Cod: 8.2011

GESTATIONAL AND LACTATIONAL TRANSFER OF FIREMASTER® 550 COMPONENTS IN DOSED WISTAR RATS: A TOXICOKINETIC CHARACTERIZATION

A. Phillips[1], A. Chen[1], K. Rock[2], B. Horman[2], H. Patisaul[2], H. Stapleton[1]
[1]*Nicholas School of the Environment, Duke University, Durham.*
[2]*Biology Department, North Carolina State University, Raleigh.*

Introduction

The voluntary phase out of pentaBDE in the United States in 2004 necessitated the development, manufacture, and application of alternative flame retardants suitable for use in polyurethane foam. One of the replacements currently used by foam manufacturers is Firemaster® 550 (FM 550), a technical mixture comprised of triphenyl phosphate (TPP), several isopropylated triarylphosphate ester isomers (ITPs), tetrabrominated ethylhexyl benzoate (TBB) and tetrabrominated diethylhexyl phthalate (TBPH). A 2011 analysis of 102 foam samples collected from residential sofas detected FM 550 more frequently in couches purchased after 2005 than in those purchased prior to 2005, suggesting that FM 550 has gained market share following the penta-BDE phase out.[1] In addition, house dust from many countries around the world has been shown to contain FM 550 components at levels ranging from 3.0 ng/g to 15,030 ng/g.[2–5] FM 550 components were also recently detected in human hair, nails, serum and breastmilk, and the urinary metabolite of TBB, tetrabromobenzoic acid (TBBA), was measured in urine analyses with detection frequencies ranging from 27-98%, indicating that human exposure to FM 550 is widespread.[6–9] Numerous studies have demonstrated the potential toxicity of the components of FM 550, identifying the mixture as endocrine disrupting and the organophosphate constituents as developmentally neurotoxic.[10,11] Despite documented human exposure and known toxicity, there have been relatively few pharmacokinetic studies involving FM 550. The present study characterizes the toxicokinetic properties of FM 550 in dosed Wistar rats, with particular attention paid to the gestational and lactational transfer of its components and their effects on thyroid hormone levels.

Materials and Methods

Materials

Gestational and lactational transfer windows were isolated from one another using two separate experiments. For each experiment, Wistar rats were obtained from Charles River (Raleigh, NC) and maintained under conditions recommended for minimizing unintended EDC exposure.[12–14] FM 550 was provided by Great Lakes Chemical (West Lafayette, IN) and dosing was blinded. Animals were dosed orally using a soy-free food treat pellet (AIN-76A Rodent Diet Test Tabs, Test Diet, Richmond, IN.)

Gestational Transfer Experiment

Adult Wistar rats (n = 18 females and 26 males) were paired and gestational day zero (GD 0) was designated when the presence of a sperm plug was observed. Three exposure groups were included (ethanol vehicle control, 1 mg/kg body weight, and 3.3 mg/kg body weight) and six dams were included per dosing groups. Pregnant dams were dosed daily from GD 9 to GD 18, totaling 10 days of cumulative FM 550 exposure. Four hours after the final dosing on GD 18, dams and fetuses were sacrificed by CO_2 asphyxiation and rapid decapitation. Dam serum and urine were also collected at this time.

Lactational Transfer Experiment

The parameters for impregnation and dosing groups were identical to the gestational transfer experiment. Pregnant dams were dosed daily from PND 3 to PND 12, totaling 10 days of cumulative FM 550 exposure. Four hours after the final dosing on PND 12, dams and pups were sacrificed by rapid decapitation. Dam and pup urine and serum were also collected at this time.

Chemical Analyses

TPP, ITPs, TBB and TBPH levels were analyzed in whole fetus (GD 18) and whole pup (PND 12) extracts. Fetus and pup homogenates were Soxhlet extracted in dichloromethane and purified using

Florisil®.[15] Dam and pooled pup serum was also analyzed for the presence of FM 550 parent components. Serum samples were acidified using formic acid and extracted using ultrasonification. Analytes were isolated from serum extracts using SPE with Waters Oasis HLB columns and extracts were cleaned using SPE with Waters Sep-Pak silica columns.[16] Dam and pup urine were analyzed for metabolites of FM 550, including TBBA, diphenyl phosphate (DPP), and isopropylphenyl phenyl phosphate (ip-PPP). Organophosphate metabolites were extracted from urine using formic acid followed by purification using Phenomenex StrataX-AW SPE columns.[17] TBBA was extracted from urine using acetic acid followed by purification using Agilent SampliQ OPT SPE columns.[8] FM 550 parent compounds were quantified according to previously published GC-MS methods; FM 550 metabolites and thyroid hormones were quantified using previously published LC-MS/MS methods.[5,17–19]

