementation (Hubbard et al., 2013; Perrier et al., 2014; Reid et al., 2014; Sutherland and Payden, 2017).

Step 5: Establish mechanisms for ongoing support of WSPs

*This step includes continuing to advocate for WSPs, scaling up WSP training, establishing utility-utility partnerships, and developing and disseminating tools and resources (*WHO and IWA, 2010*).*

Several countries have already moved beyond the implementation of WSPs demonstration projects and continued advocating for WSPs through multinational collaborations. In Central and South America, for example, the “Latin America and the Caribbean (LAC) WSP network” was created in 2008 facilitating the process of advocacy and resource dissemination (Hubbard et al., 2013) albeit their webpage seems to be inactive nowadays. Another well-known platform for exchanging materials and experiences on the international level is the “Water Safety Portal” by WHO and IWA (accessible at https://wsportal.org/). Moreover, IWA together with WHO regularly organises international conferences on water safety. In addition, utility-utility partnerships have proven successful in Bangladesh, where a forum for experience and information sharing was established (Mahmud et al., 2007).

Building a national knowledge base for ongoing support requires trained staff for both water utilities and regulators, including health authorities, regular trainings and workshops. Nationwide implementation of such training programs at the local level for experts and new staff members was reported, *inter alia*, in Iceland (Gunnarsdottir et al., 2015), South Africa (Viljoen, 2010) and several countries in South-East Asia (Sutherland and Payden, 2017). WSP facilitators were trained in Tajikistan, and a field guide for implementation published in Tajik and English, together with an awareness raising brochure outlining the in-country experience in pilot projects, including benefits (WHO EURO, 2013; WHO EURO, 2014a). Based on this, further scaling up trainings and WSPs were implemented (WHO EURO, 2014a). In addition, at a workshop for small-scale systems in the pan-European region in Dessau (Germany) in 2018, representatives from Ireland and Italy also informed about WSP trainings organized in their countries (WHO EURO, 2018). In order to support scaling up of training activities internationally, WHO conducted a Training of Trainers (ToT) event in Thika,

Kenia, in 2017, and published global (WHO, 2012) and regional (WHO SEARO, 2015) training materials.

Besides training programs for staff in the water and health sector, developing locally appropriate tools and disseminating them to facilitate widespread WSP implementation is vital. Examples can be found around the world. In Uganda, a regionally applicable "Model WSP" can be used as a starting point for individual WSPs, which saves time and cost (Howard et al., 2005). In Canada, regulators developed an Excel-based workbook containing around 200 pre-described common risks for this area for all steps of water supply – source, treatment, network, and consumer (Perrier et al., 2014). A similar approach was used in the Republic of Korea (Baum and Bartram, 2018), guidelines for implementation in urban and rural systems were published in Ethiopia (MoWIE, 2015b; MoWIE, 2015c), and guidance documents translated into Georgian for national implementation (WHO EURO, 2014a). In the Netherlands, a risk assessment software was developed using a multi-stakeholder approach, which makes audits and validation easier and more comparable (van den Berg et al., 2019). Finland, Ireland, and Luxembourg also highlighted the use of web-based tools for small scale systems (WHO EURO, 2018). In the same line, the UK Drinking Water Inspectorate developed a WSP-monitoring tool for local health authorities for monitoring small private water supplies that are required to implement a risk-assessment approach (WHO EURO, 2017b).

Step 6: Establish policy and regulatory instruments to support WSP implementation

*For implementation of this step, institutions should be empowered, legislative and regulatory instruments issued, and financial instruments secured (*WHO and IWA, 2010*).*

Apart from training programs and tools, a key outcome of discussions at a European workshop concluded that "the most effective way to ensure broad implementation of WSP-type approaches is certainly a *regulatory push*" (WHO, UBA and IWA 2014, p. 6; own emphasis). According to a global survey, at the time policy or regulatory instruments that require or promote WSPs were in place in 46 countries and under development in another 23 countries (WHO and IWA, 2017). In a reporting exercise of the WHO European Region, 18 of 28 countries stipulated having a national policy or regulation requiring WSPs or a similar approach in place (UNECE and WHO EURO, 2019). Generally, the literature reviewed revealed mostly descriptions of legislative and regulatory instruments which were in place in several countries at the national level. Less often described are examples of how countries empowered their institutions and how the needed financial instruments for the regulatory push have been provided.

WSPs or similar approaches are anchored in national legislations in a growing number of countries around the world (Roeger and Tavares, 2018). Iceland (1995), Australia (2003), and New Zealand (2007) were among the first countries to include the WSP approach in their national legislations (Baum and Bartram, 2018). Those early adopters were followed by several other countries, as described in the literature for South Africa (Ncube and Pawandiwa, 2013), Hungary (Davidovits, 2014), Belgium, Switzerland and the United Kingdom (WHO EURO, 2014a), Brazil, Costa Rica and Peru (Hubbard et al., 2013), Canada (Bereskie et al., 2018), and the Netherlands (van den Berg et al., 2019). Many countries in the EU already integrated or are planning to include WSPs in their national legislation (WHO EURO, 2018), and the Portuguese regulator encourages WSP without an enforceable legislation being in place (WHO EURO, 2014a). Croatia shifted its obligatory HACCP approach for water suppliers towards a required WSP approach, allowing adaptation periods of between 5 and 10 years (WHO EURO, 2017a).

Examples for financial instruments were identified for Ireland, the Republic of Moldova, Scotland, and South Africa. In South Africa, the introduction of an incentive-based regulation called the Blue Drop Certification program, whereby municipalities can win awards for their water management, was highlighted as a key enabler (Ncube and Pawandiwa, 2013). WSPs in rural areas in Kyrgyzstan were linked to investment planning strategies in areas of poor water quality and resulting low willingness to pay of consumers (WHO EURO, 2014). In the Republic of Moldova, a WSP could be used as the basis for funds provided by the World Bank (WHO EURO, 2014a). In Ireland, having a WSP in place is a prerequisite for receiving funding from a private well grant, and the government grant scheme for small supplies in Scotland may require WSPs as a prerequisite in the future (WHO EURO, 2018).

Step 7: Implement WSPs and verify their effectiveness

*Continuing to implement WSPs and verification that they are effective confirms that they are appropriately developed (*WHO and IWA, 2010*).*

In order to verify the effectiveness of WSPs it is necessary to set up an auditing system at the national or sub-national level. WSP audits may also provide a mechanism of continued implementation support, especially for small systems.

In South-East Asian countries, the most common approach used is the external informal audit. Continuously improving this process, trainings for potential auditors are organized in order to build a pool of "master trainers" with the aim of advancing WSP implementation in the region (Sutherland and Payden, 2017). In Hungary, WSPs are subject to approval by the public health authorities (WHO EURO, 2018). Australia and New Zealand both rely on formal external audits conducted by certified auditors that have to fulfil strict requirements (WHO and IWA, 2015). A similar approach is used in South Africa, where under the "Blue Drop Certification Program" formal, external audits of the WSP are undertaken by the Department of Water and Sanitation (Ncube and Pawandina, 2013).

External audits are conducted every two years or less often in Australia, and every two years in South Africa, however, time intervals can also vary between countries (WHO and IWA, 2015). An example from a water supplier in Portugal shows that a tiered approach including different kinds of audits is also possible: an internal informal audit every six months, an internal formal audit by an external consultant once a year, and an annual formal external audit by an independent certified auditor. The WHO proposes periodic audits every six to 12 months depending on the form (internal or external, formal or informal) (WHO and IWA, 2015).

Step 8: Review overall WSP experiences and share lessons learned

*Reviewing the countrywide WSP experiences and sharing lessons learned leads to others being able to benefit and avoid identified pitfalls (*WHO and IWA, 2010*).*

Reviewing the overall WSP experiences continually is also crucial. In Alberta, Canada, for instance, the Environment and Sustainable Resource Development decided to review their drinking-water safety plan template after the initial completion date (Reid et al., 2014). Highlighting the shortcomings identified in a European survey, Iceland identified the need to improve guidance and control by the central government as well as the need to supply all relevant information both to the authorities and the public (Gunnarsdottir et al., 2015). Kumpel et al. (2018) published a study measuring the impacts of WSPs in the Asia-Pacific region highlighting several positive benefits but also main challenges. As a result of a WSP pilot project in Tajikistan, an awareness-raising brochure was published which contains templates to be used for future scaling up as well as concrete information on the experiences from the water supplies in the pilot districts (WHO EURO, 2013). This field guide was ultimately adapted for application in other countries (WHO EURO, 2014b). Sharing experiences and lessons learned is not exclusively done on the individual case study basis. It can also be region-wide through regional workshops (WHO EURO, 2014a), international conferences, and building networks as seen in Latin America and the Caribbean or the online platforms mentioned above, like the Water Safety Portal of WHO and IWA. The LAC WSP network's focus is to "increase advocacy, facilitate communication, support research, and build capacity", thus providing a platform for sharing knowledge to improve the region-wide implementation of WSPs

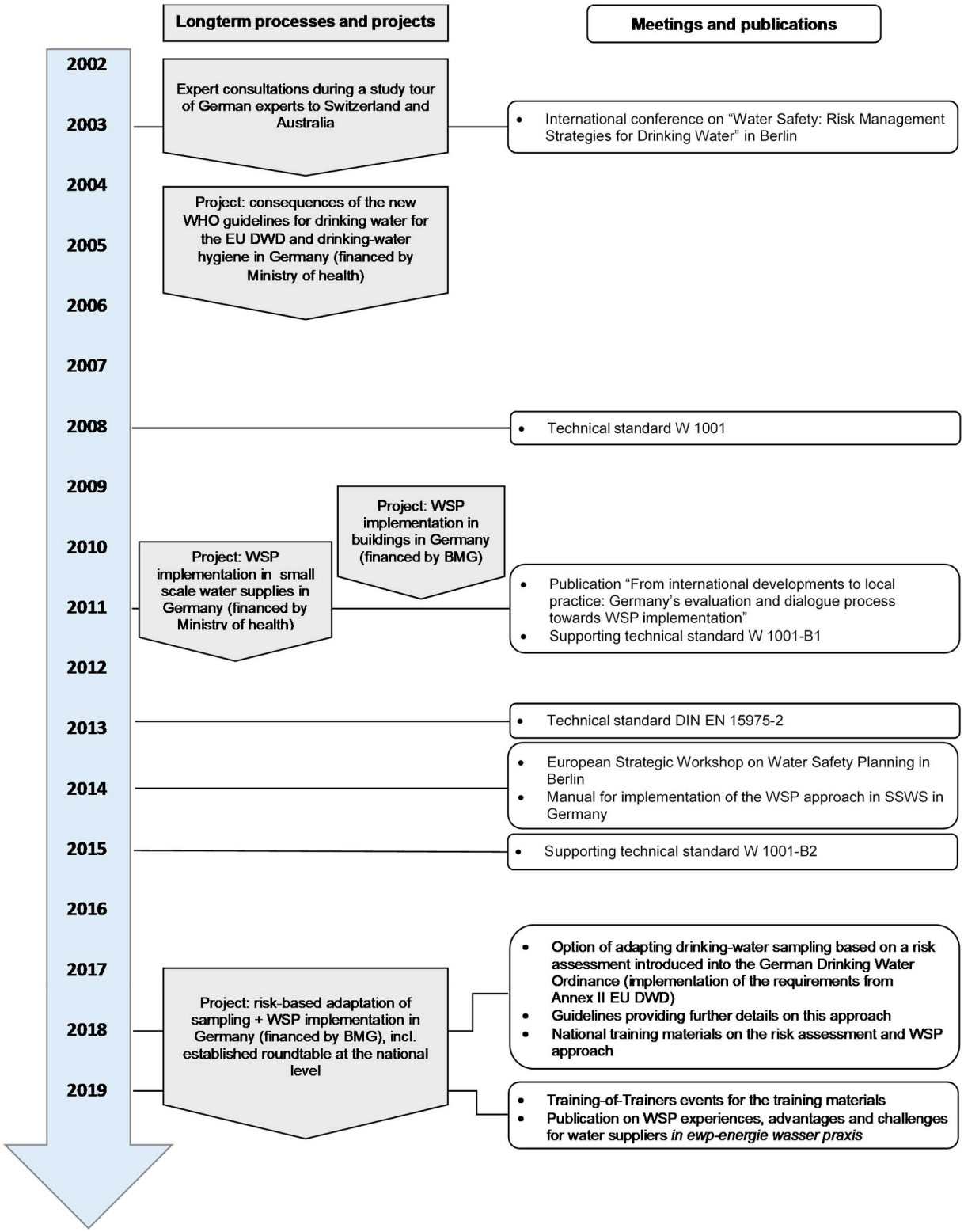

Fig. 3. Selected milestones of the German experience with RA/RM approaches.

(Rinehold et al., 2011).

3.3. Experience with creating an enabling environment in Germany

In addition to the first systematic literature review on the German experience, the following milestones were also identified (see Fig. 3), including continuous activities such as demonstration projects as well as one-off activities such as events or publication of documents related to the risk-based approach. Some of the milestones contribute to implementation of several WSP road map steps (Fig. 3).

Understanding and appreciating the benefits of the WSP approach was initiated in Germany through conducting a study tour to other countries already applying it, and through implementing a number of demonstration projects as well as collaboration and alliances with other

stakeholders (**WSP road map step 1**). The projects were financed by the Ministry of Health (MoH – in German: BMG), showing their commitment to exploring this new approach for the national context. In addition to the BMG and UBA, the projects included a number of stakeholder groups, e.g. technical associations, water suppliers, and local health agencies. Furthermore, an international conference on "Water Safety - Risk Management Strategies for Drinking Water" was organized in Berlin, Germany, as early as 2003, providing a major impulse for the national discussion (UBA, 2003).

In a first demonstration project with five utilities and the local health agencies responsible for their surveillance, WSPs were found to provide a number of benefits. These include:

- systemising long-established practices,
- stimulating an approach of continuous improvement,
- helping identify weak points,
- supporting diligent performance,
- facilitating the use of generally acknowledged codes of good practices or technical standards of the DVGW,
- providing better transparency of reasons for investments,
- strengthening organisational reliability,
- supporting avoidance of complacency, and
- improving exchange of experience and communication (Schmoll et al., 2011).

Benefits found in the literature review for the German context and reported in the expert interviews and the online survey furthermore include:

- a better understanding of the system and processes in the own water supply (expert interviews: 42%; online survey: 40%),
- increased operational safety (expert interviews: 50%), and
- improved internal and external communication (expert interviews: 42%; online survey: 75%).

The BMG and UBA started discussing a national WSP strategy in 2013. As in 2015 the focus shifted towards national implementation of the revised Annex II of the EU DWD which introduced risk-based sampling, this strategy was not published, and no national steering committee for WSP implementation has been established (**WSP road map steps 2 and 4**). However, within the scope of the national implementation of the risk-based approach to sampling, several workshops were organized in order to discuss this approach with a wide range of stakeholders, including the Federal States, local health agencies, other ministries, water suppliers' associations and the general public. As part of this process, a multi-stakeholder working group was established that developed guidelines to provide further details to those wishing to apply this risk-based approach.

Practical WSP experience has been gained through a number of demonstration projects. As the online survey has shown, in addition to the water suppliers covered by the demonstration projects in Germany, water supplies have either implemented WSPs (**WSP road map step 3**) or a "Technical Risk Management" (a WSP-like approach tailored to the German context; Mälzer et al., 2010). At an early stage, the DVGW reviewed its set of technical standards as part of the first demonstration project to assess to what extent the approach was already covered. As a result, they published the separate technical standard W 1001 (DVGW 2008) (now replaced by DIN EN 15975–2; Deutsches Institut für Normung (DIN) 2013) on how to apply the WSP approach in German water supplies (Schmoll et al., 2011), complemented by supporting technical standards W 1001-B1 (DVGW 2011) and W 1001-B2 (DVGW 2015).

The need to develop training materials, particularly easy-to-understand guidance for small water supplies, was already identified in the first demonstration project (Schmoll et al., 2011). One of the following projects had a particular focus on small-scale water supplies, as a relatively large number of such systems exists in Germany (approximately 7150 small public supplies serving 50–5000 persons, and many additional private wells).

Creating an enabling environment and facilitating the WSP implementation in the German context has been achieved through several factors: the development and dissemination of national training materials, the promotion of the risk-based approach in general, and training a group of national facilitators from different stakeholder groups in two ToT events in 2019 in how to apply the training materials, including public health agencies, water suppliers, consultants and water supply associations (**WSP road map step 5**). In addition to the training materials, national tools include the technical standards, a WSP manual for small supplies (UBA and TZW, 2014) and guidelines for conducting risk-assessments as a basis for risk-based sampling (UBA, 2018). Although only the aspect of risk-based sampling had been introduced in legislation, the national training materials also cover aspects of WSPs not yet legally introduced, in order to establish a basis for future scaling up of the application of this holistic approach. Almost 80% of the online survey responses stated that future training opportunities would facilitate implementation of RA/RM approaches, and 42% of interviewees, both small-scale water suppliers and both local health agencies, mentioned the importance of external expertise and exchange with other water suppliers as the most important aid. The ToT events showed that increased collaboration, for example through joint trainings for both water suppliers and local health agencies, is beneficial in order to develop a common understanding and vision of the implementation of this new approach. Anecdotal feedback indicates that such joint trainings are currently being planned. A perceived lack of awareness among all stakeholders was reported by several experts during their interviews (50% of interviewees). Such trainings can help to sensitize, motivate and raise awareness among all stakeholder groups in the German drinking-water sector regarding risk assessment-based approaches. The expert interviews and online survey responses also confirmed the positive assessment of involving both water suppliers as well as local health agencies in the implementation of risk-based approaches.

