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Urinary biomarkers of flame retardant exposure among collegiate U.S. gymnasts

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Abstract

Flame retardants are widely used in polyurethane foam materials including gymnastics safety equipment such as pit cubes and landing mats. We previously reported elevated concentrations of flame retardants in the air and dust of a U.S. gymnastics training facility and elevated PentaBDE in the serum of collegiate gymnasts. Our objective in this pilot study was to compare urinary biomarkers of exposure to other flame retardants and additives of polyurethane foam including tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), triphenyl phosphate (TPHP) and 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB) in samples collected from 11 collegiate gymnasts before and after a gymnastics practice (n=53 urine samples total). We identified a 50% increase in the TPHP biomarker (p=0.03) from before to after practice, a non-significant 22% increase in the TDCIPP biomarker (p=0.14) and no change for the EH-TBB biomarker. These preliminary results indicate that the gymnastics training environment can be a source of recreational exposure to flame retardants. Such exposures are likely widespread, as we identified flame retardants in 89% of foam samples collected from gyms across the U.S.

Graphical abstract

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Keywords

flame retardants; organophosphorous compounds; environmental exposure; gymnastics

1. Introduction

Flame retardants are chemical additives used to meet flammability standards required for various types of materials including polyurethane foam (PUF). They are ubiquitous in the indoor environment due to their use in consumer products such as upholstered furniture as they are not chemically bonded to PUF and migrate into the air and dust of indoor environments.^{1, 2} PentaBDE is a flame retardant mixture of polybrominated diphenyl ethers (PBDEs) that was used widely in PUF until concerns regarding its persistence and toxicity caused it to be banned in the European Union (2004) and phased out of production in the U.S. (2005). Flame retardants that replaced PentaBDE in foam include Firemaster® 550 (FM550) and tris(1,3-dichloro-2-propyl)phosphate (TDCIPP).³ FM550 is a mixture of brominated and phosphorylated compounds: triphenyl phosphate (TPHP), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB), bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (BEH-TEBP) and a mixture of isopropylated triphenylphosphate isomers¹ (abbreviations in this manuscript are as described in Bergman et al. 2012).⁴ While TPHP is part of the FM550 mixture it can also be used in PUF with the PentaBDE mixture as well as in a variety of applications as a plasticizer.⁵

The primary metabolites of these compounds are excreted primarily in urine as diphenyl phosphate (DPHP), bis(1,3-dichloro-2-propyl) phosphate (BDCIPP) and tetra-bromo benzoic acid (TBBA), respectively (Table A1 of Appendix I).⁶⁻⁹ High frequencies of detection for these urinary metabolites among U.S. adults suggest that exposure to TDCIPP and components of the FM550 mixture is widespread.^{10, 11} While the human half-lives of the PentaBDE congeners are estimated to be on the order of years,¹² the human half-lives of TPHP, TDCIPP and EH-TBB are not known but are likely on the order of hours based on animal studies.^{6, 9, 11, 13} We did not attempt to measure a biomarker for BEH-TEBP as a major metabolite has yet to be detected in urine or serum.⁸

TDCIPP and components of the FM550 mixture are endocrine disrupting chemicals that can disrupt thyroid hormone action in the body.¹⁴⁻¹⁷ Dams exposed to the FM550 mixture had increased serum concentrations of thyroxine (T₄), while their offspring experienced advanced puberty (in females), cardiac hypertrophy (in males), significant weight gain (both sexes) and increased anxiety.¹⁵ TPHP, a component of the FM550 mixture, is a suspected

obesogen as it can initiate adipocyte differentiation by binding and activating PPAR γ , a nuclear receptor that regulates fatty acid storage and glucose metabolism, and can also antagonize osteogenesis.¹⁸ The brominated components of FM550, EH-TBB and BEH-TEBP, can adversely affect fecundity, as suggested by in vitro studies that found decreased cumulative egg production among exposed Japanese medaka¹⁹ and increased multinucleated germ cells in exposed fetal rat testes.²⁰ In addition to its impact on circulating levels of thyroxine, TDCIPP is considered a genotoxic carcinogen by the State of California,²¹ and it is a suspected neurotoxicant that was found to have similar or greater potency as the developmental neurotoxicant organophosphate pesticide chlorpyrifos when tested in PC12 cell lines,²² an experimental model.^{23, 24}

To protect gymnasts from falls, gymnastics training facilities rely on safety equipment containing PUF, such as landing mats and the loose foam pit. We previously reported elevated concentrations of PentaBDE congeners, particularly BDE153, in the serum of 11 collegiate U.S. gymnasts compared to the general U.S. population.²⁵ The loose foam pit, a landing area used for learning new skills safely, was identified as a source of PentaBDE to the gym environment as components of the PentaBDE mixture were found in foam from the loose foam pit and elevated concentrations were measured in the air and dust of the gym. Additionally, hand-wipes collected after practice contained higher concentrations of the PentaBDE congeners compared to those collected before practice.

