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=0.02) 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 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.

Keywords: flame retardants, organophosphorous compounds, environmental exposure, gymnastics

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: methylated 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

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[1-3] as they are not chemically bonded to PUF and migrate into the air and dust of indoor environments.[4-7] 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).[1-2] However, other flame retardants continue to be used including Firemaster® 550 (FM550) and tris(1,3-dichloro-2-propyl)phosphate (TDCIPP). 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 isomers[3] (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]

Exposure to TDCIPP and components of the FM550 mixture is widespread in the U.S. with urinary metabolites of these flame retardants detected in over 75% (EH-TBB) and 96% (TPHP and TDCIPP) of adults examined in several studies.[6-10] Children have higher exposures, as they have higher intake rates per body weight than adults.[9] While the human half-lives of the PentaBDE congeners are estimated to be on the order of years,[11] 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.[10,12,13] 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).[12,14-16] An appropriate biomarker for BEH-TEBP exposure has not been identified, as metabolism appears to be slow to minimal and its suspected primary metabolite was not detected in *in vitro* studies with human hepatic S9 fractions.[16]

TDCIPP and components of the FM550 mixture are endocrine disrupting chemicals that can disrupt thyroid hormone action in the body.[6,17,18] Dams exposed to the FM550 mixture had increased serum concentrations of thyroxine (T_4), while their offspring experienced advanced puberty (in females), cardiac hypertrophy (in males), significant weight gain (both sexes) and increased anxiety.[17] TPHP, a component of the FM550 mixture, is a suspected obesogen as it can initiate adipocyte differentiation by binding and activating PPARγ, a nuclear receptor that regulates fatty acid storage and glucose metabolism, and can also antagonize osteogenesis.[19,20] 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 medaka[21] and increased multinucleated germ cells in exposed fetal rat testes.[22] In addition to its impact on circulating levels of thyroxine,[6,18,23] TDCIPP is considered a genotoxic carcinogen by the State of California[24] 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,[25] an experimental model.[26,27]

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.[28] 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) as they were identified at elevated concentrations in air and dust of the gym and in foam from the loose foam pit. 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. 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.[28] 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.[10,14] 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 x 2.0 mm, 2.5 µm particle size, Phenomenex). 2,3,5-triiodobenzoic 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.[29] 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 previously published methods[1,2] 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-isobutlyated triphenyl phosphate (TBPP), a mixture of methylated phenyl phosphates (MPP) and V6. 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.[30]: C_{SG}=(1.024-1)/(SG_i-1) where C_{SG}=SG normalized urinary metabolite concentration and SG_i= SG for an individual sample. 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.[31]

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 borderline significant increase in urinary DPHP among samples collected in the half hour after practice compared to those collected before practice (p=0.07, 50% or 4.43 ug/L increase) as well as compared to the other after practice categories (Table 1). We also observed a non-significant decrease across the after-practice categories. There was a significant change in urinary BDCIPP 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), a non-significant decrease across the after-practice categories and a significant decrease between the first and third after-practice time-point (p=0.01, 38% or 0.23 ug/L decrease). We observed no change in urinary TBBA across the time-points.

In a secondary analysis restricting the number of categories we identified a statistically significant increase in urinary DPHP among samples collected in the half hour after practice compared to those collected before practice (p=0.03).

We observed marked increases in urinary metabolite concentrations for some participants from before to after practice; whereas for others there was little change or a slight decline (Figure 1). 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 µg/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[31] 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 reported[28] resulting in a total of 28 samples from 11 gyms (Table 2). 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 organophophate 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. In contrast, the increase in urinary BDCIPP was smaller and not-statistically significant while no change in urinary TBBA was observed. Urinary metabolites of TDCIPP and EH-TBB thus appear to be influenced by additional factors or other sources of exposure. The stronger finding for DPHP is consistent with the higher concentrations of TPHP previously measured in the gym air (40 to 80 ng/m^3 at two locations in the gym) compared to EH-TBB (5 to 26 ng/m^3) or TDCIPP (8 to 12 ng/m^3),[28] suggesting inhalation may be an important route 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.[28] 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.[32]

Compared to other measured populations in the U.S., our gymnast participants had higher GM concentrations of urinary DPHP and TBBA, but not BDCIPP (Table 3).[7-10] However, care should be taken in comparing our findings to other populations; for example, 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.[32,33] 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,[7,10,34,35] 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.[36-38]