Although WSPs are not legally required in Germany, the aspect of risk-based sampling has been included in the Ordinance on The Quality of Water Intended For Human Consumption (Drinking Water Ordinance; Ordinance on The Quality of Water Intended For Human Consumption 2016) as an option in 2018, and the technical standard DIN EN 15975–2 (DIN 2013), as well as additional technical standards, describing the application of the WSP approach (**WSP roadmap step 6**). Legal anchoring was also considered absolutely necessary by half of the stakeholders during their interviews in order to ensure nationwide implementation. Interestingly, only one small-scale water supplier (defined as supplying water to less than 5000 people) indicated that WSPs should not become a legal requirement. Already in 2011, Germany was considered well-prepared in case legally binding WSP requirements were introduced through the EU DWD (Schmoll et al., 2011). Within the current revision of the EU DWD, it is anticipated that additional legal requirements with respect to RA/RM will need to be applied in Germany.

As part of the stakeholder participation process of the legislative changes, there were intense discussions about responsibilities for developing as well as externally assessing and approving the risk assessments and WSPs, as concern was voiced regarding the human resources required for this (**WSP road map elements 6 and 7**). Nevertheless, there was consensus that the main responsibility for the process of ensuring drinking-water safety and related surveillance and oversight should remain with the water suppliers and the local health agencies, and external expertise should be involved as support. No additional financial resources are currently foreseen for implementation of risk-based approaches. However, it is recognised that eventually the application of a risk-based approach can lead to a more efficient use of existing resources, particularly in the long term. The national training materials are available free of charge. The current practice with respect

Table 1
Assessment of the WSP road map elements implemented in Germany.

WSP road map step		Status of implementation in Germany
Step 1.	Understand and appreciate the benefits of WSP	(+ +) Implementation fully covered
Step 2.	Establish preliminary WSP vision	(+ +) Implementation fully covered
Step 3.	Attain practical WSP experience	(+ +) Implementation fully covered
Step 4.	Establish national strategy to scale up WSP	(−) Limited implementation
Step 5.	Establish mechanisms for ongoing support of WSPs	(+ +) Implementation fully covered
Step 6.	Establish policy and regulatory instruments to support WSP implementation	(+) Implementation partly covered
Step 7.	Implement WSPs and verify their effectiveness	(−) Limited implementation
Step 8.	Review overall WSP experiences and share lessons learned	(+) Implementation partly covered

to risk-based sampling is that the local health agency has to approve the adaptation of the sampling scheme for each individual water supply. To date, only a limited number of WSPs have been implemented in Germany, and anecdotal evidence indicates that the number of applications for risk-based sampling adaptations with the local health agencies is quite low. The currently ongoing project on risk-based adaptation of sampling and WSP implementation in Germany explores feasible options for externally assessing and approving WSPs.

The review of the overall WSP experiences and sharing of lessons learned (**WSP road map element 8**) has taken place in Germany through the expert interviews as well as the online survey and within the scope of the publication of the evaluation of Water Safety Plan implementation in Germany in general (Schmoll et al., 2011; Zügner et al., 2019) and for buildings (Schmidt et al., 2019). As in Germany waterborne diseases are not monitored separately from other infection routes, and the level of compliance with values for those parameters which are regulated in the Drinking Water Ordinance is already very high at 99–100% (Bundesministerium fur Gesundheit and UBA, 2017, 2018), showing concrete improvements with respect to drinking-water quality and public health will continue to remain challenging in the future. An interesting development that may be anticipated is that the introduction of risk-based monitoring may indeed lead to increased results showing exceedances, either of parametric values regulated legally or showing the occurrence of parameters additionally monitored: this is almost inevitable as the focus shifts towards analysing samples and parameters for which risks are presumed and exceedance is therefore more likely while parameters and sites that have shown compliance in the past will be analysed less frequently. This anticipated development is likely to pose major public communication challenges.

As shown in Fig. 3, activities increased over time after the added value of WSP implementation was appreciated and first experiences were gathered which could be used as the basis for further policy activities such as introducing first risk-based aspects into legislation. After the introduction of a technical standard at the EU level and legislative changes, national activities increased again as there was a legislative push for increasing action. Table 1 gives a summarising assessment of the implementation of the WSP road map elements in Germany.

4. Discussion

The German enabling environment for scaling up WSPs is strongest in WSP road map steps 1–3, and further activities may be informed by experiences from other countries for the other steps, in particular steps 4 and 7. In the international literature, most information was documented for steps 1 and 3 whereas the least was found for step 7 and 8.

As suggested by the WSP road map and shown in the international experience, collaboration and partnerships, as well as experience exchange also occupied a central role in how German stakeholders started to understand and appreciate the benefits of a WSP approach (WSP road map step 1). The benefits identified in Germany mainly mirrored those highlighted in the literature. However, the main advantage of the WSP approach for the German context lies in the “soft” benefits, e.g.

- deeper system and process understanding,
- identification of weaknesses within the system or increased awareness of responsibilities and due diligence, and
- improved documentation

with less focus on improvement with respect to water-related diseases (possibly not detected due to lack of data gathering and collating) or the compliance with existing standards (levels of which are already very high) as they are more difficult to document.

While in Germany, the Drinking Water Ordinance is the main legislation on drinking-water at the national level, responsibility for the drinking-water quality lies with the 16 Federal States, and the local health agencies are legally required to regularly perform surveillance activities of drinking-water supplies, making coordination between stakeholders particularly important. Initially, a loose alliance was formed including Federal agencies and other stakeholder groups. This did not, however, lead to the establishment of a formal national steering committee, as suggested in step 2 of the WSP road map. In Germany as well as other countries, the WSP approach was initially compared against existing practices and standards in order to identify overlaps and added values. At the same time, several demonstration projects were set up (WSP road map step 3). Identified benefits and added values were documented and communicated. Challenges in the German context – as well as internationally – were flagged for the catchment areas of drinking-water suppliers as they are often outside the influence of the supplier. In part, this issue is already addressed at the legislative level of the EU DWD through requiring information about the catchment and providing it to water suppliers as a basis for the risk assessment, and within the current revision, particular attention is given to RA/RM in catchments of drinking-water supplies (Council of the European Union, 2020).

Despite not having a formal steering committee, the BMG and UBA still started to discuss a national WSP strategy which would have represented a pro-active initiative even at times without a regulatory push from the EU level, which was however not published. Elements mentioned in the WSP road map step 4 for developing the strategy and promoting multi-stakeholder buy-in were however implemented; they just did not result in establishing a formalised strategy. Experience from Portugal, a country which is also subject to EU legislation, confirms the benefits of establishing a strategic framework as a basis for monitoring WSP implementation and names prioritizing core components and stipulating stakeholders' responsibilities as key success factors (Vieira, 2011). Ethiopia developed a strategy including information on how implementation can be monitored and financed (MoWIE, 2015a). In the German context, the importance of dialogue and communication processes with key stakeholders was highlighted (Schmoll et al., 2011), and the importance for stipulating stakeholders’ responsibilities was also flagged for Portugal (Vieira, 2011). Further activities may build on existing standards and regulations as described in the literature (Baum and Bartram, 2018; Gunnarsdottir et al., 2015), for example stipulating strategic goals by when these should be implemented by a certain percentage of the water suppliers, such as the targets set under the Protocol on Water and Health (UNECE and WHO EURO, 2019).

Several aspects of step 5, i.e. establishing mechanisms for ongoing support of WSPs, are already implemented in Germany. In support of the legislative changes of the German Drinking Water Ordinance in 2018, UBA published guidance for the new risk-based approach to support the water suppliers with practical advice, and a handbook for WSP in small supplies and technical standards on WSP implementation were published previously. Utility-utility partnerships in the context of risk assessment have been realized successfully in several WSP demonstration projects. Exchange between water utilities, particularly larger ones, often takes place within networks and associations, which may also include exchange of experiences on application of RA/RM. Existing partnerships, including for example municipal associations of small water supplies, could be used for future implementation of risk assessment-based approaches.

In order to promote the implementation process of WSPs and to build capacity and knowledge in the water and health sector, UBA released free-of-charge workshop materials in 2018 covering the German approach and WSPs in general and organized several ToT workshops in 2019. This is a profound basis for future workshops in which staff from water utilities and health authorities can be trained together, with the potential of improving their cooperation in the implementation process. WSPs have been integrated into university/higher learning curricula in a number of other countries (Ferrero et al., 2019), as recommended in the WSP road map. However, no national overview exists of the extent to which this topic is already being taught at universities in Germany, as responsibility for education lies with the 16 Federal states and not with the national level.

Implementing step 6 (i.e. establishing regulatory and policy instruments) at the national level in Germany is less advanced than the previous WHO road map steps. In contrast to most documented international experience, German regulation does not require WSPs but merely offers to adjust the monitoring scheme based on a risk assessment of the whole utility, as introduced by the EU DWD. Establishing policy and regulatory instruments has thus been realized only for implementing risk-assessments on a voluntary basis, but not as mandatory requirements or compulsory element of risk management. The fact that data collection on the experience with the approach in Germany showed that only a small number of the German water suppliers (26 online survey responses compared to several thousand public water supply areas) has implemented the WSP approach so far on a voluntary basis confirms the finding in the literature that a regulatory push is most effective for broad WSP implementation (WHO, UBA and IWA 2014). While there is no such legal requirement in Germany, a comprehensive set of approximately 300 technical standards exists in addition to drinking-water legislation. These describe requirements for elements of drinking-water supplies (from the protection zone to the tap). In addition, DIN-EN 15975–2 introduces central elements of the WSP-approach and risk management under normal operating conditions. The German Drinking Water Ordinance refers to these standards in numerous articles. No separate financing instruments for WSP development or risk assessments for adapting the sampling and monitoring scheme are in place. This situation appears to be common also as described in the international literature, with few examples documented, particularly for requiring RA/RM approaches as a basis for grants for small supplies (WHO EURO, 2018). The majority of countries mentioned having released some kind of guidance, but information about financial support is very rare.

Regarding WSP road map step 7 (implementing WSP and verifying effectiveness), the reviewed literature focused mainly on the auditing aspect; the literature review revealed no further published information on continued support for the WSP implementation. Although the WSP road map claims that not all steps will be similarly relevant in all countries, and that they do not necessarily have to be followed in a certain order, for WSP road map steps 7 and 8 it is a prerequisite that WSP implementation has at least started in the respective country, as no audits can be conducted if WSPs are not yet implemented. No formal audits of WSPs are conducted in Germany to date. However, if water suppliers choose to implement a risk assessment for adapting their monitoring, this has to be approved by the local health agency. In the literature, audits being required at the national level were only found in countries in which WSPs are legally required, e.g. Australia or Hungary. With EU legislation and respective national requirements probably anticipated to require WSPs in the future, it is likely that audits will also be regulated at the national level in Germany. It is then also likely that models relying on formal audits, like in Australia, New Zealand and South Africa, or the Portuguese approach of a combination of internal and external audits, will be more relevant for inspiring further action in Germany than those approaches building on informal audit approaches. In case RA/RM should be applied at a larger scale also for small supplies in Germany, it is likely that a more supportive approach would be feasible for such systems in order to improve operators' understanding of WSP principles and for capacity- and technical support to WSP teams. International experience shows different responsibilities for conducting the audits. As the local health agencies in Germany are responsible for surveillance of water supplies, they could be considered for conducting the external audits, however, options for further support may need to be explored, particularly for those agencies responsible for surveillance of a large number of small supplies. Currently, UBA is compiling information, including on experiences from other countries, on options for verifying appropriateness of WSPs in order to inform the German context.

WSP road map step 8 (review of experience and sharing lessons learned) is only partly implemented in Germany at a larger scale as WSPs are not legally required. Moreover, information about implemented WSPs and their assessment is rare at the national level. However, the first "formal" assessment was carried out in 2011 (Schmoll et al., 2011) which was quite early in the national WSP implementation process. This has been followed by continually reviewing experience at the national level, including the literature review, the expert interviews and the online survey. The survey questionnaire was designed to be easily replicated and revised for future surveys. Furthermore, an evaluation of the successfully conducted WSP trainings is planned.

Overall, WHO road map steps 7 and 8 were both not commonly described in the peer reviewed literature. One reason might be that authors were mainly from universities and not from national level regulation authorities. This fact could have led to the underrepresentation of steps 7 and 8 in the literature. However, one could argue that publishing an article on national experiences represents implementation of parts of step 8 in itself. Furthermore, literature reviewed was limited to publications in English or German, thus possibly missing those addressing national stakeholders in local languages. For the same reason, a recent publication in German described experiences with and advantages of WSP implementation in order to encourage increased implementation within the German water supply sector (Zügner et al., 2019).

In the literature, information appears to be scarce particularly on developing a national strategy, securing financial instruments, activities to support continual implementation of WSPs and on review of the overall WSP experiences and sharing lessons learned, and the German situation mirrors the international experiences. Therefore, only limited recommendations for further action on those elements can be drawn from the literature review for the German context.

Reported blockers for successful national WSP scale-up include lack of awareness and recognition, complacency, competing priorities, lack of resources and skills, as well as poor internal relationships (Summerrill et al., 2011). The absence of specific legislative frameworks and the lack of appropriate tools have also been highlighted (Roeger and Tavares, 2018; Hubbard et al., 2013).

Large similarities in global and German experiences were also revealed in several further aspects: the identification of benefits of WSPs through collaboration and alliances; the review of the existing

regulatory framework in light of added value of the WSP approach; the implementation of demonstration projects; and the development of locally appropriate training materials and workshops have both been described in the literature as well as addressed in WSP implementation in Germany. Stakeholder engagement through collaboration, experience exchange, and discussions has been at the center of the German approach and in this context, existing utility-utility partnerships as a specificity of the German context have proven useful. Also, the thorough reviewing of experiences and lessons learned regarding the enabling environment of WSP scaling-up, currently underrepresented in the literature, is a good starting point for further action in this domain. Aspects from the literature review that could inspire further action at the national level particularly relate to considering to establish a formal strategy relating to road map step 4 (e.g. like in Portugal), and information on how to shape a system for external quality assurance of WSPs in the future tackling road map step 7 (e.g. Australia, Hungary, New Zealand, Portugal, and South Africa).

5. Conclusions

Since the publication of the WSP road map by WHO and IWA in 2010, the individual/national experience on creating an enabling environment for WSP scaling up has been published for a number of countries. However, information appears to be scarce particularly on developing a national strategy, securing financial instruments, activities to support continual implementation of WSPs and on review of the overall WSP experiences and sharing lessons learned. In order to support uptake in countries wishing to scale up WSPs, collating and providing respective information would be very beneficial.

Comparing German national experiences of WSP activities to the WSP road map and international experience helped to contextualize the own country situation and to identify helpful approaches, which can be used for creating or improving the enabling environment for future country-wide scaling up of WSP implementation, such as described details and advantages of developing a national scaling up strategy. For roadmap steps still to be fully implemented, such as auditing WSPs, examples from the different international approaches can help shaping future national implementation. As the WSP roadmap claims that the steps described therein may have differing importance and the order in which they are implemented may differ, it is important to consider whether and to which extent the description of experiences from other countries is comparable to the own national context. The German experience confirmed this approach which a number of national activities relating to several WSP roadmap steps and running in parallel and not necessarily in the order given in the WSP roadmap.

The assessment of the German context with the international experiences showed large similarities. The development of an enabling environment in Germany provides information from a context of a country with a large number of water supplies, including small ones, with no formal legal WSP requirements but technical standards covering WSP aspects. Furthermore, national approaches are influenced by Germany being bound to EU legislation, which is expected to increase importance of WSP-like approaches in the near future, thus providing a “regulatory push”, as highlighted to be vital by many stakeholders.

Declaration of competing interest

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Appendix A. Supplementary data

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Estimation of the time-varying reproduction number of COVID-19 outbreak in China

Chong You[a,1], Yuhao Deng[b,1], Wenjie Hu[b], Jiarui Sun[b], Qiushi Lin[b], Feng Zhou[c], Cheng Heng Pang[d], Yuan Zhang[e], Zhengchao Chen[f], Xiao-Hua Zhou[a,c,*]

[a] Beijing International Center for Mathematical Research, Peking University, China
[b] School of Mathematical Sciences, Peking University, China
[c] Department of Biostatistics, School of Public Health, Peking University, China
[d] Faculty of Science and Engineering, University of Nottingham Ningbo China, China
[e] National Research Institute for Health and Family Planning, China
[f] Beijing Obstetrics and Gynecology Hospital, Capital Medical University, China

A B S T R A C T

Background: The 2019 novel coronavirus (COVID-19) outbreak in Wuhan, China has attracted world-wide attention. As of March 31, 2020, a total of 82,631 cases of COVID-19 in China were confirmed by the National Health Commission (NHC) of China.