Similar to PentaBDE, the loose foam pit appeared to be a source of exposure to components of the FM550 mixture (i.e., TPHP and EH-TBB), which were identified at elevated concentrations in air and dust of the gym and in foam from the loose foam pit.²⁵ TDCIPP was not identified in foam from the loose foam pit but may have been in other foam equipment (i.e., landing mats) as it was present in gym air and dust at concentrations similar to other indoor environments.²⁵ While our previous study provided serum measurements of the PentaBDE congeners among gymnasts as a biomarker of internal dose, we did not report biomarkers of exposure to these other flame retardants.

Therefore, the objective of this pilot study was to measure urinary metabolites of TPHP, EH-TBB and TDCIPP in samples collected from the same 11 collegiate gymnasts before and after a gymnastics practice. We hypothesized that samples collected after practice would have higher concentrations of urinary metabolites compared to those collected before practice.

2. Methods

2.1 Study Design

As previously reported, we recruited a convenience sample of 11 female gymnasts from one collegiate gym with a loose foam pit (Gym 1) in the Eastern United States.²⁵ To be eligible for participation, gymnasts had to be older than 15 years in age and practice at least 3 hours/week. The Boston University Medical Center Institutional Review Board approved the study protocol. All participants gave their informed consent prior to participation. Sampling centered around one Friday practice during the spring of 2012. Prior to practice, each

participant filled out a questionnaire regarding her demographics, habits, gymnastics history and gym use.

2.2 Urine

Each participant provided a sample of urine before (~2 pm) and soon after (~4:45 pm) practice as well as from her next three urinations. Urine samples were immediately placed in a cooler with ice for storage and transport. Upon receipt, samples were cold and ice packs were still frozen. Three field blanks were collected by transferring deionized water from one urine cup to another at the location of sample collection. Samples were measured for specific gravity (SG) and stored at -20°C within 24 hours of collection.

Urine samples were analyzed for DPHP, BDCIPP and TBBA using previously published methods.^{11, 26} Briefly, BDCIPP and DPHP were extracted and cleaned up with solid phase extraction StrataX-AW (60 mg, 3 mL, Phenomenex, Torrance, CA, USA). Deuterated BDCIPP (d10-BDCIPP) and d10-DPHP were spiked as the surrogate standard to quantify BDCIPP and DPHP, respectively. BDCIPP and DPHP extracted from urine were analyzed by LC/MS-MS (Agilent 6410 Triple Quad LCMS). Chromatographic separation of the extracts (5 µL injection) was performed on a Kinetex XBC18 column (100 × 2.1 mm; 2.6 µm; Phenomenex) maintained at 45 °C. BDCIPP and DPHP were detected by atmospheric pressure chemical ionization (APCI) operating in negative ionization mode using multiple reaction monitoring (MRM).

A standard of TBBA was synthesized by the Small Molecule Synthesis Facility at Duke University (Durham, NC). Urine samples were analyzed for TBBA as previously described.¹³ Briefly, 10 mL urine samples were extracted with a novel liquid-liquid extraction technique using hexane and analyzed by LC/MS-MS in negative ESI (electrospray ionization) mode using MRM. The separation was performed using a Synergi Polar-RP column (50 × 2.0 mm, 2.5 µm particle size, Phenomenex). 2,3,5-triodobenzoic acid (TIBA, Sigma-Aldrich, St. Louis, MO) was used as the internal standard. In a matrix spike recovery evaluation, the average recovery of TBBA was 79±9 % in 10 urine samples. The method detection limit (MDL) of DPHP of 0.106 (µg/L) was calculated as 3 times the standard deviation of the laboratory blanks; the average concentration of DPHP in these blanks was 0.131 µg/L (0.10 to 0.19 µg/L). BDCIPP and TBBA were not detected in field or laboratory blanks, therefore their MDLs of 0.01, and 0.003 µg/L, respectively, were estimated using the instrument detection limit.