Consistent with reports from rodent studies and a recent investigation of human exposure to TPHP in nail polish,[39] concentrations of urinary metabolites returned to baseline within hours of peak exposure, suggesting relatively rapid absorption and metabolism.[12,15,40] 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 the 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.[39] 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.[10] Similar delays have not been observed for organophosphate flame retardants such as TDCIPP.[12,15]

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 compared to the homes of four coaches.[41] Future research should seek to further quantify these exposures and explore interventions for reducing gymnast 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 PPARγ. [42,43] Several types of halogenated flame retardants including PBDEs,[44,45] TDCIPP[6] and components of the FM550 mixture[17,22] are endocrine disrupting chemicals that can impact thyroid homeostasis, 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.[46,47] 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,[28] 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.

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Appendix I. Tables A1-A2 and Figure A1 are available free of charge via the Internet.

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Figure 1. Urinary metabolites measured in samples collected before and after practice, normalized for specific gravity. Each line represents repeated measures for one individual. For urinary TBBA, note a smaller unit of measure (ng/L) and that one participant with consistently very high levels (> 300 ng/L) is off scale.

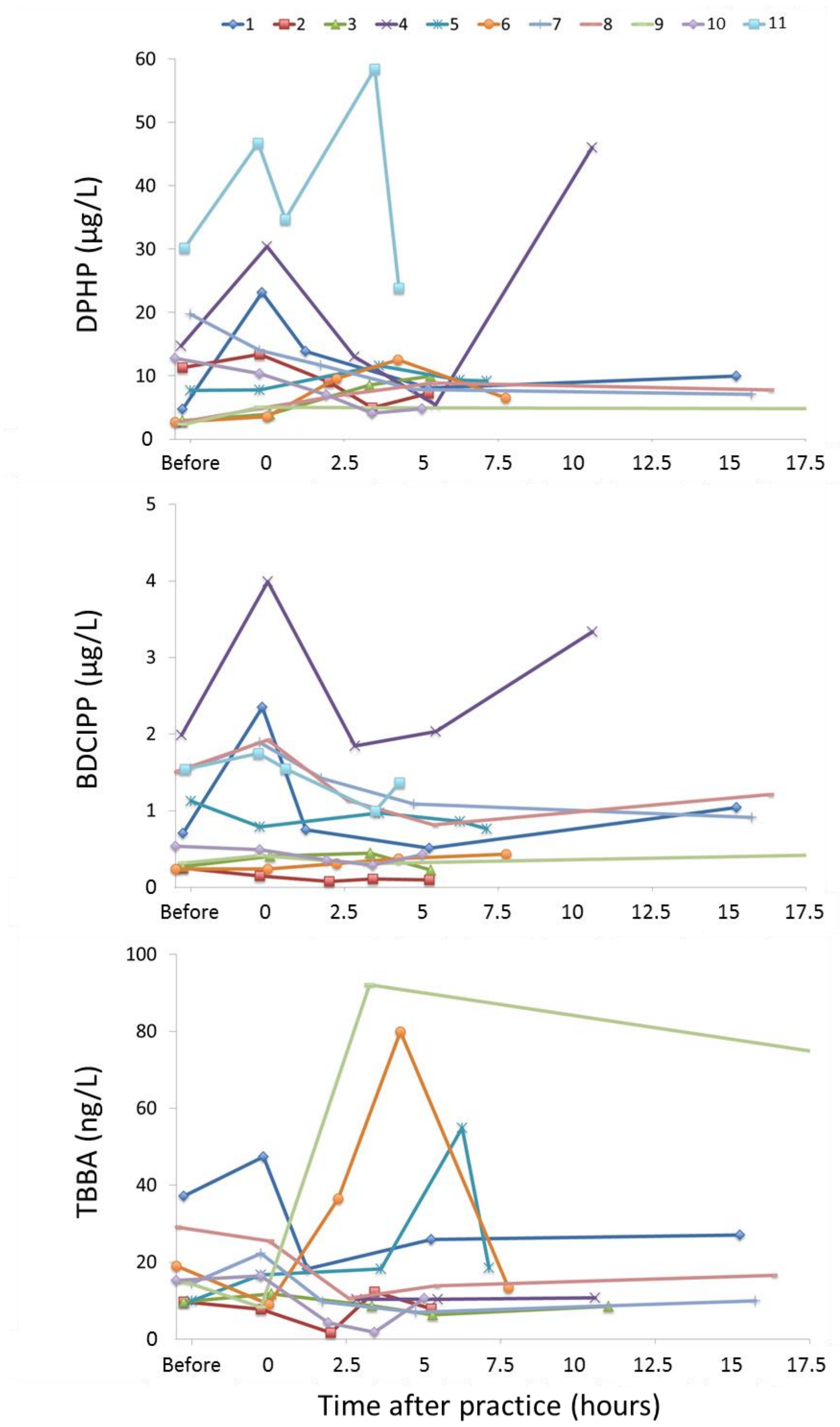
1
2
3
4
5
6
7
8
9
10
11
DPHP (µg/L)
0
10
20
30
40
50
60
BDCIPP (µg/L)
0
1
2
3
4
5
TBBA (ng/L)
0
20
40
60
80
100
Before
0
2.5
5
7.5
10
12.5
15
17.5
Time after practice (hours)