Methods: Three approaches, namely Poisson likelihood-based method (ML), exponential growth rate-based method (EGR) and stochastic Susceptible-Infected-Removed dynamic model-based method (SIR), were implemented to estimate the basic and controlled reproduction numbers.

Results: A total of 198 chains of transmission together with dates of symptoms onset and 139 dates of infections were identified among 14,829 confirmed cases outside Hubei Province as reported as of March 31, 2020. Based on this information, we found that the serial interval had an average of 4.60 days with a standard deviation of 5.55 days, the incubation period had an average of 8.00 days with a standard deviation of 4.75 days and the infectious period had an average of 13.96 days with a standard deviation of 5.20 days. The estimated controlled reproduction numbers, R_c, produced by all three methods in all analyzed regions of China are significantly smaller compared with the basic reproduction numbers R_0.

Conclusions: The controlled reproduction number in China is much lower than one in all regions of China by now. It fell below one within 30 days from the implementations of unprecedent containment measures, which indicates that the strong measures taken by China government was effective to contain the epidemic. Nonetheless, efforts are still needed in order to end the current epidemic as imported cases from overseas pose a high risk of a second outbreak.

1. Introduction

On December 29, 2019, four cases of pneumonia with unknown etiology were reported in Wuhan, the capital city of Hubei Province in Central China (Li et al., 2020). Since then, the outbreak has dramatically worsened over a short span of time and has received considerable global attention. On January 7, 2020, the pathogen of the current outbreak was identified as a novel coronavirus (2019-nCoV), and its gene sequence was quickly submitted to the WHO (The coronavirus was renamed COVID-19 by the WHO on February 12, Huang et al., 2020, Wang et al., 2020). On January 30, the WHO announced the listing of this novel coronavirus-infected pneumonia (NCP) as a "public health emergency of international concern". As of March 31, 2020, the National Health Commission (NHC) of China had confirmed a total of 82,631 cases of COVID-19 in China, including 3,321 fatalities and 76,415 recoveries.

Since January 19, 2020, strict containment measures, including travel restrictions, contact tracing, entry or exit screening, non-hospital isolation, quarantine and awareness campaigns have been implemented by the Wuhan municipal government and quickly adopted by other cities within China with the aim to minimize virus transmission via human-to-human contact. In 2009, similar measures were employed in China in response to the outbreak of H1N1 virus breakout.

This article investigates the change in the basic reproduction number R_0 and controlled reproduction number R_c since the outbreak of COVID-19. We have found that the estimated controlled reproduction numbers R_c in all different regions are significantly smaller compared with the basic reproduction numbers R_0, which indicates that the containment measures carried out by Chinese government was effective and efficient.

* Corresponding author. Beijing International Center for Mathematical Research, Peking University, China.
E-mail address: azhou@math.pku.edu.cn (X.-H. Zhou).
[1] Joint first authors.

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Table 1
Numerical summaries of serial intervals, infectious period, and incubation period.

	Minimum	1st quartile	Median	Mean	3rd quartile	Maximum
Serial intervals	−13.00	1.00	4.00	4.60	7.00	21.00
Infectious period	0.00	11.00	13.50	13.96	17.50	29.00
Incubation period	0.00	4.50	7.00	8.00	10.00	23.50

2. Data

A form was designed to collect and standardize information from each individual confirmed case. There were two parts in the designed form:

(A) Part A directly retrieved pubic available data from provincial or municipal health commissions as well as apps and websites managed by local governments in China. The following details were included on each confirmed case: case ID, region, age, gender, first sign of symptoms, date of symptom onset, date of diagnosis, history of travel or previous residency in Hubei Province, contact history with other confirmed cases and others. See more detail information collected in Part A of the form in Supplement.
(B) Part B includes probable transmission chains which were *inferred* based on history of travel to or previous residency in Hubei and other related information. The transmission chain was inferred as follows: (1) if the individual X has not recently been to Hubei Province, but has been in close contact with an imported case Y from Hubei, then the individual X was determined to be infected by this imported case Y and hence formed a transmission chain; (2) if the individual X has not recently been in Hubei province, but has been in close contact with a local case Y who was clearly infected before the contact, then this individual X was determined to be infected by the local case Y. Note: if the individual has been to Hubei Province, the transmission history would not be recorded despite the existence of contact tracing information. See Part B of an empty form in Supplement.

The date of infection was not included in the form but some can be inferred from the form as follows:

(1) If the individual has not been to Hubei Province recently, but was exposed within a three-day period (i.e., the individual had contact with confirmed cases within three consecutive days), then the corresponding date of infection is inferred as the middle of the exposure period;
(2) If the individual previously traveled to Hubei Province but returned within three days, then the date of infection is inferred as the middle of the travel period.

All data collection and processing were done manually except that the data from Shenzhen was semi-automated collected (a spreadsheet can be downloaded from an official website for Part A, however information in Part B still needed to be inferred and entered manually). Data collectors were trained and divided into 6 groups according to the regions of confirmed cases with 2 collectors in each group to ensure the efficiency and accuracy of data collection. Data sources, typically the website address, and names of the collectors were also recorded in the form of each case for quality control purposes. The collected data were consistently monitored and spot checked by two assigned supervisors. As of March 31, 2020, we have collected a total of 14,829 confirmed cases detected outside Hubei Province in China.

3. Inference about the generation time, incubation period, serial interval and infectious period

The generation time is the time difference between dates of infection of successive cases in a transmission chain while the serial interval is the difference in dates of symptoms onset between a pair of a primary case and its secondary case. The incubation period is defined as time difference between contraction of the disease and symptoms onsets. The infectious period is the duration of which an infected individual can transmit pathogens to a susceptible host. In this study, the infectious period is defined as the time difference between date of infection and date of diagnosis as there is strong evidence showing that a diseased individual remains infectious even during the incubation period, and would be immediately isolated upon positive diagnosis hence losing the transmissibility (Rothe et al., 2020). All are key quantities that depict an epidemic and are essential to estimate the basic/controlled reproductive number, R_0/R_c. Among the 645 chains of transmission identified from 14,829 confirmed cases recorded outside Hubei Province as of March 31, 2020, very few of them have their dates of infection acquired, but 198 of them have their dates of symptoms onset available. Hence, we only calculate the serial interval but not the generation time.

We can see that some serial intervals are negative, which suggests that COVID-19 is infectious during incubation and negative values were caused by different lengths of incubation period between individuals. The average of the serial intervals is 4.60 days and the standard deviation is 5.55 days (see Table 1). Note that the serial interval of SARS-nCoV in Hongkong was 8.4 days on average (Lipsitch et al., 2003). In fact, the distribution of serial interval may be biased for estimating generation time, especially when the disease is infectious during incubation, in that the variance of generation time could be overestimated (Britton, 2019). In addition, a total of 169 cases in the collected data were able to identify the dates of infection according to the method described in previous section. The histogram of infectious period is in Fig. 1 while the numerical summary is in Table 1. We acknowledge that isolation could occur before the date of diagnosis and suspected cases could be isolated without the formal positive results, hence infectious period could be potentially overestimated. Furthermore, incubation periods of these 169 cases can be identified. See Fig. 1 and Table 1 for the histogram and numerical summary for the incubation period.

We found that there were no significant demographical differences between the subset of cases used to estimate serial interval and infectious period and the cases in the full dataset. Therefore, the inference made on serial interval, incubation period and infectious period based on the corresponding subsets should be able to represent the full dataset.

4. Estimation of basic/controlled reproduction number

4.1. Definition

The reproduction number R_0 is defined as the (average) number of new infections generated by one infected individual during the entire infectious period in a fully susceptible population (Anderson et al., 1992). The basic reproduction number reflects the ability of an infection spreading under no control. When the size of susceptible

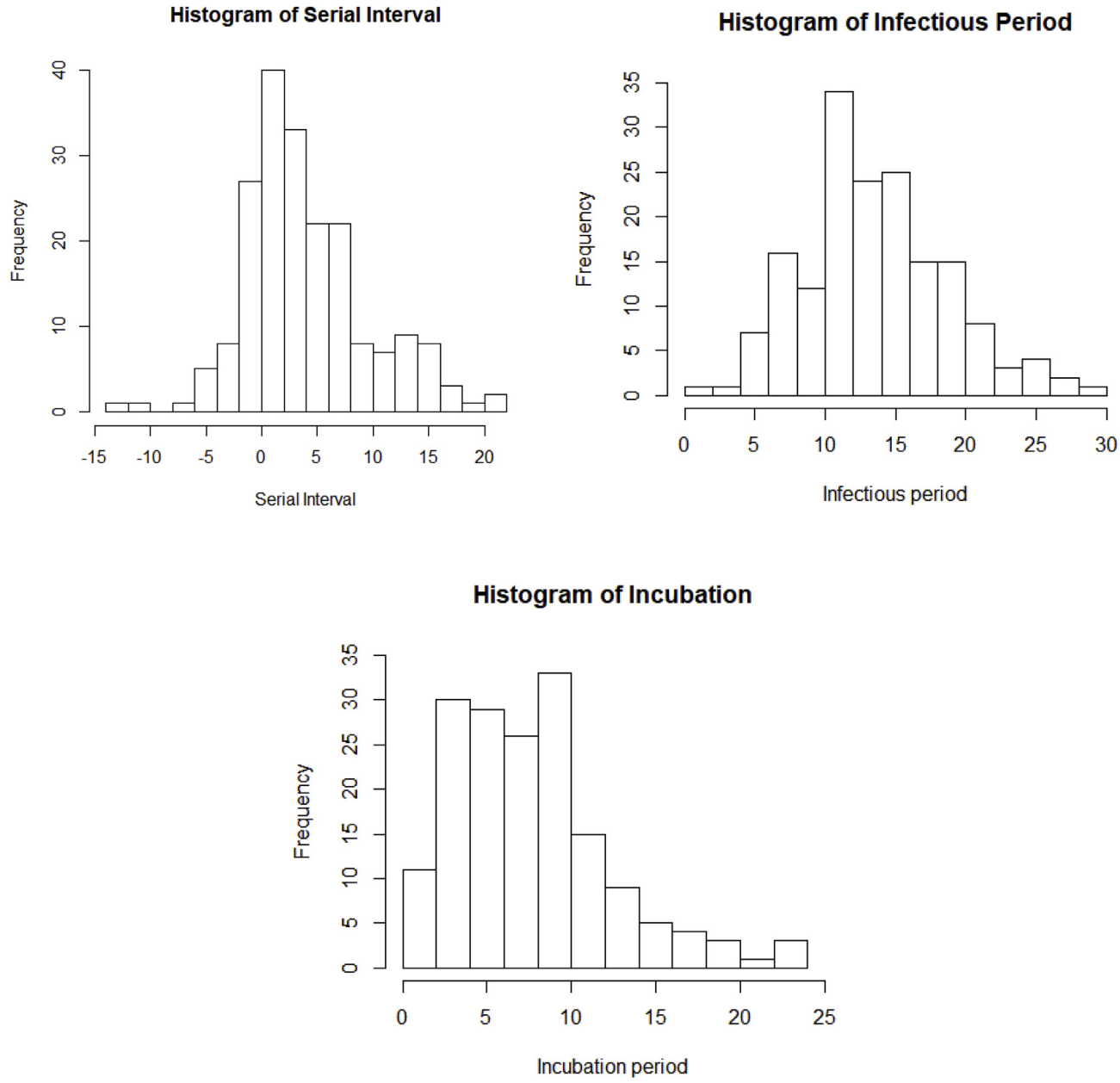

Fig. 1. Histograms of serial interval with the median of 4.0 days, infectious period with the median of 13.5 days, and incubation period with the median of 7.0 days.

population is limited, the quantity, effective reproduction number R_e, is used instead of R_0. Similarly, the quantity, controlled reproduction number R_c, should be used to describe the ability of disease spreading when interventions (such as quarantine, isolation, or traffic control) are taking place. Hence a good measure of any intervention is to reduce R_c. Note that the disease will decline and eventually die out if $R_c \leq 1$.

4.2. Methods

The basic reproduction number can be estimated through a variety of models (Nikbakht et al., 2019). In this section, we have compared three most popular estimates of R_0 or R_c as shown below.

4.2.1. Poisson likelihood-based (ML) method

This method assumes that the total number of secondary cases infected by a single primary case follows a Poisson distribution. The number of individuals infected on Day t is usually approximated by the number of new reported confirmed cases on Day t, and the generation time is approximated by its corresponding serial interval. Let N_t be the number of reported new confirmed cases on Day t. Suppose that the serial interval has a maximum of k days and the number of new cases generated by an infected individual is assumed to follow a Poisson distribution with parameter R (Forsberg White and Pagano, 2008). The probability that the serial interval of an individual lies in j days is w_j, which can be estimated from the empirical distribution of serial interval or by setting up a discretized Gamma prior on it. Note only the non-negative values of serial interval are used here. Thus, the likelihood function can be reduced into a thinned Poisson

$$L(R, w) = \prod_{t=1}^{T} \frac{e^{-\mu_t} {\mu_t}^{N_t}}{N_t!}$$

where

$$\mu_t = R \sum_{j=1}^{\min\{k,t\}} N_{t-j} w_j.$$

The reproduction number $R.$ can be estimated by maximizing the likelihood function. Note that if the empirical distribution of serial interval is used or w_j's is given, then

$$\hat{R.} = \frac{\sum_{t=1}^{T} N_t}{\sum_{t=1}^{T}\sum_{j=1}^{\min\{k,t\}} N_{t-j} w_j}.$$

4.2.2. Exponential growth rate-based (EGR) method

At the early period of an epidemic, the number of infected cases rises exponentially. Similar to the ML method, the number of individuals infected on Day t is approximated by the number of new reported confirmed cases, and the generation time is approximated by its corresponding serial interval. Suppose the exponential epidemic growth rate (Malthusian coefficient) is r, which can be estimated by fitting a least square line to the daily number of reported new confirmed cases in a log-scale, namely, $\log(N_t)$. Let $f_G(t)$ denote the probability density function of serial interval. Hence the reproduction number can be calculated according to the Euler-Lotka equation in a moment generating form (Wallinga and Lipsitch, 2007)

$$\hat{R.} = \frac{1}{\int_0^{\infty} e^{-rt} f_G(t)dt}.$$

4.2.3. Stochastic dynamic model-based method

Here we consider a stochastic Susceptible-Infected-Removed (SIR) model rather than a standard deterministic one. The major advantage of using a stochastic dynamic model is that it affords improved accounting for real variabilities and increases opportunity for quantifying uncertainties (King Aaron et al., 2015). Here we denote $S(t)$, $I(t)$ and $R(t)$ as the number of susceptible, infected but not lab-confirmed cases (including those in incubation period) and removed population (including recoveries, fatalities and confirmed cases) at time t respectively, and note that $N = S(t) + I(t) + R(t)$ is a constant. There was some evidence indicating that the COVID-19 is infectious during its incubation period (Rothe et al., 2020). Due to this unique nature of COVID-19, individuals in state I is contagious during even the incubation period. With the assumption of equal transmissibility during the whole infectious period, individuals in state I pass pathogens to susceptible population with a constant transmission rate β. The removed individuals are no longer infectious since they have been isolated in hospital. Suppose that the infectious period of an individual is a random variable T~Exp(γ), then the reproduction number $R. = \beta E(T) = \beta/\gamma$, where γ and β are the removing rate and transmission rate. Note that the following mean-field Differential Equation System serves as a deterministics counterpart of the stochastic model,

$$\frac{d\tilde{S}}{dt} = -\frac{\beta\tilde{I}\tilde{S}}{N},$$

$$\frac{d\tilde{I}}{dt} = \frac{\beta\tilde{I}\tilde{S}}{N} - \gamma\tilde{I},$$

$$\frac{d\tilde{R}}{dt} = \gamma\tilde{I},$$

where $\tilde{S}(t)$, $\tilde{I}(t)$ and $\tilde{R}(t)$ are the deterministic counterparts of $\tilde{S}(t)$, $\tilde{I}(t)$ and $\tilde{R}(t)$.

The maximum likelihood method is used to estimate model parameters where the likelihood is obtained by sequential Monte Carlo method, and parameters are estimated using the Iterated Filtering algorithm (IF2) implemented as mif in the R package pomp (Ionides et al., 2015, King et al., 2010). Here we set $S(0)$ equals the population of the region, $R(0) = 0$, $I(0)$ is 14 times the average number of confirmed cases from Day 0 to Day 7, and $\gamma = 1/13.96$, the inverse of mean infectious period, obtained from the collected data described before.

It is arguable that the transmission rate β is a constant over the whole infectious period, some studies indicate that β may vary and possibly peak on or before symptoms onset (He et al., 2020). However, it is beyond the scope of this study. In addition, this model does not consider asymptomatic and undocumented case, quarantine measures and human mobility, a more sophisticated and realistic model to reflect unique features of COVID-19 is worth of another paper itself. Nonetheless, this SIR model should at least provide some useful insight into reproduction numbers and is a better alternative of the popular SEIR model which assumes noninfectious incubation period.