2.3 Pit Cube Survey

Between September 2014 and May 2015 we collected samples of pit cube foam from U.S. gymnastics training facilities and screened them for the presence or absence of 7 common flame retardants found in PUF through a testing service provided by the Duke University Superfund Research Center.²⁷ The protocol was approved by the Duke University Institutional Review Board. Participants were asked to cut a small piece of foam (about the size of a marble) from the pit cube, wrap in aluminum foil and seal in a zip bag and to indicate the approximate year of purchase. Samples were tested using GC-MS under previously published methods^{3, 28} for the presence or absence of seven different types of

flame retardant chemicals that are commonly applied to polyurethane foam including PentaBDE, components of Firemaster ® 550 (FM550; containing TPHP, a mixture of isopropylated aryl phosphates, EH-TBB and BEH-TEBP), TDCIPP, tris (1-chloro-2-propyl) phosphate (TCPP), tris-isobutylated triphenyl phosphate (TBPP), a mixture of methylated phenyl phosphates (MPP) and V6. Field and laboratory blanks were prepared during sample preparation. All the data generated were background noise corrected using field blank samples. Positive detection of a flame retardant was defined as greater than 0.1% by weight. Each participant received the results for his/her own sample(s), as well as an information sheet on common flame retardants found in furniture.

2.4 Data Analysis

DPHP concentrations were blank-corrected using the average of the laboratory blanks (0.131 µg/L). Data were approximately log-normally distributed; therefore we used non-parametric methods (i.e., Spearman correlation) or natural log transformation in statistical analysis. For calculation of GMs and use in statistical tests, unquantified concentrations <MDL were substituted with MDL/2.

To adjust for urinary dilution we normalized to SG using the mean SG (1.024) as described by Pearson et al.²⁹: $C_{SG} = C * [(1.024 - 1) / (SG_i - 1)]$ where C_{SG} = SG normalized urinary metabolite concentration, C = urinary metabolite concentration and SG_i = SG for an individual sample. All analyses used SG normalized urinary metabolite data. Urine results were collected immediately before and within 0.5 hours after practice. Because the time between the three subsequent urinations differed between gymnasts they were divided into two categories: 1 to 3 hours and >3 to 17 hours after practice. We fit a multivariate generalized linear mixed model with random intercepts to test for differences in urinary metabolites between time categories that allowed for the use of multiple outcome observations per individual while accounting for within-person correlations in outcomes. These models are appropriate for an unbalanced design (e.g., different number of urine samples contributed per participant). Beta estimates of log-transformed data were exponentiated to provide the multiplicative difference from the referent. Non-linear mixed effect pharmacokinetic models were employed to estimate half-lives.³⁰

One sample could not be analyzed for DPHP and BDCIPP due to the formation of a gel layer upon extraction. Two participants had insufficient volume for analysis of TBBA at one time point, before and soon after practice respectively, reducing the number of urine samples to 53. All statistical analyses were performed using SAS (version 9.3; SAS Institute Inc., Cary, NC) with statistical significance defined as $\alpha = 0.05$.

3. Results

3.1 Study population

Each participant had been a gymnast for at least 12 years, was a female of 18–22 years of age and currently trained on the same collegiate team. On average, they reported training 19 hours/week during the last competitive season (September–May) and 10 hours/week during the prior off-season (June–August). Practice on the sampling day lasted 2.5 hours.

3.2 Urinary metabolites

Detection frequencies for DPHP and BDCIPP were 100% and ranged from 90 to 100% for TBBA. In our primary analysis we identified a 50% increase in urinary DPHP among samples collected in the half hour after practice compared to those collected before practice that approached statistical significance ($p=0.07$, 3.52 ug/L increase) (Figure 1). There was also a non-significant decrease across the after-practice categories. We conducted a secondary analysis that restricted the number of categories in order to improve statistical power. In this analysis we identified a significant 50% increase in urinary DPHP among samples collected in the half hour after practice compared to those collected before practice ($p=0.03$). For urinary BDCIPP, there was a significant change across categories ($p=0.03$), a non-significant increase in the half hour after practice compared to before ($p=0.14$, 22% or 0.15 ug/L increase), and a significant decrease between the first and third after-practice time-point ($p=0.01$, 38% or 0.23 ug/L decrease). There was no change in urinary TBBA across the time-points. Results were similar using urinary metabolite data not adjusted for SG (See Appendix I: Tables A2-A3 and Figure A2).