Figure 1.

Table 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.

Urinary Metabolite	Before Practice (n=11)	After Practice		
		< 0.5 hour (n=11)	> 1 to 3 hours (n=15)	> 3 to 17 hours (n=18)
DPHP	7.02 (4.38, 11.24)	10.5 (6.57, 16.9)*	9.55 (6.1, 14.94)	8.99 (5.81, 13.92)[a]
BDCIPP	0.69 (0.39, 1.22)	0.84 (0.47, 1.48)	0.58 (0.33, 1.02)	0.61 (0.35, 1.07)[a,b]
TBBA	0.021 (0.010, 0.045)	0.019 (0.009, 0.041)[1]	0.018 (0.009, 0.037)[a]	0.019 (0.009, 0.038)

[*] Significantly different from before practice (p=0.03).

[a] One sample excluded due to missing metabolite data.

[b] Significantly different from <0.5 hour after practice (p=0.01).

Table 2. Flame retardants in pit cubes from gymnastics training facilitates in the U.S. Gymnasts in the current study trained in Gym 1.

Sample	Gym	Year of Purchase[a]	Flame Retardant						None
			PentaBDE	TPHP	FM550	TDCIPP	TBPP	Mixture 1	
1	1	1990	🟦						
2	1	2001		🟧	🟥				
3	2	1991	🟦	🟧					
4	2	1991	🟦	🟧					
5	2	2001	🟦	🟧					
6	2	2001	🟦			🟩			
7	3	2001	🟦	🟧					
8	4	2007		🟧	🟥				
9	4	2007		🟧	🟥				
10	4	2007		🟧	🟥				
11	4	2007		🟧				🟦	
12	D[b]	2010		🟧	🟥				
13	D[b]	2010				🟩			
14	D[b]	2010				🟩			
15	5	2012		🟧		🟩	🟪		
16	6	2013		🟧			🟪		
17	6	2013		🟧			🟪		
18	7	2013		🟧		🟩		🟦	
19	7	2013		🟧		🟩	🟪		
20	8	NR		🟧	🟥				
21	8	NR		🟧	🟥				
22	9	NR		🟧	🟥	🟩			
23	9	NR		🟧	🟥				
24	10	NR		🟧	🟥	🟩			
25	10	NR	🟦						
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]

Table 3. GM (95% CI) concentrations of urinary metabolite (µg/L) in gymnasts and other populations, specific gravity normalized.

Population	Reference	DPHP	BDCIPP	TBBA
Gymnasts (n=54[a])	Current study	9.0 (7.7-11.1)	0.67 (0.52-0.85)	0.018 (0.01-0.02)
Toddlers (n=26)	Butt et al. 2014[9]	3.0 (1.9-4.9)	5.6 (3.2-9.7)	0.007 (NR)
Mothers (n=22)	Butt et al. 2014[9]	1.9 (1.1-3.4)	2.4 (1.5-3.7)	NR (NR)
Office Workers (n=29)	Carignan et al. 2013[7]	1.9 (1.2-3.0)[c]	0.41 (0.28-0.59)	NR
North Carolina adults (n=53[b])	Hoffman et al. 2015,[8] 2014[10]	1.7 (1.36-2.18)[d]	0.63 (0.49-0.81)[d]	0.006 (0.004-0.008)[d]

NR=not reported due to low detection frequency (27%).

All metabolite concentrations are normalized to specific gravity to adjust for urinary dilution using the same formula.

[a] Number of samples. Includes available data from all five time points among 11 participants, n=53 for TBBA.

[b] n=52 for TBBA

[c] Previously unpublished data obtained from the authors.

[d] Obtained from the authors as comparable statistics.