5. Results

In this section we have estimated the basic reproduction number R_0 and the controlled reproduction number R_c. Since January 19, 2020, various containment measures have been strictly implemented, especially after the State Council agreed to include COVID-19 into the Management of the Infectious Diseases Law and the Health and Quarantine Law on January 20. Wuhan was locked down on January 23, and the inter-provincial flow of people was greatly reduced (https://qianxi.baidu.com). which effectively controlled the exportation of infected individuals. Based on an average 13.91-day infectious period estimate from our collected data, we expect a flatter rate of increment starting from the end of January. Fig. 2 plots the number of daily new cases on a log-scale against date, and, as anticipated, the trend supports this estimate. Therefore, the basic reproduction number R_0 and the early-phase controlled reproduction number R_c, are estimated based on collected data in two separate periods, i.e., from January 21 (the starting date of daily updates of confirmed cases nationwide) to January 28, and from January 29 to February 5 respectively.

The estimates of R_0 and R_c by Poisson likelihood (ML), exponential growth rate (EGR) and Susceptible-Infected-Removed (SIR) model in selected regions of China are listed in Table 2 and Table 3. Despite the disagreement between different estimation methods, all three methods indicate notable reductions from R_0 to R_c which suggests an improvement from January 21 to February 5. This is possibly due to the effective interventions and prompt actions by the local and central governments to minimize further spreading. We also notice that EGR yields smaller estimates of R_c compared to other methods. This might be because the number of infected patients does not grow exponentially after such strict containment measures, hence EGR is only recommended to estimate R_c in early stage of an epidemic.

Furthermore, the time-varying controlled reproduction number $R_c(t)$ can be estimated through the Poisson likelihood (ML) method where t is from February 1 to 29, 2020. Note that there were very few new cases confirmed outside Hubei Province since March 1, 2020, hence we only updated $R_c(t)$ up to February 29, 2020. For each Day t, the number of daily reported new cases from Day $t-9$ to Day t is used to estimate $\hat{R}_c(t)$. Fig. 3 plots the estimated controlled reproduction number $\hat{R}_c(t)$ along with its 95% confidence interval (CI) for selected regions of China. Note that the estimated $\hat{R}_c(t)$ reflects the average spreading ability of the epidemic in a short period prior to Day t. As a result, the real-time $R_c(t)$ might be overestimated if the general trend of $R_c(t)$ is declining.

6. Conclusion

Despite the continuous increase in new confirmed cases between January 21, 2020 and February 5, 2020, the estimated controlled reproduction numbers R_c produced by all three methods in all analyzed regions are significantly smaller compared with the basic reproduction numbers R_0. We can see that outside Hubei Province, $R_c(t)$ dropped below 1 around February 11, 2020 while in Hubei $R_c(t)$ fell below 1 around February 19, 2020, which indicates that the containment measure carried out by Chinese government was effective and efficient. The controlled reproduction numbers R_c is now much lower than one in all regions of China for quite a time, however, it has yet reached zero. A possible explanation is that the number of foreign imported cases has

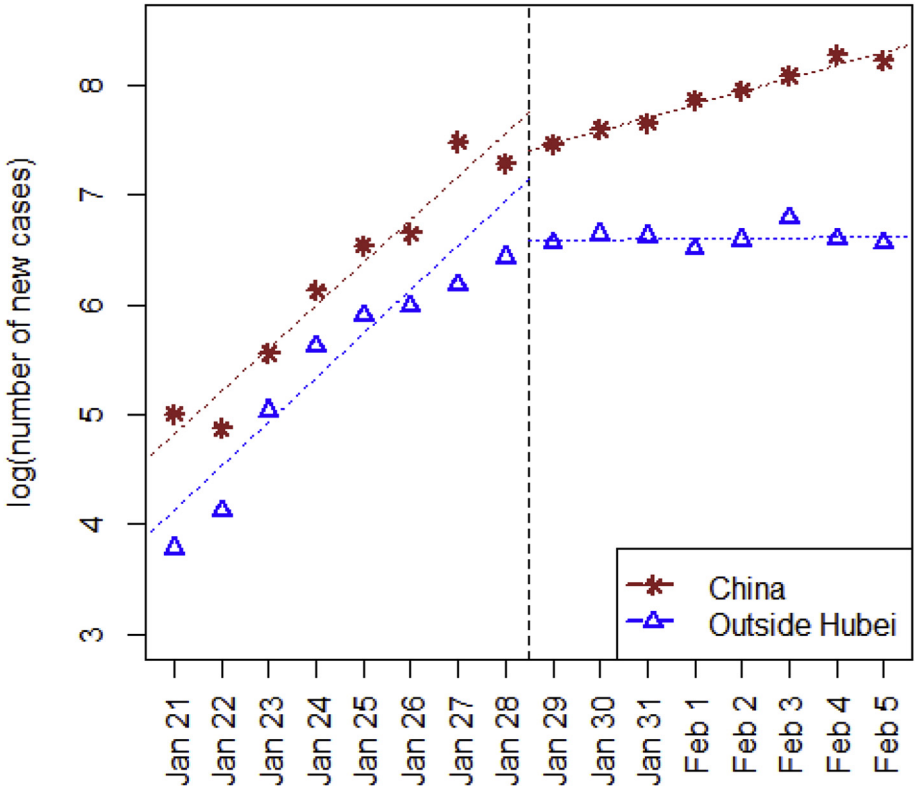

Fig. 2. Visualization of daily numbers of new confirmed case along with date, as obvious change of rate occurred on January 29 as expected.

Table 2
Estimates and 95% confidence intervals of basic reproduction number in some selected provinces (or cities) of China, from Jan 21 to Jan 28, 2020.

	ML	EGR	SIR
Hubei	2.73 (2.06, 3.61)	3.63 (2.56, 5.22)	6.8 (5.1, 8.5)
Beijing	2.13 (1.53, 2.81)	1.74 (0.57, 3.26)	2.7 (1.0, 4.6)
Shanghai	2.24 (1.56, 2.95)	1.92 (0.88, 3.30)	2.8 (1.0, 4.5)
Guangdong	2.44 (1.82, 3.15)	2.77 (1.59, 4.40)	4.5 (3.2, 6.0)
Zhejiang	2.67 (2.02, 3.48)	3.03 (1.43, 5.26)	6.2 (3.9, 8.7)
Henan	2.38 (1.80, 3.10)	3.41 (1.45, 6.03)	7.8 (4.1, 12.8)

Table 3
Estimates and 95% confidence intervals of controlled reproduction number in some selected provinces (or cities) of China, from Jan 29 to Feb 5, 2020.

	ML	EGR	SIR
Hubei	2.34 (1.93, 2.83)	1.78 (1.40, 2.27)	3.3 (3.0, 3.7)
Beijing	1.96 (1.53, 2.45)	1.29 (0.33, 2.61)	2.3 (0.9, 4.0)
Shanghai	1.78 (1.39, 2.21)	0.77 (0.40, 1.18)	1.1 (0.4, 2.2)
Guangdong	1.88 (1.54, 2.25)	0.92 (0.52, 1.38)	1.1 (0.5, 2.0)
Zhejiang	1.56 (1.27, 1.86)	0.58 (0.21, 1.03)	0.8 (0.1, 1.6)
Henan	1.93 (1.58, 2.36)	1.08 (0.75, 1.47)	1.6 (1.0, 2.3)

grown significantly since late February, and posed a high risk of a second outbreak. Efforts are needed in order to end the current epidemic, especially improving quarantine measures at the border.

7. Discussion

In this study, we estimated the reproduction number of COVID-19 in China based on three approaches, namely Poisson likelihood-based method (ML), exponential growth rate-based method (EGR) and stochastic Susceptible-Infected-Removed dynamic model-based method (SIR). The EGR method can be only used at the early period of an epidemic, when the number of confirmed cases grows exponentially. The SIR method is not able to provide a time-varying reproduction number, and as existing literature on COVID-19 showed that SIR model is likely to overestimate the basic reproduction number since a large proportion of susceptible cases were isolated due to the strong control measures implemented (Wu et al., 2020, Tang et al., 2020a, Tang et al., 2020b). Hence, ML method is preferred in this study. Note that this study omits the effect of human mobility before the lockdown starting from January 23, 2020, which may cause an overestimation of the basic reproduction outside Hubei. A more realistic transmission model with spatial spread such as metapopulation disease model can be used but this is beyond the scope of this study (Balcan et al., 2010).

The dataset used in this study is based on the confirmed cases reported by the NHC of China. However, during our period of data collection, the official guidelines for diagnosis and treatment of COVID-2019 underwent six updates. The criteria of confirmation have evolved from the original “whole genome sequencing of the respiratory excretion” to “positive viral nucleic acid results by the RT-PCR of the respiratory excretion or viral gene sequence” in the 5th edition, and, as of now, the inclusion of positive nucleic acid results of the blood sample. The confirmation process has been simplified by the removal of the accreditation process by the national expert committee for confirmed cases. The fourth edition of the official guidelines for diagnosis and treatment granted the accrediting authority to municipalities (National Health Commission of, 2020). In addition, the medical resources in Hubei Province especially in Wuhan received a remarkable boost from early February 2020. All of these changes might result in a temporary surge of confirmed cases and lead to an overestimation of R_c during mid of February, especially in Hubei Province.

The current containment measures in China mainly aim to cut the transmission from human to human via respiratory droplets and have received a significant success by reducing the reproduction number below one in 30 days from the implementation of measures (namely February 19, 2020). However, other transmission pathways, including fecal-oral transmission and aerosol transmission, could not yet be

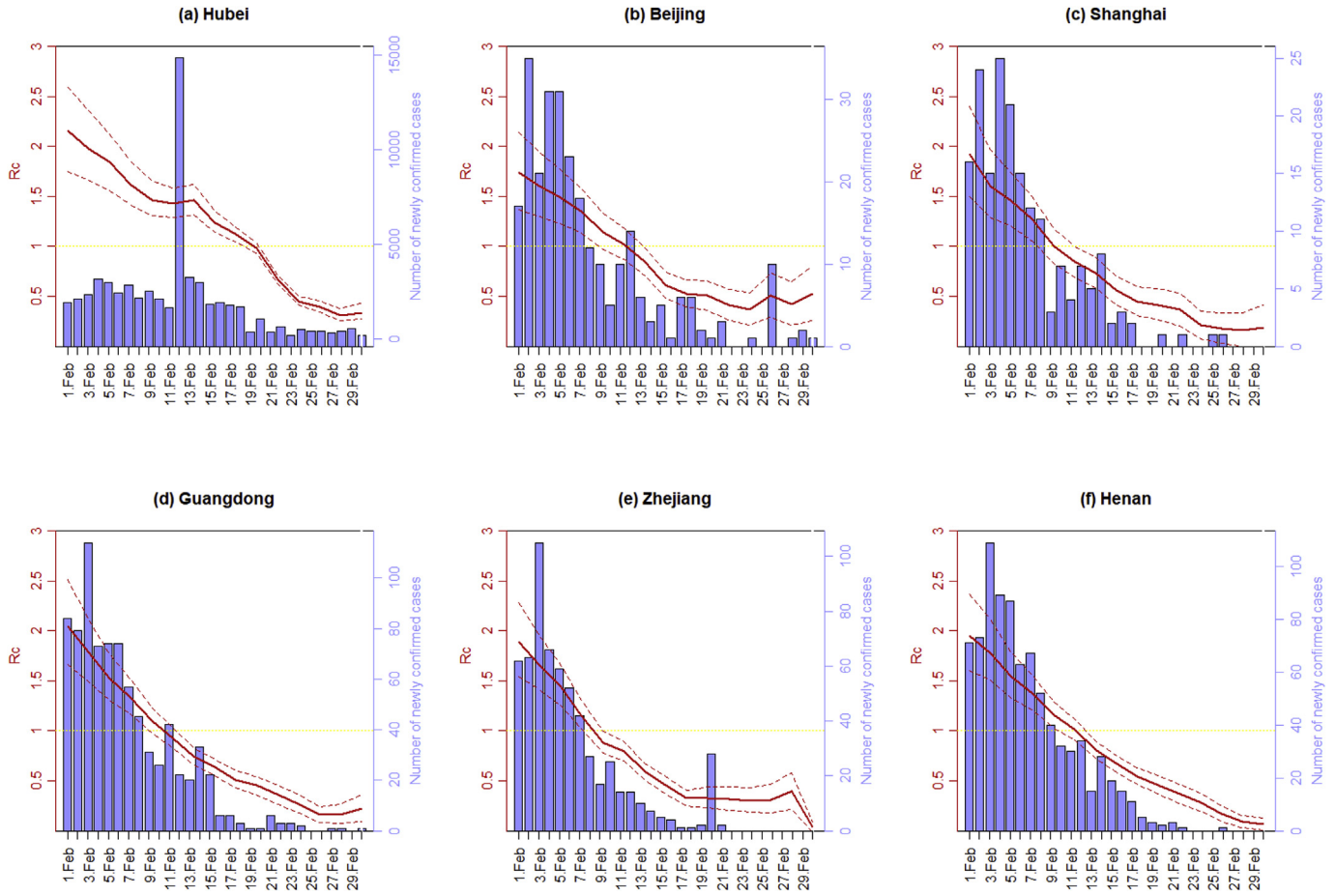

Fig. 3. The number of daily confirmed cases and estimated controlled reproduction number in (a) Hubei, (b) Beijing, (c) Shanghai, (d) Guangdong, (e) Zhejiang, and (f) Henan. The dashed line is the 95% confidence interval.

excluded based on current evidence. If other transmission mechanisms do exist, the R_c values could increase in the future unless further measures would intersect these transmission pathways. China NHC have started to report the number of asymptomatic cases since April 1. The asymptomatic cases are typically hard to detect. If asymptomatic cases are not isolated, there may be a second chance of outbreak. Note the incubation period, serial intervals and infectious period estimated in this study only apply to symptomatic patients. Furthermore, despite that the outbreak has been effectively contained currently in China, the number of imported cases will potentially grow with the development of global pandemic. As reported by China NHC, there are totally 788 confirmed imported cases and 4 domestic cases related to these imported cases during March 2020. In brief, the main goal of epidemic prevention in China has shifted from preventing the transmission of domestic confirmed cases to prevention of the spread of asymptomatic cases and foreign imported cases.

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Ethics committee approval

Not required.

Authors' contributions

Chong You: data collection, writing. Yuhao Deng: writing, data analysis. Wenjie Hu: data analysis. Jiarui Sun: data analysis. Qiushi Lin: data collection. Feng Zhou: data collection. Cheng Heng Pang: writing. Yuan Zhang: writing. Zhengchao Chen: wrting. Xiao-Hua Zhou: overall design.

Declaration of competing interest

We have no financial relationships (regardless of amount of compensation) with any entities. There is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijheh.2020.113555.

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Per- and polyfluoroalkyl substances in blood plasma – Results of the German Environmental Survey for children and adolescents 2014–2017 (GerES V)

Anja Duffek[a,*], André Conrad[b], Marike Kolossa-Gehring[b], Rosa Lange[b], Enrico Rucic[b], Christoph Schulte[c], Jörg Wellmitz[a]

[a] German Environment Agency, Laboratory for Water Analysis, Corrensplatz 1, Berlin, 14195, Germany
[b] German Environment Agency, Toxicology, Health-related Environmental Monitoring, Corrensplatz 1, Berlin, 14195, Germany
[c] German Environment Agency, Department Water and Soil, Wörlitzer Platz 1, Dessau-Roßlau, 06844, Germany

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ABSTRACT

The 5th cycle of the German Environmental Survey (GerES V) investigated the internal human exposure of children and adolescents aged 3–17 years in Germany to per- and polyfluoroalkyl substances (PFAS). The fieldwork of the population-representative GerES V was performed from 2014 to 2017.

In total, 1109 blood plasma samples were analysed for 12 PFAS including perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and perfluorohexane sulfonic acid (PFHxS). PFOS was quantified in all and PFOA in almost all samples, demonstrating ubiquitous exposure. The highest geometric mean concentrations measured were 2.49 ng/mL for PFOS, followed by PFOA (1.12 ng/mL) and PFHxS (0.36 ng/mL), while concentrations of other PFAS were found in much lower concentrations. The 95th percentile levels of PFOS and PFOA were 6.00 and 3.24 ng/mL, respectively.