Examined on an individual basis, trends in urinary metabolite concentrations were highly variable between the participants (Figure 2). The percentage of participants with any increase was 82%, 64% and 50% for DPHP, BDCIPP and TBBA, respectively. The highest concentrations of DPHP and BDCIPP occurred immediately after practice, whereas peak concentrations of TBBA occurred 3 to 7 hours later. Urinary TBBA was notably elevated in all samples for one participant, with concentrations ranging from 0.281 to 0.375 ($\mu\text{g/L}$ and no apparent trend with time. This outlier is off scale of Figure 1, but is re-scaled for inclusion in Figure A1 of Appendix I.

Our non-linear mixed effect pharmacokinetic models did not converge, likely due to the limited statistical power; therefore we were unable to reliably estimate half-lives. However, the declines of urinary concentrations following the peaks (Figure 1) suggest that human half-lives may be on the order of hours. Full summary statistics of urinary metabolites by time point are provided in Table A2 of Appendix I, with and without SG normalization.

3.3 Pit Cube Survey

We collected eighteen pit cube foam samples from a convenience sample of 8 U.S. gyms. Results were combined with those previously reported²⁵ resulting in a total of 28 samples from 11 gyms (Table 1). The majority (89%) of pit cube samples contained at least one flame retardant. FM550 and PentaBDE were detected in 36% and 25% of samples respectively. A smaller proportion of samples contained TDCIPP (18%), TBPP (7%) or Mixture 1 (4%, proprietary mixture with some similarities to FM550), and none contained TCPP, MPP or V6. TPHP was identified as a component of 71 % of the samples. The year of purchase was reported for 67% ($n=19$ of 28) of the samples and ranged from 1990 to 2013. PentaBDE was identified in significantly ($p=0.0001$) more samples purchased prior to 2005 (86%) compared to after 2005 (0%).

4. Discussion

These preliminary results add to the evidence that the gymnastics training environment can be a source of exposure to flame retardants used in polyurethane foam and that pit cubes can commonly contain both brominated and organophosphate flame retardants. We found evidence that the gym is a source of exposure to TPHP, based on the significant increase in urinary DPHP from before to after practice. A similar trend was observed for TDCIPP exposure, with a significant after practice decline in urinary BDCIPP. In contrast, no change in urinary TBBA was observed, suggesting the influence of additional factors such as delayed excretion or other sources of exposure. While the loose foam pit was identified as a source of TPHP and EH-TBB (but not TDCIPP), other foam-containing equipment such as landing mats may also be important sources of flame retardants to the gym.²⁵ In addition, the vinyl fabric of landing mats may be a source of TPHP as it has reportedly been used as a plasticizer in this material.³¹

GM urinary DPHP among gymnasts (7.02 to 10.5 µg/L from before to after practice) was higher than among an occupationally exposed population of aircraft maintenance technicians (n=5, median pre-shift 2.9 µg/L, post-shift 3.5 µg/L)³² and initial peak concentrations among gymnasts (~20-50 µg/L) were higher than observed in a nail polish exposure study (~10-20 µg/L).³³ Compared to other samples in the U.S., gymnasts had higher GM concentrations of urinary DPHP and TBBA, but not BDCIPP (Table 2).^{7-10,34} Gymnast concentrations of urinary DPHP and BDCIPP were higher compared to a sample from Norway³⁵ and lower compared to pooled samples from Australia for urinary for DPHP but similar for BDCIPP.³⁶ Note that comparisons should be interpreted with caution where methodologies differ.

Additionally, care should be taken in comparing our findings to non-athlete populations as gymnasts may metabolize and excrete these compounds differently than others due to the effects of exercise and leanness on biotransformation enzymes, blood distribution and urinary clearance.^{37, 38} In addition, primary exposure routes may be different during a gymnast practice than in other indoor environments. For example, inhalation exposure may be pronounced due to elevated ventilation rates during exercise. Gymnast exposure via incidental dust ingestion and dermal absorption, important exposure pathways for flame retardants in other indoor environments,^{39, 40} may also be higher than the general population due to suspension of dust particles during training activities, increased dermal loading due to direct dermal contact with foam and dust, and increased dermal absorption due to increased permeability from perspiration. Also, primary exposure routes are expected to differ by class of flame retardant; for example inhalation and dermal exposures may be more important for TPHP and TDCIPP due to higher vapor pressures and dermal permeability compared to PBDEs and EH-TBB.⁴¹⁻⁴³

Consistent with reports from rodent studies and a recent investigation of human exposure to TPHP in nail polish, concentrations of urinary metabolites returned to baseline within hours of peak exposure, suggesting relatively rapid absorption and metabolism.³³ We also found marked increases immediately after practice for some participants but not others, suggesting that additional factors may have influenced individual exposure during practice. One such factor is training activity, as we observed that some of the gymnast participants dismounted

into the loose foam pit during practice whereas others used the floor exercise or balance beam. For example, we might expect increased exposure via incidental ingestion and dermal absorption when training into the loose foam pit.