The results document a still considerable exposure of the young generation to the phased out chemicals PFOS and PFOA. The observed exposure levels vary substantially between individuals and might be due to different multiple sources. The relative contribution of various exposure parameters such as diet or the specific use of consumer products need to be further explored. Although additional investigations on the time trend of human exposure are warranted, GerES V underlines the need for an effective and sustainable regulation of PFAS as a whole.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of more than 4700 substances (OECD/UNEP, 2018) known for making materials and products stain- and water-resistant - including packaging materials and textiles. PFAS have been widely used in various consumer products and industrial applications (Kantiani et al., 2010; Kedikoglou et al., 2019). Until the substitution by other PFAS, perfluorooctanoic acid (PFOA) was used to manufacture certain fluoropolymers like PTFE commonly used in many applications including aerospace, automotive, building and construction, healthcare and semiconductors (https://fluorocouncil.com/). Due to their wide use and the extreme chemical stability, PFAS have been detected in the natural environment since the early 2000s (e.g. Houde et al., 2006; Ahrens et al., 2014; Zareitalabad et al., 2013) and humans worldwide (e.g. Lau et al., 2007; Lindstrom et al., 2011; Schoeters et al., 2012). Along with air, contaminated drinking water and consumer products containing PFAS, diet is assumed to be a major exposure source (Jian et al., 2018). Once taken up by the human body, PFAS will bind especially to blood proteins and bioaccumulate (Cheng et al., 2018; Ng et al., 2014). The half life determined for the human body is 2.3–3.8 years for PFOA, and 4.8–5.4 years for perfluorooctane sulfonic acid (PFOS) (Bartell et al., 2010; Brede et al., 2010; Olsen et al., 2007). During pregnancy, certain maternal PFAS are transferred from the blood through the placenta resulting in prenatal exposure of the fetus (Gützkow et al., 2012). Breastfeeding has also been shown to be a substantial route of exposure in children (Mogensen et al., 2015).

Against this background, the health-relevance of human exposure to PFAS is of particular concern. Health effects of PFAS have been studied in a large number of toxicological studies especially in animals, and in epidemiology, mostly focusing on PFOS, PFOA, perfluorohexane sulfonic acid (PFHxS) and perfluorononanoic acid (PFNA). The Agency for Toxic Substances and Disease Registry has published a comprehensive draft toxicological profile (ATSDR, 2018). The available

* Corresponding author.
E-mail address: anja.duffek@uba.de (A. Duffek).

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epidemiological studies identified links between certain PFAS exposure and liver effects, changes in the lipid metabolism like increases in serum lipid levels, particularly total cholesterol and LDL cholesterol, endocrine effects, impairment of the immune response shown as decreased antibody responses to vaccines, developmental effects like small decreases in birth weight, and increased risk of decreased fertility determined as prolonged time to pregnancy. There is also epidemiological evidence for an association between serum PFOA and PFOS and pregnancy-induced hypertension and/or pre-eclampsia. A recent systematic review on health effects of PFAS exposure and childhood health outcome observed generally consistent evidence for PFAS' association with dyslipidemia, immunity including vaccine response and asthma, renal function, and age at menarche (Rappazzo et al., 2017).

Due to the risk for people working in the producing facilities, a major producer started voluntarily to phase-out PFSO-based materials in 2000. The regulation of PFAS was initiated in 2006, with several OECD reports, Canadian regulations, and especially the PFOA stewardship program of US-EPA with the intention to phase out PFOA and related PFAS from manufacturing and production by 2015. The program was supported by eight major fluorochemical companies (US-EPA, 2020b, US-EPA, 2020a). In 2009, PFOS has been added to the Stockholm Convention's list of globally restricted Persistent Organic Pollutants (POP) (Commission Regulation EU No 757/2010, 2010). In 2019 PFOA was also added, and a proposal for PFHxS is under consideration. Restrictions related to PFOA manufacture and placement on the market will enter into force in 2020 (REACH, Commission Regulation EU 2017/1000, 2017), others are in preparation. In the US, EPA released an ambitious action plan in February 2019 to manage all remaining PFAS (US-EPA, 2019).

According to Annex VI of the European Regulation on Classification, Labelling and Packaging (CLP, (EC) 1272/2008) which is based on the United Nations' Globally Harmonised System (GHS), PFOA, PFNA, PFDA, PFOS and certain of the salts are legally classified as carcinogenic Cat. 2 (suspected of causing cancer), and toxic for reproduction Cat. 1B (may damage the unborn child). In addition, IARC (2017) classified PFOA as possibly carcinogenic to humans (group 2B, testicular and liver cancer).

Despite these regulations and further mitigation measures, human exposure and especially exposure of children and adolescents to the group of PFAS is still of concern. In a joint approach PFAS were identified as top priority substances of concern in the frame of the European Human Biomonitoring Initiative HBM4EU (Ganzleben et al., 2017, www.hbm4eu.eu). Representative biomonitoring studies of PFAS concentrations in children are rare.

For these reasons, the German Environmental Survey (GerES) carried out from 2014 to 2017 (GerES V) investigated the exposure to PFAS of the German population of Children and Adolescents. GerES is a cross-sectional population study, repeatedly carried out in Germany since the mid-1980s. These surveys yield extensive data on the internal and external human exposure to environmental stressors and exposure factors (Schulz et al., 2007). This paper presents results on representative blood plasma concentrations on 12 PFAS. In addition, possible exposure sources, pathways and routes linked to the children's internal exposure are discussed.

2. Materials and methods

2.1. Study design and sampling

2294 children aged 3–17 years from 167 sampling locations in Germany took part in GerES V. This survey comprised Computer Assisted Personal Interview (CAPI) and self-administered questionnaires to collect information on exposure factors, such as food consumption, exposure-relevant behaviors and various aspects of the residential environment (Schulz et al., 2017). GerES V was carried out as a subsample of and in close co-operation with the second follow-up to the German Health Interview and Examination Survey for Children and Adolescents (KiGGS Wave 2), performed by the Robert Koch Institute. The Ethics Committee of the Hannover Medical School approved KiGGS Wave 2 (No. 2275–2014) (Mauz et al., 2017). GerES V received approval of the Ethics Committee of the Berlin Chamber of Physicians (No. Eth-14/14).

This co-operation allows for combining data on health and sociodemographics (KiGGS Wave 2) and exposure (GerES V) for participants of both studies. PFAS have been analysed in 1,109 blood plasma samples of GerES V participants that have been collected by the Robert Koch Institute during KiGGS Wave 2. In addition, drinking water samples have been collected in the households of GerES V participants. A limited subset of 62 drinking water samples has been analysed for PFAS. These have been selected from all available drinking water samples taking into account variation in blood plasma concentrations aiming for including participants with low, medium and high internal PFAS exposures. Therefore, drinking water samples have been divided into three subgroups based on PFOA and PFOS in plasma of the respective participant. These categories represented low (below or near the l), medium (near the geometric mean) and high (highest including maximum concentrations) PFOA and PFOS plasma levels. For each category approximately 20 drinking water samples have been selected.

2.2. Chemical analysis

Drinking water samples have been analysed for PFAS according to DIN 38407–42. The analysis of the blood plasma samples was performed by liquid chromatography tandem mass spectrometry (LC-MS/MS) after protein precipitation with acetonitrile and membrane filtration.

The analytes and mass-labelled internal standards according to Table 1 were obtained from Wellington Laboratories as mixtures in

Table 1
List of substances.

Analyte	Abbreviation	CAS-No.	Internal Standard
Perfluoro-n-butanoic acid	**PFBA**	375-22-4	Perfluoro-n-[$^{13}C_4$]butanoic acid
Perfluoro-n-pentanoic acid	**PFPeA**	2706-90-3	Perfluoro-n-[1,2–$^{13}C_2$]hexanoic acid
Perfluoro-n-hexanoic acid	**PFHxA**	307-24-4	Perfluoro-n-[1,2–$^{13}C_2$]hexanoic acid
Perfluoro-n-heptanoic acid	**PFHpA**	375-85-9	Perfluoro-n-[1,2,3,4–$^{13}C_4$]octanoic acid
Perfluoro-n-octanoic acid	**PFOA**	335-67-1	Perfluoro-n-[1,2,3,4–$^{13}C_4$]octanoic acid
Perfluoro-n-nonanoic acid	**PFNA**	375-95-1	Perfluoro-n-[1,2,3,4,5–$^{13}C_5$]nonanoic acid
Perfluoro-n-decanoic acid	**PFDA**	335-76-2	Perfluoro-n-[1,2–$^{13}C_2$]decanoic acid
Perfluoro-n-undecanoic acid	**PFUdA**	2058-94-8	Perfluoro-n-[1,2–$^{13}C_2$]undecanoic acid
Perfluoro-n-dodecanoic acid	**PFDoA**	307-55-1	Perfluoro-n-[1,2–$^{13}C_2$]dodecanoic acid
Perfluoro-1-butanesulfonate	**PFBS**	29,420-49-3	Perfluoro-n-[1,2–$^{13}C_2$]hexanoic acid
Perfluoro-1-hexanesulfonate (linear isomer)	**PFHxS**	108,427-53-8	Natrium-perfluoro-1-hexane[$^{18}O_2$]sulfonate
Perfluoro-1-octanesulfonate (linear isomer)[a]	**PFOS**	45,298-90-6	Natrium-perfluor-1-[1,2,3,4–$^{13}C_4$]octanesulfonate

[a] Branched isomers were evaluated with the linear isomer.

methanol (PFAC-MXB, MPFAC-MXA, 2000 ng/mL each). HPLC-grade methanol and HPLC-grade water was purchased from Fluka, HPLC-grade acetonitrile, ammonium acetate (> 98%) and acetic acid (100%) from Merck.

All parts used in sample preparation and measurement were made of polypropylene. 200 µL water, 200 µL blood plasma, 50 µL internal standard solution (containing 40 ng/mL of each component) and 550 µL acetonitrile were added consecutively into a 1.5 mL micro tube, then vortex-mixed and centrifuged at 2000 g for 10 min. The supernatant was membrane filtered (0.2 µm) into a HPLC vial and analysed by LC-MS/MS.

Samples were analysed using a Prominence UFLC liquid chromatograph (Shimadzu) connected to an API 4000 QTrap mass spectrometer (AB SCIEX) operated in negative ion electrospray ionization (ESI) mode. 20 µl of the sample solution were injected on a ZORBAX Eclipse XDB-C18 column, 150 mm × 2.1 mm, 3.5 µm particle size (Agilent). The mobile phases were (A) water with 5 mmol/L ammonium acetate and (B) methanol with 0.05% acetic acid with a flow of 0.2 mL/min at 40 °C. The separation started with 35% (B), changed to 95% (B) in 20 min and was kept constant for another 10 min.

Quantification was conducted using precursor-product ion multiple reaction monitoring (MRM) transitions. For all precursor compounds an additional product ion was recorded for confirmation, where possible.

PFAS concentrations were calculated using an internal standard approach by the measurement of eight calibration solutions with concentrations between 0.1 and 5.0 ng/mL and with linear correlation coefficients $r > 0.98$.

Limits of quantification (LOQ) in plasma (Table 2) were verified prior to the analysis by multiple measurement of spiked matrix samples at the concentration levels of LOQ according to ISO/TS 13530 Annex A.

2.3. Quality assurance

Internal quality control was performed by analyzing a blank sample, two control samples with known concentrations (ring test samples from AMAP, see below) and a random duplicate analysis of one sample in every analytical batch of about 20 samples. Blank values were always below the LOQs. The control samples ($n = 123$) showed relative standard deviations between 9 and 17% depending on the analyte and arithmetic means in the range for a z-score < 2. Duplicates had relative differences of smaller 20% for almost all samples well above LOQ.

External quality control consisted of three participations per year in ring tests for “Persistent Organic Pollutants in Human Serum” in the Arctic Monitoring and Assessment Program (AMAP; Quebec, Canada). The z-scores achieved for the six PFAS offered in the program (PFHxA, PFOA, PFNA, PFUdA, PFHxS and PFOS) were always < 2.

2.4. Statistical analysis

The drawing of sample points was biased in favor of former East Germany, for a higher precision in estimates for this region. Moreover, an oversampling was performed for participants without German nationality to compensate for the expected higher share of quality neutral losses and the lower response rate in this segment of the population (Kamtsiuris et al., 2007; Hoffmann et al., 2018). In order to correcting for design and non-response bias and for ensuring the representativeness according to age, sex, former East/West Germany as well as community size, GerES data are weighted on the basis of population data provided by the German Federal Statistical Office as of December 2013 for all statistical analyses (Hoffmann et al., 2018).

Characteristics of the frequency distribution of the measured concentrations were calculated (10th, 90th, 95th, and 98th percentiles, 50th percentile (median), maximum value, arithmetic mean (AM), and geometric mean (GM) and its 95% confidence interval). All concentrations below their respective LOQ were set to LOQ/2 for data treatment. All statistical calculations were performed with SPSS for Windows, Versions 20 and 25. Statistical characteristics are described for the total sample, and stratified by selected variables that are suspected to be associated with PFAS concentrations. Statistical significance of differences in geometric means between subgroups was tested by one-way ANOVA, if at least 50% of measurements were at or above LOQ. The socioeconomic status (SES) was derived in KiGGS Wave 2 based on parental levels of education, occupational status and income (Lampert et al., 2018).

3. Results and discussion

We analysed 12 PFAS in 1109 blood plasma samples from children and adolescents, of which 543 were females and 566 males. PFOS was quantified in all and PFOA in almost all samples, demonstrating ubiquitous exposure (Table 2). Similar to results of prior studies, PFOS was the substance with the highest plasma concentrations showing a geometric mean of 2.49 ng/mL, followed by PFOA (1.12 ng/mL) and PFHxS (0.36 ng/mL), while PFNA and PFDA showed substantially lower concentrations. The concentrations of the other PFAS were mostly below the LOQ of 1.0 and 0.25 ng/mL, respectively.

For further statistical analysis, we considered only PFOS, PFOA and PFHxS for which at least 50% of concentrations were at or above the LOQ. The distributions of the concentrations for these three compounds were substantially right-skewed. All three compounds correlated statistically significant with each other. Correlation was strongest for PFOS and PFHxS (Spearman $\rho = 0.511$). In Table S1 – S3 in the supplemental file descriptive statistics using all considered stratification parameters are summarized for these three compounds measured in blood plasma.

In Fig. 1 geometric mean concentrations in plasma are displayed for

Table 2
Summary of measured plasma concentrations [ng/mL].

Analyte	LOQ	N < LOQ	% ≥ LOQ	P10	P50	P90	P95	P98	MAX	AM	GM	CI GM
PFBA	1.0	1109	0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	–
PFPeA	0.25	1109	0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	–
PFHxA	0.25	1105	0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.58	< LOQ	< LOQ	–
PFHpA	0.25	1101	1	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.67	< LOQ	< LOQ	–
PFOA	0.50	157	86	< LOQ	1.27	2.70	3.24	3.78	6.33	1.427	1.124	1.075–1.176
PFNA	0.50	997	10	< LOQ	< LOQ	0.56	0.66	0.88	3.54	< LOQ	< LOQ	–
PFDA	0.25	1000	10	< LOQ	< LOQ	< LOQ	0.35	0.49	3.00	< LOQ	< LOQ	–
PFUdA	0.25	1099	1	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.78	< LOQ	< LOQ	–
PFDoA	0.25	1108	0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.96	< LOQ	< LOQ	–
PFBS	0.25	1109	0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	–
PFHxS	0.25	290	74	< LOQ	0.38	0.84	1.26	2.02	34.1	0.542	0.355	0.339–0.372
PFOS	0.25	0	100	1.41	2.41	4.43	6.00	8.09	129	3.019	2.487	2.413–2.563

N = 1109; LOQ = Limit of Quantification; PX = Xth Percentile; AM = Arithmetic Mean; GM = Geometric Mean; CI GM = 95% Confidence Interval of Geometric Mean (only calculated if GM ≥ LOQ).

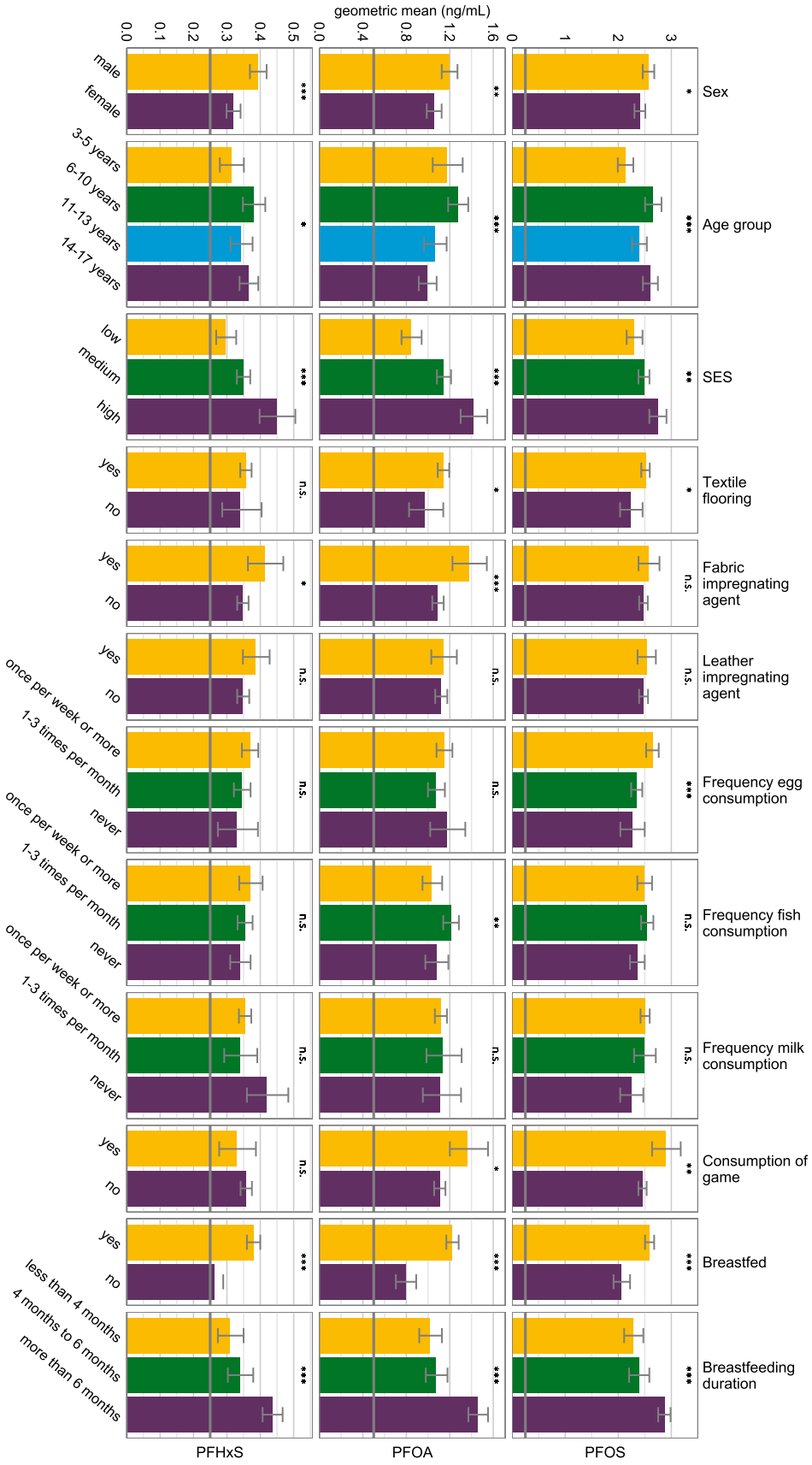

Fig. 1. Geometric Mean of relevant variables for PFOS, PFOA, PFHxS. Bar plots represent the Geometric Mean (GM) and its confidence interval. The horizontal grey lines represent the LOQ. Asterisks denote the significance level for differences between groups (* = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; n. s. = not significant; one-way ANOVA).

PFOS, PFOA and PFHxS stratified by twelve relevant aspects of exposure (sex, age group, SES, textile flooring at home, use of impregnating agents, food consumption and breastfeeding). The geometric mean concentrations show statistically significant differences by sex. Statistically significant lower concentrations in girls' plasma samples might be partly related due to elimination via menstruation (Wong et al., 2014), physiological difference such as plasma protein levels and/or differences in behaviours including nutritional habits. Highest concentrations for PFOS, PFOA and PFHxS were observed in the age group 6–10 years, whereas for the youngest age group of 3–5 years lowest concentrations for PFOS have been detected. In the U.S. National Health and Nutrition Examination Survey (NHANES) 2013–2014, age-dependency was observed for PFHxS and PFOA, and differences in sex for PFHxS. Multivariate regression analysis revealed that PFOA levels were statistically significantly higher in older children (6–11 years), but only in boys and not in girls (Ye et al., 2018). Age-related differences are not consistent among different studies. A positive trend between PFOS and PFHxS concentration and age was observed in an Australian study, whereas in two Asian studies PFOS and PFHxS concentrations were higher in younger children (Kim et al., 2014; Toms et al., 2019; Zhang et al., 2017). In First Nation children from a Canadian study PFOS and PFOA serum concentrations were statistically significantly higher in the 6–11 year age group compared to the younger or older age groups (Caron-Beaudoin et al., 2019). Differences between males and females were not observed in two Asian studies (Kim et al., 2014; Zhang et al., 2017). The youngest age-group of GerES V participants has been born after the regulation of most uses of PFOS in the EU. This was followed by a slow decrease of the environmental concentrations and can therefore be assumed to be less exposed to PFOS as also observed in several other studies (Land et al., 2018). Moreover, older children might have been exposed to a higher extent and over a longer period of time. On the other hand, PFAS exposure normally decrease with age due to growth dilution (Winkens et al., 2017; Koponen et al., 2018). Against this background, a detailed regression analysis of GerES V data is planned in order to further elucidate associations with age and other exposure determinants.

As shown in Tables S1–S3 (supplemental file), exposure regarding PFHxS is statistically significantly higher in former West Germany with a GM of 0.37 ng/mL compared to 0.29 ng/mL in former East Germany. Reasons for this regional difference in internal exposure, such as a possible link to local sources, nutritional habits or the use of products containing PFAS, will be subject to further research.

Frequently associated sources of human exposure to PFAS are food, drinking water, house dust, outdoor and indoor air (Winkens et al., 2017; Colleen et al., 2017; Sunderland et al., 2019). As a first step in GerES V data analysis, human dietary exposure to PFOS, PFOA, and PFHxS was stratified by self-reported consumption frequencies of eggs, fish, milk, and game in the last four weeks before sampling (Fig. 1). No consistent pattern for these foods emerged. However, children show lower PFAS concentrations if no fish was consumed. PFOS in plasma is positively associated with the consumption of eggs and game. Also PFOA levels are statistically significantly higher in participants with reported consumption of game. This is in line with measurements of PFAS in roe deer liver samples as well as wild boar liver and muscle tissue samples from Germany, where accumulation of PFOS and PFOA could be detected in almost all samples independently from the sampling site and the land use (agricultural vs. urban-industrial, Stahl et al., 2012; Falk et al., 2019).

Breastfeeding is statistically significantly associated with the internal PFAS exposure of GerES V participants. Concentrations of PFOS, PFOA and PFHxS were higher in blood plasma of participants that have been breastfed. In addition, the reported duration of breastfeeding shows a strong positive association. This is in line with results of other studies (Kingsley et al., 2018; Mogensen et al., 2015). Exposure via breastfeeding may even result in higher total concentrations in toddlers (1–3 years) than in their mothers at delivery as shown by Papadopoulou et al. (2016).

Higher blood levels of PFAS can also be a result of longer and more frequent use of consumer products. During their lifetime, consumer products release PFAS via abrasion and volatilisation which can enter the human body via ingestion of dust, inhalation of air, or through the skin after direct product contact or due to hand/object to mouth contact (Winkens et al., 2017). Especially for children below the age of 11 years dust ingestion and hand/object mouthing activities might be specific product-related pathways in addition to food intake and inhalation (Winkens et al., 2018; Trudel et al., 2008). PFAS have been detected in numerous consumer products including treated textiles, carpets, cleaning and impregnating agents (Fiedler, 2010; Fareau et al., 2017), leather samples, baking and sandwich papers, paper baking forms and ski waxes (Kotthoff et al., 2015) as well as in cosmetic products (Schultes et al., 2018). Recently, Boronow et al., (2019) showed that flossing with PTFE-based dental floss could contribute to an individual's body burden of PFAS. In our study, the application of textile impregnating agents as well as textile flooring at home are positively associated with higher blood concentrations of PFAS (Fig. 1 and Tables S1–S3, supplemental file).

Our first statistical analysis gives no deeper insight in exposure pathways related to the presence and quantity of PFAS-containing consumer products and the frequency of use. Our questionnaire did not capture lifestyle habits such as travel and leisure activities which might be also connected to the specific use of all-weather clothing (Gremmel et al., 2016) or hunting, camping, leisure and sports equipment containing PFAS. This use may also contribute to the overall PFAS exposure.

Differences in living conditions, lifestyle and dietary patterns might be the underlying reason for our observation that a higher SES is associated to a higher exposure to PFAS (Fig. 1 and Tables S1–S3, supplemental file). A similar association of PFAS levels with SES has also be reported by Buekers et al., (2018) based on a meta-analysis including five studies. The specific causes of SES-related differences in PFAS concentrations remained unclear. Recently, Montazeri et al., (2019) published the results of a study using six mostly urban European birth cohorts observing higher childhood concentrations of PFAS in groups with a higher socio-economic position (maternal education, employment status and family affluence scale). In the US, national human biomonitoring data (NHANES, 2001–2010) indicated that increased exposure of adults to certain chemicals such as PFAS is associated with increased SES (Tyrell et al., 2013). A more detailed analysis of all our data generated through questionnaires will help to understand the effect of SES and associated factors to the PFAS exposure. In addition to the use of PFAS containing consumer products, this association might be related to differences in breastfeeding or the age of the mothers when giving birth. From other studies it is known that also the age of mothers is associated with the blood plasma levels of PFAS as observed for Danish school children (Mørck et al., 2015) or Canadian children (Workmann et al., 2019).

Our first exploratory assessment of the relevance of drinking water intake show background levels of PFAS in drinking water comparable to the results published by Lange et al., (2007). Most concentrations were below the LOQ of 1 and 3 ng/L, respectively. Maximum drinking water levels reached about 10 ng/L for PFOS and PFOA. In this comparatively small subset of GerES V data, no statistically significant correlation between concentrations in drinking water and blood plasma samples was found. This indicates that the studied population was not exposed to substantially contaminated drinking water as also observed by Domingo et al. (2019) for the regular consumers of municipal/tap water. Currently, more drinking water samples are being analysed to further elucidate the relevance of exposure via drinking water which can be of high importance in case of local contamination events.

3.1. Comparison with other studies

The blood plasma levels of PFAS in children in our study are similar to what was found recently in a regional study of children, 6–11 years of age living in Catania city, Sicily, Italy, except for median concentrations of PFOA which were 1.46 times higher in Italian children (Ledda et al., 2018). When comparing to regional studies from outside Europe (sampling between 2012 and 2017), mean PFOS, PFOA and PFHxS levels were lower in GerES V children compared to children in China and South Korea (Kim et al., 2014; Zhang et al., 2017). Total mean serum levels of PFOS are similar, whereas PFOA and PFHxS concentrations are lower in our study when compared to Australian children (Toms et al., 2019). First Nation children and adolescents from Canada, though, showed lower GM serum concentrations of PFOS and PFOA compared to GerES participants, while GM concentrations of PFHxS were comparable (Caron-Beaudoin et al., 2019). However, sample composition and size, year of sampling and age range varied and comparisons need to be done with reasonable care.

Therefore, other studies taken into account for comparison with our data were selected based on the following criteria: similar sampling period (within the years 2014–2017), similar age group (3–17 years) and national representativity of the studies. The NHANES study quantified serum concentrations of fourteen different PFAS in a nationally representative subsample of 639 3–11 year old children in the period of 2013–2014 (Ye et al., 2018). When comparing GM concentrations all values for PFAS measured were higher in US children than in German children. In particular, PFOS and PFOA concentrations for 3–11 year old NHANES participants were approximately 1.60 and 1.45 times higher than in GerES V. A more pronounced difference was observed for PFHxS, where the GM concentrations were approximately 2.4 times higher in the blood of the US children (Table 3). According to our data, German children are less exposed to the three above mentioned perfluorinated compounds than US children. The fifth cycle of the Canadian Health Measure Survey, conducted from 2016 to 2017 measured nine different PFAS in plasma samples of the Canadian population aged 3–79 years (Health Canada, 2019). Canadian children and adolescents had higher GM concentrations of PFHxS and lower GM concentrations of PFOS when compared to GerES V for all three age groups (3–5, 6–11 and 12–17/19 years). PFOA GM concentrations were similar between both populations, with slightly higher GM concentrations for the age group 3–5 year (1.3 times higher) and 0.5 times lower GM concentrations for the age group 12–19 years observed in CHMS (see Table 3 for more details). For all three substances and age groups, levels of the CHMS were lower than in NHANES.

In Europe, one other study were identified, investigating the levels of PFAS in children and adolescents on national level. In a sub-sample of the national representative French national programme (Health Study on Environment, Biomonitoring, Physical Activity and Nutrition, ESTEBAN study), conducted from 2014 to 2016, seventeen perfluorinated compounds were measured in 249 serum samples of children aged 6–17 years (Santé publique France, 2019). The GM concentration for French children and adolescents are higher for PFOA (1.4 times) and for PFHxS (2 times), but slightly lower for PFOS (0.14 times) compared to German children in the same age group (see Table 3).

In summary, the GM concentrations of PFOA and PFHxS were lower in German children and adolescents compared to other national studies, whereas for PFOS GM concentrations, higher values were observed in German children than in French or Canadian children and adolescents. Only the GM concentrations measured in U.S. children were higher compared to German children.

Compared to the decreasing plasma levels over time in young German adults (Schröter-Kermani et al., 2013; Yeung et al., 2013) mainly lower concentrations have been detected in our study. Although, this might be related to implemented phase-outs, our findings reflect long-term exposure. A pronounced decline in the concentrations of PFOS and PFOA in humans has been observed due to the effects of regulatory actions on EU-level and global scale as concluded by Land et al., (2018) based on a systematic review of human biomonitoring studies. Contrarily, the exposure to longer-chained perfluoroalkyl carboxylic acids (PFCAs) with 9–14 carbon atoms is generally increasing. In addition, indirect exposure to precursors and subsequent biotransformation to PFOS and PFCAs has to be taken into account (Gebbink et al., 2015) as well as the mixture exposure to PFAS. The substitution of regulated substances lead also to an increased diversity of molecules with ether functions, single chlorinated instead of fluorinated positions or branched molecules (Wang et al., 2017). Therefore, continued temporal trend monitoring is needed to measure the performance of past and continuing regulatory mitigation measures.

3.2. Health risk assessment

The German Human Biomonitoring Commission has derived health-related guidance values (HBM values) for the evaluation of internal exposures to PFOS or PFOA, respectively. The effects considered were reduced birth weight and developmental toxic effects, reduced fertility, reduced antibody formation after vaccination, increased cholesterol concentrations (LDL and total), and type II diabetes. The HBM-I-values were defined as 2 ng PFOA/mL and 5 ng PFOS/mL blood plasma. For women at childbearing age, the HBM-II-values are 5 ng PFOA/mL and 10 ng PFOS/mL. For all other population groups, the HBM-II-values are 10 ng PFOA/mL and 20 ng PFOS/mL (German Human Biomonitoring Commission, 2018, 2020). The HBM-I-value represents the concentration of a substance in human biological material below which – according to current knowledge – there is no risk for adverse health effects to be expected. Plasma concentrations above the HBM-I-value but below the HBM-II-value indicate an exposure at which according to the current knowledge effects can no longer be excluded with sufficient certainty. Above the HBM-II-value there is an increasing probability for effects. Both, the HBM-I- and the HBM-II-value for PFOA and PFOS are based on the assessment of the population-related risk of changes in the selected effect indicators. Thus, the HBM values should be carefully addressed at the individual level. In any case possible effects should always be evaluated together with individual preconditions and also in the context of other risk factors, e.g. lifestyle.

21.1% of the participants investigated for PFOA-levels in blood plasma are at or above the HBM-I-value. All PFOA levels are below the HBM-II-value. For PFOS, 7.1% of concentrations are at or above the HBM-I-value while staying below the HBM-II-value. For 0.2% of participants, PFOS plasma concentrations are even at or above the HBM-II-value. This observation is in line with the Scientific Opinion of the European Food Safety Authority (EFSA) concluding that PFOS and PFOA exposure of a considerable part of the population exceeds the proposed tolerable weekly intakes (TWI) for food (Knutsen et al., 2018).

4. Conclusions

The German Environmental Survey conducted between 2014 and 2017 provides for the first time population representative data on the PFAS exposure of German children and adolescents (3–17 years). The results show that the young generation in Germany is widely exposed to PFOS, PFOA and PFHxS. Our study indicates that multiple exposure sources and routes might be relevant for children and adolescents in Germany. Building on the present results, a detailed multivariate data evaluation will be carried out to further investigate associations with living conditions and behaviors such as food and drinking water consumption. After identification of highly exposed sub-populations and the most relevant sources policy recommendations will be derived.

As people are usually exposed to a mixture of PFAS and short-chain PFAS are increasingly used as replacements for long-chain PFAS, human exposure to this diverse group of substances is of ongoing concern. For a better protection of human health and for a safer future of our children, a continued monitoring of internal and external

Table 3
Comparison of levels of PFOS, PFOA and PFHxS in plasma and serum samples (ng/mL) measured in different nationally-representative studies.