Subsequent peaks during the hours following practice suggest exposure to other sources of flame retardants outside the gym. This may be from exposure to flame retardants in other microenvironments such as the car, classroom or dorm, as well as other sources such as dermal absorption from nail polish containing TPHP.³³ The delayed peaks in urinary TBBA observed for some participants may be from sources outside the gym, but may also have been caused by a delay in absorption and/or metabolism as rodent studies report peak excretion approximately three hours after a single dietary dose of EH-TBB.¹¹ Similar delays have not been observed for TDCIPP.^{6, 7} While OPFRs may be eliminated from the body quickly it is important to consider scenarios of chronic exposure. Competitive gymnasts can train for over 20 hours per week starting at a young age, thus may experience repeated high exposures during practice in addition to exposure in other microenvironments (home, school, ect). Additionally, coaches may spend up to 40 hours a week in the gymnastics training environment.

Our survey of pit cubes, while small and non-random, suggests that flame retardants are common in pit cubes from U.S. gyms and that the PentaBDE mixture was more common in pit cubes purchased prior to its discontinuation of its manufacture in 2005. The variability in pit cube composition suggests that gymnasts are likely exposed to varying mixtures of flame retardants. This is supported by another U.S. study that reported varying concentrations and composition of flame retardants in air and dust of four gyms in Seattle, Washington as well as higher concentrations of flame retardants used in foam in the gyms compared to the homes of four coaches.⁴⁴

As efforts to reduce flame retardant exposure are underway for the general population, future research among gymnasts should include interventions for reducing exposure. Future research should also evaluate cumulative effects of these mixtures, as compounds that share a common mechanism of action can have cumulative toxicity. For example, recent studies have reported that house dust, which contains a mixture of flame retardants and other semi-volatile organic compounds, can act on hormone receptors including PPAR γ .^{45, 46} Several types of halogenated flame retardants including PBDEs,⁴⁷ TDCIPP,¹⁴ and components of the FM550 mixture^{15, 20} are endocrine disrupting chemicals that can impact thyroid homeostasis,^{14 14} however little is currently known about the cumulative action of exposure to mixtures of these flame retardants.

This study is the first to report urinary metabolites of flame retardants in gymnasts. An important strength of the study is our use of repeated urine samples at multiple time points. As this was a pilot study, our conclusions were limited by low statistical power due to the small number of participants. We were unable to control for exposure outside of the gym other than by a before-practice urine. While analogous pre- and post-shift measurements are used to examine exposure in occupational studies, we cannot rule out the possibility that pre-workout exposure contributed to some of the peaks observed after practice. We were unable to examine the potential metabolic effects of exercise or inter-individual polymorphisms in

metabolic enzymes.^{48, 49} We also lacked data on individual training activity, which may have contributed to differences in individual exposure.

In combination with our previous study of PBDEs,²⁵ these preliminary results indicate that the gymnastics training environment can be a source of exposure to multiple flame retardants and that exposures can vary greatly between individuals. Such exposures are likely widespread, as flame retardants were detected in 89% of foam samples collected from gyms across the U.S.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BDCIPP	bis(1,3-dichloro-2-propyl) phosphate
DPHP	diphenyl phosphate
EH-TBB	2-ethylhexyl-2,3,4,5-tetrabromobenzoate
FM550	Firemaster® 550
MDL	method detection limit
MPP	methyalted phenyl phosphates
PBDE	polybrominated diphenyl ether
PUF	polyurethane foam
SG	specific gravity
TBBA	tetra-bromo benzoic acid
TBPP	tris-isobutlyated triphenyl phosphate
TCPP	tris (1-chloro-2-propyl) phosphate
TDCIPP	tris(1,3-dichloro-2-propyl) phosphate
TPHP	triphenyl phosphate

Highlights

- The urinary metabolite of triphenyl phosphate increased from before to after a gymnastics practice
- Triphenyl phosphate was identified in polyurethane foam cubes from the loose foam pit
- Flame retardants were detected in 89% of pit cubes collected from across the U.S.
- The gymnastics training environment can be a source of exposure to organophosphate flame retardants

Figure 1. Geometric mean urinary metabolite concentrations (95% confidence intervals) (µg/L, specific gravity normalized) for samples collected from 11 gymnasts before and after a gymnastics practice. *p=0.03 in secondary analysis with improved statistical power

Figure 2.

Urinary metabolites measured in samples collected before and after practice, normalized for specific gravity. Each line represents repeated measures for one individual. Note for urinary TBBA that participant 11, who had outlier concentrations ($> 0.25 \mu\text{g/L}$) of urinary TBBA, is off scale.

Flame retardants in pit cubes from gymnastics training facilities in the U.S. Gymnasts in the current study trained in Gym 1.

Table 1

Sample	Gym	Year of Purchase ^d	Flame Retardant						None
			PentaBDE	TPHP	FM550	TDCIPP	TBPP	Mixture 1	
1	1	1990	Blue						
2	1	2001		Orange	Red				
3	2	1991	Blue	Orange					
4	2	1991	Blue	Orange					
5	2	2001	Blue	Orange					
6	2	2001	Blue			Green			
7	3	2001	Blue	Orange					
8	4	2007		Orange	Red				
9	4	2007		Orange	Red				
10	4	2007		Orange	Red				
11	4	2007		Orange				Blue	
12	D ^b	2010		Orange	Red				
13	D ^b	2010				Green			
14	D ^b	2010				Green			
15	5	2012		Orange		Green	Purple		
16	6	2013		Orange			Purple		
17	6	2013		Orange			Purple		
18	7	2013		Orange		Green		Blue	
19	7	2013		Orange		Green	Purple		
20	8	NR		Orange	Red				
21	8	NR		Orange	Red				
22	9	NR		Orange	Red	Green			
23	9	NR		Orange	Red				
24	10	NR		Orange	Red	Green			
25	10	NR	Blue						

Sample	Gym	Year of Purchase ^a	Flame Retardant						None
			PentaBDE	TPHP	FM550	TDCIPP	TBPP	Mixture 1	
26	10	NR							
27	10	NR							
28	11	NR							
		Percentage:	25%	71%	36%	29%	14%	7%	11%

^a Approximate year of purchase

^b Samples purchased by the investigators directly from a distributor

NR=Not reported

FM550 indicates detection of TPHP, isopropylated aryl phosphates, EH- TBB and BEH-TEBP.

Mixture 1 is a proprietary mixture with some similarities to FM550

Sample results from Gyms 1-3 and D were previously reported in Carignan et al. (2013)²⁸

GM (95% CI) concentrations of urinary metabolite (µg/L) in gymnasts and other populations.

Table 2

Region (sample size)	Year	DHPH	BDCIPP	TBBA	Reference
US Gymnasts (n=11 ^a)	2012	9.0 (7.7-11.1)	0.67 (0.52-0.85)	0.018 (0.01-0.02)	This study
North Carolina (n=53)	2012	1.7 (1.36-2.18) ^b	0.63 (0.49-0.81) ^b	0.006 (0.004-0.008) ^b	Hoffman et al. (2015)
United States (n=9)	2011	3.0 (NR)	0.41 (NR)	NR	Cooper et al. (2011)
Massachusetts (n=29)	2009	1.9 (1.2-3.0) ^c	0.41 (0.28-0.59)	NR	Carignan et al. (2013)
Massachusetts (n=45)	2002-2007	0.31 (NR)	0.13 (NR)	NR	Meeker et al. (2013)
California (n=14)	2011	0.44 ^d (NR)	0.09 ^d (NR)	NR	Dodson et al. (2014)
Norway (n=48) ^e	2012	0.51 ^e (NR)	0.12 ^e (NR)	NR	Cequier et al. (2015)
Australia (n=35 and 100) ^f	2010-2013	24.4 and 63.4 ^f	1.00 and 0.66 ^f	NR	Van den Eede et al. (2015)

Normalized using a mean specific gravity of 1.024 unless otherwise indicated

NR=not reported

^aIncludes available data from all five time points among 11 participants (n=54 samples, n=53 for TBBA)

^bConfidence intervals obtained from study authors, n=52 for TBBA

^cPreviously unpublished data obtained from the authors

^dNot SG normalized, median concentrations

^eNormalized using a mean SG of 1.015, 244 urine samples total

^fNot SG normalized, 28 pools of 28 individuals and 44 pools of 7 individuals