Study/region	Study type	Sample	Year	Sample: age and size (N)	50th percentile	95th percentile	Geometric mean
PFOS[ab]							
GerES V Germany (this study)	national	plasma	2014–2017	3–11 years, N = 631	2.34	6.43	2.43
			2014–2017	3–5 years, N = 196	2.07	6.30	2.13
			2014–2017	6–11 years, N = 434	2.41	6.68	2.58
			2014–2017	6–17 years, N = 912	2.5	5.99	2.57
			2014–2017	12–17 years, N = 478	2.6	5.62	2.56
NHANES, USA (n-PFOS + branched PFOS)	national	serum	2013–2014	3–11 years, N = 639	3.75	11.0	3.88
			2013–2014	3–5 years, N = 181	3.41	8.82	3.38
			2013–2014	6–11 years, N = 458	4.02	12.4	4.15
CHMS, Canada	national	plasma	2016–2017	3–5 years, N = 491	1.6	5.5[f]	1.7
			2016–2017	6–11 years, N = 520	1.6	4.2	1.7
			2016–2017	12–19 years, N = 526	1.8	3.9	1.9
ESTEBAN, France	national	serum	2014–2016	6–17 years, N = 249	2.00	6.12	2.22
PFOA[cd]							
GerES V Germany (this study)	national	plasma	2014–2017	3–11 years, N = 631	1.37	3.50	1.25
			2014–2017	3–5 years, N = 196	1.34	4.02	1.17
			2014–2017	6–11 years, N = 434	1.37	3.43	1.29
			2014–2017	6–17 years, N = 912	1.24	3.14	1.11
			2014–2017	12–17 years, N = 478	1.15	2.82	0.98
NHANES, USA (n-PFOA)	national	serum	2013–2014	3–11 years, N = 639	1.82	4.07	1.81
			2013–2014	3–5 years, N = 181	1.72	5.32	1.87
			2013–2014	6–11 years, N = 458	1.84	3.77	1.78
CHMS, Canada	national	plasma	2016–2017	3–5 years, N = 491	1.3	3.6	1.5
			2016–2017	6–11 years, N = 520	1.2	2.4	1.3
			2016–2017	12–19 years, N = 507	1.0	1.9	1.1
ESTEBAN, France	national	serum	2014–2016	6–17 years, N = 249	1.54	2.76	1.56
PFHxS[e]							
GerES V Germany (this study)	national	plasma	2014–2017	3–11 years, N = 631	0.38	1.47	0.35
			2014–2017	3–5 years, N = 196	0.35	1.83	0.31
			2014–2017	6–11 years, N = 434	0.39	1.31	0.37
			2014–2017	6–17 years, N = 912	0.39	1.19	0.36
			2014–2017	12–17 years, N = 478	0.39	1.16	0.36
NHANES, USA	national	serum	2013–2014	3–11 years, N = 639	0.81	2.14	0.84
			2013–2014	3–5 years, N = 181	0.74	1.55	0.72
			2013–2014	6–11 years, N = 458	0.85	2.33	0.91
CHMS, Canada	national	plasma	2016–2017	3–5 years, N = 491	0.54	3.1[f]	0.61
			2016–2017	6–11 years, N = 520	0.49	–[g]	0.59
			2016–2017	12–19 years, N = 527	0.58	3.6	0.69
ESTEBAN, France	national	serum	2014–2016	6–17 years, N = 249	0.72	2.25	0.79

[a] PFOS (n-PFOS + monomethyl branched isomers): LOQ is 0.25 ng/mL, and LOD is 0.1 ng/mL in GerES V; LOD is 0.1 ng/mL in NHANES.
[b] PFOS: no specification whether single isomers were measured were given for the CHMS and ESTEBAN study. LOD is 0.43 ng/mL in CHMS. In ESTEBAN LOD is 0.03 ng/mL and LOQ is 0.1 ng/mL.
[c] PFOA (n-PFOA): LOQ is 0.5 ng/mL, and LOD is 0.2 ng/mL in GerES V; LOD is 0.1 ng/mL in NHANES.
[d] PFOA: no specification on the isomer measured for the CHMS and ESTEBAN study. LOD is 0.066 ng/mL in CHMS. In ESTEBAN LOD is 0.02 ng/mL and LOQ is 0.05 ng/mL.
[e] PFHxS: LOQ is 0.25 ng/mL, and LOD is 0.1 ng/mL in GerES V; LOD is 0.1 ng/mL in NHANES; LOD is 0.063 ng/mL in CHMS; LOD is 0.05 ng/mL and LOQ is 0.19 ng/mL in ESTEBAN.
[f] According to the CHMS Report, data shall be used with caution.
[g] According to the CHMS Report, the data is too unreliable to publish.

exposure (from multiple pathways) is needed to evaluate regulatory mitigation measures and support future risk assessment and decision-making.

Declaration of competing interest

None.

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Appendix A. Supplementary data

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Urine glyphosate level as a quantitative biomarker of oral exposure

Otmar Zoller*, Peter Rhyn, Jürg A. Zarn, Vincent Dudler

Federal Food Safety and Veterinary Office (FSVO), Risk Assessment Division, Schwarzenburgstrasse 155, 3003, Bern, Switzerland

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ABSTRACT

Background: Since the classification of glyphosate as a Group 2A substance "probably carcinogenic to humans" by the IARC in 2015, human health concerns have been raised regarding the exposure of operators, bystanders, and consumers. Urine measurement studies have been conducted, but since toxicokinetic data on glyphosate in humans is lacking, a meaningful interpretation of this data regarding exposure is not possible.

Objective: This study aims to determine the fraction of glyphosate and AMPA excretion in urine after consuming ordinary food with glyphosate residue, to estimate dietary glyphosate intake.

Methods: Twelve participants consumed a test meal with a known amount of glyphosate residue and a small amount of AMPA. Urinary excretion was examined for the next 48 h.

Results: Only 1% of the glyphosate dose was excreted in urine. The urinary data indicated the elimination half-life was 9 h. For AMPA, 23% of the dose was excreted in urine, assuming that no metabolism of glyphosate to AMPA occurred. If all of the excreted AMPA was a glyphosate metabolite, this corresponds to 0.3% of the glyphosate dose on a molar basis.

Conclusion: This study provides a basis for estimating oral glyphosate intake using urinary biomonitoring data.

1. Introduction

N-(phosphonomethyl)-glycine (glyphosate, CAS RN® 1071-83-6) is a systemic herbicide that competitively inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, blocking plant biosynthesis of aromatic amino acids.

The main environmental biodegradation product of glyphosate is aminomethylphosphonic acid (AMPA, CAS RN® 1066-51-9). Glyphosate is poorly metabolised in animals and plants, and AMPA is the main metabolite (EFSA, 2015).

Human health concerns have been raised regarding the exposure of operators, bystanders, and residents to glyphosate-based pesticides during spraying and consumer exposure to glyphosate residues in food crops (Myers et al., 2016). The International Agency for Research on Cancer (IARC, 2015) recently concluded that "Glyphosate is probably carcinogenic to humans (Group 2A)". According to the European Chemicals Agency's (ECHA) Committee for Risk Assessment (RAC), glyphosate does not meet the classification criteria as a carcinogen, a mutagen, or for reproductive toxicity (ECHA, 2017). The reasons for the diverging conclusions regarding glyphosate's carcinogenic hazard were recently reviewed (Portier et al., 2016; Tarazona et al., 2017). The European Food Safety Authority (EFSA) recommended an acceptable daily intake (ADI) and an acute reference dose (ARfD) of 0.5 mg kg^{-1} body weight (bw) per day (EFSA, 2015). The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) recommended an ADI of 0–1 mg kg^{-1} bw per day and considered an ARfD unnecessary due to glyphosate's low acute toxicity (FAO/WHO, 2017). Both bodies concluded that glyphosate and AMPA have similar toxic potency and the maximum residue levels set for glyphosate and expected consumer exposure to food crop residues are safe. Swiss consumer exposure to glyphosate was demonstrated in a limited survey (RTS, 2015). In approximately 40% of 40 participants, glyphosate was detected in the urine at levels of 0.1–1.5 ng mL^{-1}. Analyses in Germany confirmed low levels of glyphosate and its degradation product AMPA in consumers' urine (Conrad et al., 2017). In approximately 30–50% of the urine samples, glyphosate and AMPA were above the LOQ of 0.1 ng mL^{-1}. The median levels of both glyphosate and AMPA were below the LOQ or up to 0.2 ng mL^{-1} and the maximum values were 2.8 and 1.9 ng mL^{-1}, respectively. The highest quantification rates and highest values were reported in 2012 and 2013. There was a moderate correlation between glyphosate values and AMPA. Based on this urinary data, glyphosate exposure in Switzerland and Germany appear to be similar.

An analysis of glyphosate and AMPA in food commodities in Switzerland, identified cereals and pulses (e.g., beans, lentils, chickpeas) as the main contributors for consumers' exposure (Zoller et al., 2018). Although only approximately 20% of an orally administered

* Corresponding author.
E-mail address: otmar.zoller@blv.admin.ch (O. Zoller).

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Abbreviations

ADI	acceptable daily intake
AMPA	aminomethylphosphonic acid
ARfD	acute reference dose
bw	body weight
E_{max}	maximum excretion rate
F	female
LOD	limit of detection
LOQ	limit of quantification
M	male
T_{max}	time to excretion peak

glyphosate dose is absorbed in laboratory animals (EFSA, 2015), urine levels of glyphosate are considered to be good exposure markers because glyphosate does not bioaccumulate and is poorly metabolised and exclusively to AMPA. Based on rat absorption rates, a preliminary dietary risk assessment of glyphosate and AMPA levels was conducted and no unacceptable health risk was identified (Zoller et al., 2018). The validity of this dietary risk assessment was limited by using rat oral absorption rates as a proxy for the human oral absorption rate. However, animal absorption rates are not reliable to predict human oral absorption (Musther et al., 2014) and may compromise risk assessments based on oral exposure. Using the animal oral absorption rate is especially problematic if it is low, as in the case of glyphosate. If the unknown human absorption rate is significantly higher, the risk to consumers is underestimated. If urinary excretion represents the systemically available dose, this uncertainty can be reduced by comparing human urinary levels to a known intake level.

The scope of the present study was to correlate urinary glyphosate and AMPA excretion with a known oral glyphosate and AMPA dose in a natural matrix. This allows the use of urinary glyphosate and AMPA levels as reliable quantitative biomarkers of oral exposure to obtain a more refined human exposure assessment.

2. Materials and methods

2.1. Study design and protocols

The four-day trial was designed as follows: after a two-day wash-out period, glyphosate was administered via a test meal followed by a two-day urine sampling period. During the trial, the participants' glyphosate intake was kept as low as possible to enable the observation of the excretion of the administered glyphosate.

The participants received two lists (see Supplementary Information S1), one that has foods that are assumed to have low glyphosate residue levels, which were recommended for consumption and the other with products to avoid because of possible glyphosate contamination. A few days later, shortly before the trial started, the participants were individually instructed on the study protocol and the recommended foods were discussed in detail. The participants were instructed to eat according to the food lists during the four-day trial. They were also instructed to eat as similar and monotonous meals as possible over the trial period and to keep a detailed log of their food and beverage consumption. During the two-day urine sample collection period, each micturition was logged and collected in a separate 1000 mL polypropylene wide-mouth bottle (Semadeni, Ostermundigen, Switzerland), with the time and date recorded on the label, and stored in a refrigerator. The samples were transferred to the laboratory within a few hours or at a maximum, within 24 h. In the laboratory, the volume and pH of each sample was measured. Several 5 mL aliquots were taken and either used for immediate analysis or stored in the freezer at −20 °C until analysis.

The test meal was eaten in the morning of a workday at the laboratory, so the most important excretion period after the meal was during the day. The participants were instructed to log the exact time of micturition of the first morning urine at home, but not to collect it. They were told to drink a little and eat little to nothing, to ensure they would be able to consume the test portion of approximately 150 g of falafel. Shortly before the test meal, the first urine was collected. Most of the participants also drank water while eating the test meal.

The participants were instructed to urinate regularly once every 1.5 h after the test meal to obtain enough data points during the first 6 h after consumption of the glyphosate dose. They were instructed to drink enough to allow sufficient urination during the day but to avoid excessive consumption of water that could hamper the glyphosate analysis with too strong dilution. After the initial 6-h period, the participants were free to urinate as needed. Each urine sample was collected in a separate bottle until the first morning urination two days after the test meal.

The study protocol was approved by the Ethics Committee of Bern (BASEC ID 2018–00110). Participation was voluntary and informed consent was obtained from all of the participants.

2.2. Study participants

Office employees working near the laboratory were recruited as volunteers. All of the participants received a voucher worth CHF 200 for a local retailer. A short walking distance to the laboratory was defined as the selection criterion for ease of handling of the urine samples. Twelve healthy adults, six males (M) and six females (F) participated.

2.3. Test meal

The test meal was a homemade falafel dish. Each participant was instructed to eat approximately 150 g of the falafel, corresponding to an intake of 196.8 μg of glyphosate and 1.67 μg of AMPA. The falafel's ingredients met all the legal requirements. The food used was not spiked with glyphosate or AMPA. Both substances were present as residues in the gram flour (chickpea flour) used (see Supplementary Information S2). The falafel dish was prepared ready for consumption. All portions of the dish were frozen. For the trial, a portion of the dish was thawed and reheated in a combi steamer. The ingested glyphosate and AMPA dose were calculated before reheating. The falafel's homogeneity and stability were checked during the reheating process of the meal preparation (see Supplementary Information S3).

2.4. Analysis methods

The glyphosate and AMPA in the gram flour and falafel were analysed using a previously established method (Zoller et al., 2018). To analyse the glyphosate and AMPA in the urine samples, the same method was used with minor modifications (see Supplementary Information S4). The limit of quantification (LOQ) was defined as a signal to noise (s/n) ratio of 10:1 for the quantifier transition and the limit of detection (LOD) was 3:1. The LOQ for the glyphosate and AMPA was 0.05 and 0.1 ng mL^{-1}, respectively, and the LOD was 0.02 and 0.04 ng mL^{-1}, respectively. Values between the LOD and LOQ were also used for calculations. For values below the LOD, the following procedure was applied. If there was an integrable signal, this was used for calculation. Otherwise, the value was set to 0.01 ng mL^{-1}. Creatinine was analysed at an external clinical laboratory using the Jaffé Gen.2 method (cobas, Roche Diagnostics, Mannheim, Germany).

3. Results

All of the participants followed the study protocol and successfully

completed the experiment. The experimental design showed to be suitable for obtaining sufficiently low background levels of glyphosate, as the concentration in the urine at time zero (before consumption of the test meal) was below 0.1 ng mL^{-1} for all participants. The experimental data is summarised in Table 1 and the raw data is available in Excel file S5. The administered dose range per participant was 125–247 μg for glyphosate and 1.06–2.08 μg for AMPA, which corresponds to the consumption of 95–188 g of falafel. The range of the applied dose in relation to body weight was 2010–4360 ng kg^{-1} for glyphosate and 17.0–39.2 ng kg^{-1} for AMPA. The highest urinary glyphosate concentration was 5.6 ng mL^{-1}, which was measured in the urine sample at the time interval of 9.92–11.50 h from participant 15M. The highest excretion rate of glyphosate was 250.6 ng h^{-1}, measured during the time interval of 8.25–9.92 h from participant 15M. The lowest and highest measured urine output rates were 13 mL h^{-1} and 569 mL h^{-1}, respectively. The mean urine sampling duration was 45.3 h within a range of 37–51 h.

The range of total urinary excretion in relation to the administered dose was 0.57–1.68% for glyphosate and 9.8–32.6% for AMPA when referring to the AMPA dose, or 0.087–0.276% when referring to the glyphosate dose. These results are based on the measured raw data without any data fitting. For participants 17F and 18F, one and two samples (micturitions), respectively, were missing. To estimate the magnitude of the error on glyphosate excretion, a simple linear interpolation with the adjacent excretion values was conducted to generate the missing data. This correction showed that the excretions were underestimated by approximately 3% and 9% for 17F and 18F, respectively. As this error was marginal, the data was used without correction.

The results showed no indication that the glyphosate and AMPA excretion rates increased due to higher urine output.

The participants' cumulative glyphosate and AMPA excretion are depicted in Fig. 1a and b. Most of the glyphosate and AMPA excretion was completed within 10–20 h after ingestion. After 45 h, the excretion of all of the participants reached a plateau. The cumulative excretion of glyphosate and AMPA expressed as a percentage of the dose was not significantly different in the male and female participants, according to the two-sample *t*-test (unequal variances). The p-values of glyphosate and AMPA were 0.31 and 0.14, respectively.

To calculate the toxicokinetic parameters, a one-compartment absorption model was applied. This model, shown in Fig. 2, assumes that both the oral absorption (Ka) and elimination rate (Ke) of glyphosate, followed a first-order kinetic (Hacker et al., 2009). The measured excretion values were fitted against the equation using the non-linear least squares method. This simple model fitted the experimental data well for all of the participants (see Fig. 3) and the excretion rates were accurately assessed. The calculated toxicokinetic parameters are summarised in Table 1. A very consistent elimination half-life value was obtained. The half-life was approximately 9 h for both the male and female participants, but the variation differed. For the male participants, the observed half-life range was between 6.34 and 9.96 h whereas it varied from 5.21 to 14.49 h for the female participants. For almost all of the participants, the time to excretion peak (T_{max}) was reached within approximately 5 h. Two male participants had a shorter and a longer T_{max} of 2.99 h and 9.14 h. The maximum glyphosate excretion rate (E_{max}) varied from 45.1 to 212.45 ng h^{-1} with a median value of 94.85 ng h^{-1}. The E_{max} was slightly lower in the females than the males, but the females also received a lower dose on average.

Due to the very low AMPA concentration in the urine (see Fig. 3), it was not possible to calculate the toxicokinetic parameters of each individual participant. The combined data showed the absorption and elimination of AMPA, which followed similar kinetics as that of glyphosate (see Fig. 4a). The average calculated parameters are provided in Table 2.

4. Discussion

4.1. Glyphosate

The literature on glyphosate's toxicokinetics is surprisingly scant, especially in view of the public interest of the possible risk associated with oral consumption of pesticide residues due to consumption of food treated with glyphosate-based pesticides. Specifically, there are no publications discussing urinary glyphosate levels in relation to defined oral exposure. Only one paper explored glyphosate's half-life in human urine samples from amenity horticulture workers using glyphosate-based pesticides (Connolly et al., 2019). A symposium abstract also reported preliminary results of a kinetic study (Faniband et al., 2017). Glyphosate was administered at a dose level of 25% of the ADI (0.125 mg kg^{-1} bw) to one male and female subject and their urinary excretion was investigated for 100 h.

To the best of the author's knowledge, the present paper is the first to investigate glyphosate's urinary excretion characteristics in humans

Table 1
Doses of glyphosate given to individual participants and the observed excretion and excretion parameters. Excreted dose corresponds to the effective measured amount without any data fitting. C_{max} are the effectively measured maximum concentrations in relation to the creatinine concentration. T_{max} and E_{max} are the time to excretion peak and the maximum excretion rate, respectively. $T_{1/2}$ is the elimination half-life. These three parameters were calculated using the model shown in Fig. 2. Codes with M represent male participants and codes with F represent female participants.

Participant code	Glyphosate							AMPA	
	Urine sampling time period (h)	Ingested Dose (ng)	Excreted Dose (%)	C_{max} (ng/g creatinine)	T_{max} (h)	E_{max} (ng/h)	$T_{1/2}$ (h)	Ingested Dose (ng)	C_{max} (ng/g creatinine)
11M	44.42	188928	0.74	1876	5.08	90.6	7.46	1598	542
12M	46.08	216480	1.28	1865	5.71	140.2	9.96	1832	534
13M	46.50	205984	0.64	1471	5.60	72.3	9.16	1743	422
14M	44.77	173184	0.98	2120	4.19	92.8	9.23	1465	287
15M	47.00	246656	1.68	4256	9.14	212.5	6.34	2087	326
22M	36.92	233536	0.99	2673	2.99	165.6	8.09	2076	497
16F	44.25	245344	0.57	2105	4.40	69.2	14.49	1055	575
17F	45.42	124640	1.30	2825	4.97	114.9	5.21	1277	448
18F	45.92	150880	0.65	1084	4.76	45.1	10.05	1376	524
19F	45.58	162688	0.87	3006	4.50	109.6	5.59	1343	524
20F	46.33	158752	0.76	1484	4.57	56.4	13.18	1665	528
21F	50.58	196800	0.95	2157	4.99	96.9	9.21	1976	524
Median all	45.75	192864	0.91	2112	4.87	94.9	9.19	1632	524
Median M	45.43	211232	0.98	1998	5.34	116.5	8.63	1788	460
Median F	45.75	160720	0.82	2131	4.67	83.1	9.63	1360	524

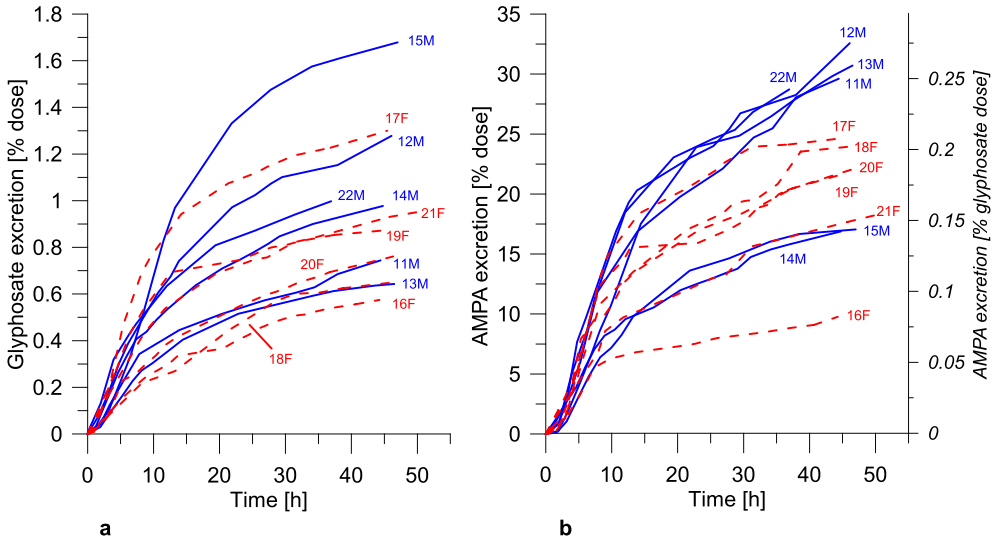

Fig. 1. Cumulative excretion of glyphosate (a) and AMPA (b) by male [blue, solid line] and female [red, dashed line] subjects expressed in percent of the oral dose.

$$Excretion = F\left(\frac{k_a}{k_a - k_e}\right) * (e^{-k_a * t} - e^{-k_e * t})$$

Fig. 2. One-compartment absorption model. F is a constant related to the bioavailability of the oral dose, k_a and k_e, the first-order absorption and elimination rate constant, respectively.

after oral consumption of a defined dose. In this study, to create a scenario as similar as possible to the consumer situation, glyphosate was administered as a real residue present in a foodstuff. Each urine sample was collected individually, so the precise time course could be evaluated. This data indicates that human urinary levels are a reliable indicator of oral dietary exposure to glyphosate residues. As the median urinary excretion was 0.91% (mean = 0.95%) of the administered dose, it is reasonable to assume that the urine levels indicated approximately 1% of dietary glyphosate exposure. Common dietary risk assessments compare the dose ingested — and not the dose systemically available after absorption — with a reference dose such as ADI or ARfD. Using nominal dietary exposure (glyphosate level in food multiplied by its consumption) instead of the systemically available dose, the procedure implicitly assumes 100% oral absorption. For most compounds, probably including glyphosate, this is an overestimation of the absorption and hence provides a certain margin of conservatism.

The observed median urinary excretion of 0.91% was 22 times lower than reported in animal studies, which showed a 20% excretion (EFSA, 2015). However, in animal studies, the dose was administered via gavage and not by feeding; thus, absorption might be lower. The range of the urinary excretion in different studies was from 11% to 43% (EFSA, 2014; WHO, 1994). Only one study administered glyphosate by diet (WHO, 1994). In this 14-day oral study in rats, the observed total excretion in urine was ≤10%. In the human study of Faniband et al. (2017), the dose was 30–60 times higher than in our study and the urinary excretion was similar. The total dose recovered as glyphosate in urine was approximately 1% and 0.4% in the female and male volunteers, respectively.

Dietary glyphosate is likely to be very poorly absorbed in humans and lower than in rats exposed by gavage. As the blood concentration was not measured in our study, this precluded any quantitative comparison of the toxicokinetic characteristics between rats and humans. However, the low urinary excretion level identified in our study (approximately 1% of dietary exposure) suggests that intake estimations calculated from human urine data systematically underestimated

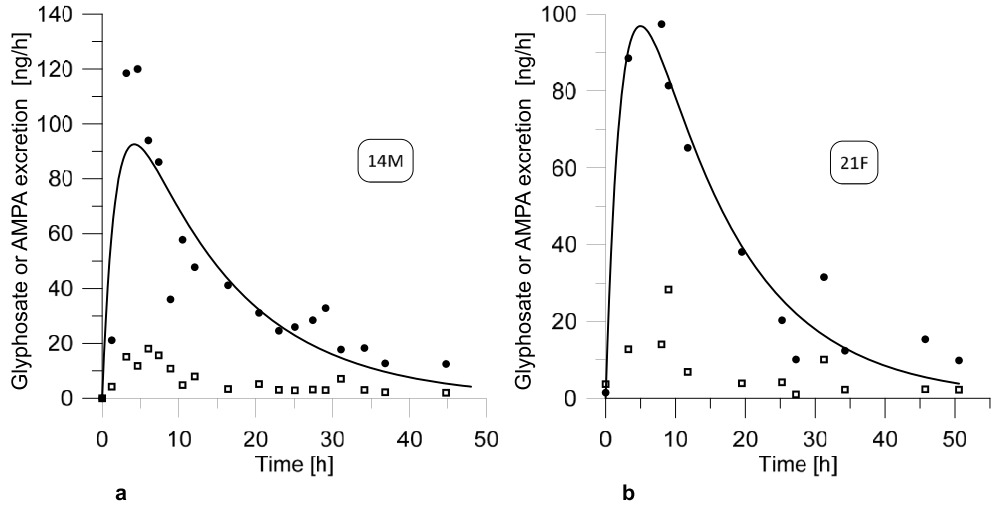

Fig. 3. Glyphosate (●) and AMPA (□) excretion rate as a function of time for participants 14M (a) and 21F (b). Glyphosate data points are best fitted (solid line) with a one-compartment model, first-order absorption and elimination kinetics.

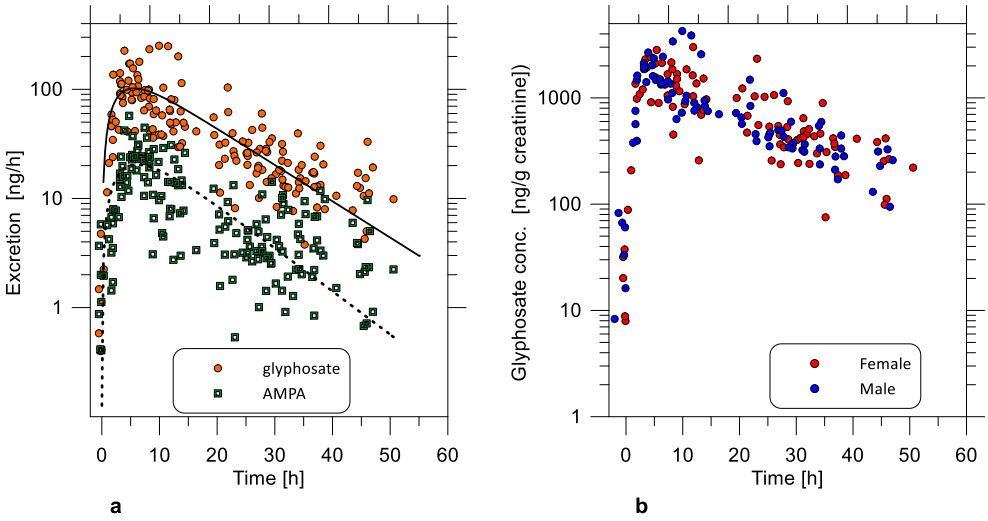

Fig. 4. a: observed excretion rates of glyphosate (◯) and AMPA (□); values are fitted (lines) with the model shown in Fig. 2 b: creatinine adjusted concentration of glyphosate for the male (blue) and female (red) subjects.

Table 2
Kinetics parameters for glyphosate and AMPA excretion calculated using the participants' data sets. F is a constant related to the bioavailability of the oral dose, K_a and K_e, the first-order absorption and elimination rate constant, respectively. T_{max} and E_{max} are the time to excretion peak and the maximum excretion rate, respectively. $T_{1/2}$ is the elimination half-life.

	Glyphosate	AMPA
F (ng/h)	156.89	35.67
K_a (h^{-1})	0.34	0.27
K_e (h^{-1})	0.08	0.09
T_{max} (h)	5.62	6.13
E_{max} (ng/h)	102.01	20.39
$T_{1/2}$ (h^{-1})	9.05	7.60

exposure (Conrad et al., 2017; Niemann et al., 2015).

The median urinary excretion of 0.91% of the dose has some uncertainties. The mean dose per person of 192 μg of glyphosate was quite low but comparable to assumed regular intake of glyphosate. During the 4-day experiment, the participants were free to consume recommended foods that were likely to contain only very low amounts of glyphosate. Their food consumption was not fully controlled, only recorded. Since the foods they ingested were not tested for glyphosate, there is a potential of consuming additional small amounts of glyphosate that could cause the underestimation of the dietary exposure. However, all of the participants had very low glyphosate concentrations after the wash-out period, indicating that the diet regimen was adequate. Additionally, the cumulative excretion curves (Fig. 1a) do not provide evidence of further relevant glyphosate sources. There is a potential that the sampling period was too short and that a small amount of the excreted glyphosate and AMPA could have been missed and the total excretion was underestimated. Although, these two minor issues have opposing shortcomings, which could cancel each other out.

The human elimination half-life of 9 h in our study is comparable to that of animal (rat) studies. In one rat study, a half-life of approximately 9.5 h was described after oral administration by gavage, 10.9 h in male rats and 8.1 h in female rats (FAO/WHO, 2017). Anadón et al. (2009) reported a half-life of 10.0 h after intravenous and 14.4 h after oral administration in rats. Connolly et al. (2019) found a half-life of 7.25 h in humans by calculating with urinary excretion rates and a range of 5.5 h up to 10 h when considering all calculation models. Faniband et al. (2017) reported first-order kinetics and a two-phase excretion with a half-life range for the rapid phase of 4–17 h. Overall, the published elimination half-lives have a good concordance.

4.2. AMPA

There is even less data available for AMPA than glyphosate. In our study, a low AMPA level was identified in the test meal consumed by the participants and therefore, they ingested a small amount of 1–2 μg of AMPA. If the amount of glyphosate in the test meal is assumed to be 100%, the fraction of AMPA concentration was only 0.85% (1.29% on a molar basis). After intravenous administration of glyphosate in rats, no transformation to AMPA was reported (EFSA, 2014; FAO/WHO, 2017; WHO, 1994). After oral administration in rats, glyphosate is mostly excreted unmetabolised with only small levels of AMPA observed (EFSA, 2014). Only 0.2–0.4% of a glyphosate dose of 10 mg kg^{-1} bw were found in the excreta as AMPA (FAO/WHO, 2017; WHO, 1997). In a study of bile duct cannulated rats, who received an oral dose of glyphosate of 10 mg kg^{-1} bw, only 0.07–0.19% of the dose was excreted in urine as AMPA (EFSA, 2014). In one rat study (Anadón et al., 2009), a relatively high transformation rate of glyphosate to AMPA concentration of 6.49% was found (9.88% on a molar basis). The data was measured in plasma and calculated by comparing the area under the concentration-time curves. Data on urinary excretion was not reported in this study. A small amount of human data is available from poisoning cases with herbicides. In seven cases of acute human intoxication, the ratio of urine concentrations of glyphosate/AMPA ranged from 243 to 7863 (Zouaoui et al., 2013). Only one study was available concerning the kinetics of AMPA alone. Wistar rats were fasted for 4 h and then given a single oral dose of 6.7 mg kg^{-1} bw AMPA by gavage. Overall, 20% of the dose was excreted in their urine within 120 h (18% within 24 h) (WHO, 1997).

Considering this data, the following two options are probable:

Option 1: There is a certain transformation rate of glyphosate to AMPA and all or most of the AMPA excreted in urine is a glyphosate metabolite. If all urinary AMPA stems from metabolised glyphosate then approximately 0.2% of the glyphosate dose on a concentration basis is excreted in urine as AMPA (see Fig. 1b, y axis on the right side). This corresponds to approximately 0.3% of the dose on a molar basis.

Option 2: There is no transformation to AMPA and the AMPA excreted in urine originated exclusively from the ingested AMPA. If this was the case, then AMPA is absorbed and excreted to a much higher degree than glyphosate. If no metabolism is assumed, approximately 23% of the AMPA dose is excreted in urine (see Fig. 1b, y axis on the left side). It is improbable that AMPA is so much more highly absorbed than glyphosate.

It is more probable that it is a combination of both options.

In this study, it was not possible to deduce where the urinary excreted AMPA originated. It could have been a metabolite of glyphosate

and/or unchanged absorbed AMPA. Further studies administering pure glyphosate alone and pure AMPA alone are necessary.

The AMPA concentration in urine is often only slighty lower than that of glyphosate (Niemann et al., 2015). To date, there was no convincing explanation for this observation as it is generally agreed that glyphosate residues are higher than those of AMPA in a European diet. The intake of AMPA from other sources, e.g. as a breakdown product of certain detergents such as (nitrilotris(methylene))triphosphonic acid, has been postulated. Although this is the most plausible source, no studies to date have demonstrated this as a source or route of human exposure. The result of our experiment can contribute to partially explain these observations, either because of the higher than expected transformation rate of glyphosate to AMPA or the relatively better AMPA absorption.

4.3. Estimating glyphosate intake via urine sample measurement

Our data suggest that the glyphosate concentration in relation to the creatinine concentration and the glyphosate excretion rate are equally good markers for glyphosate intake (see Fig. 4a and b). To calculate the excretion rate, the sampling duration and urine volume must be known. Therefore, to interpret spot urine samples, it would be easier to use the glyphosate concentration in relation to the creatinine concentration. Of course, in both cases, it is also important to know the time interval since the suspected exposure.

5. Conclusion

In humans, the proportion of urinary excretion of glyphosate is only approximately 1% and therefore much lower than previously assumed based on animal data. Consequently, the glyphosate intake could be approximately 20 times higher than previously assumed when using urinary data. However, human systemic availability is most likely also 20 times lower than in rats, which gives the risk assessment a certain margin of conservatism.

Further human toxicokinetic studies should be performed individually. In these studies, the determination of blood concentrations is necessary to improve human bioavailability data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2020.113526.

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