Results and Discussion

TBB body burdens (total mass in whole body) were 200-300-fold higher in pups than in fetuses, signifying that transfer of TBB occurs to a greater extent during lactation than during gestation (**Figure 1**). Similar results have been demonstrated for PCB 153 and p,p'-DDE, which, like TBB, have $\log K_{OW}$ values near 7.[20,21] There is evidence that suggests that very low density lipoproteins (VLDL) play a role in the redistribution of lipophilic compounds during lactation by providing molecular binding sites for these chemicals.[22,23] As such, TBB may enter milk by passive diffusion and by binding to sites on VLDL and later be transferred from dam to pup during nursing. The relatively low levels of TBB in fetuses may be explained by a lipid gradient present during pregnancy that deters the movement of lipophilic compounds from maternal plasma to fetal plasma.[24]

Urinary TBBA concentrations were 5-7-fold higher in gestating rats compared to lactating rats. It is possible that more TBB is cleared from dams through the urine during gestation than during lactation due to lower amounts of VLDL present in gestational compared to lactational stages of pregnancy. It is also possible that this urinary difference results from simple dilution, as the water intake of rats has been shown to vary over pregnancy and nursing.[25]

Interestingly, TBPH body burdens in pups were only 13-14 times higher than body burdens in fetuses. It is likely that TBPH does not partition from serum to milk as readily as TBB, because compounds with higher molecular weights have been shown to exhibit lesser blood to milk partitioning than compounds of the same class with lower molecular weights.[26]

TPP and ITPs were <MDL in both the fetus and pup homogenates, indicating that they did not undergo gestational or lactational transfer. However, DPP and ip-PPP were detected in the urine of dams and displayed a dose response, supporting the rapid metabolism of TPP and ITPs by the dam. No differences in DPP and ip-PPP urinary levels were observed in dams from the gestational and lactational exposures. This is the first study to confirm ip-PPP as a urinary metabolite of ITPs in a controlled study, confirming its utility as a biomarker of FM 550 exposure.

TBB and TBPH were detected in the serum of dams in the high dose group only, and TPP and ITPs were <MDL in dam serum across dosing groups. None of the components of FM 550 were detected in pup serum.

Pup thyroid hormone levels were unaffected as a result of FM 550 exposure; however, changes in thyroid hormones were observed in lactating dams. Compared to controls, total triiodothyronine (TT3) levels in the serum of lactating dams in the low dose group were significantly elevated. A suggestive hyperthyroid trend was also observed for total thyroxine (TT4) in the serum of dams exposed to FM 550 during lactation. Interestingly, this result was not observed for dams exposed to FM 550 during gestation.

Acknowledgements

The authors are grateful to the staff of the NCSU Biological Resource Facility for their supervision of animal care and husbandry, and Meghan Rebuli and Sheryl Arambula for their assistance with tissue collection. FM 550 was provided by Chemtura.

References
1. Stapleton, H. M. et al. Environ. Sci. Technol. 46, 13432–9 (2012).
2. Ali, N., Harrad, S., Goosey, E., Neels, H. & Covaci, A. Chemosphere 83, 1360–1365 (2011).
3. Al-Omran, L. S. & Harrad, S. Emerg. Contam. (2015). doi:10.1016/j.emcon.2015.10.001
4. Dodson, R. E. et al. Environ. Sci. Technol. 48, 13625–13633 (2014).
5. Stapleton, H. M. et al. Environ. Sci. Technol. 42, 6910–6916 (2008).
6. Liu, L.-Y., He, K., Hites, R. A. & Salamova, A. Environ. Sci. Technol. 50, acs.est.5b05073 (2016).
7. Zhou, S. N. et al. Environ. Sci. Technol. 48, 8873–80 (2014).
8. Hoffman, K. et al. Environ. Health Perspect. 122, 963–9 (2014).
9. Butt, C. M., Congleton, J., Hoffman, K., Fang, M. & Stapleton, H. M. Environ. Sci. Technol. 48, 10432–8 (2014).
10. Behl, M. et al. Neurotoxicol. Teratol. 52, 181–93 (2015).
11. Pillai, H. K. et al. Environ. Health Perspect. (2014).
12. Hunt, P. A., Susiarjo, M., Rubio, C. & Hassold, T. J. Biol. Reprod. 81, 807–13 (2009).
13. Li, A. A. et al. Neurotoxicology 29, 504–19 (2008).
14. Richter, C. A. et al. Reprod. Toxicol. 24, 199–224
15. Van den Eede, N., Dirtu, A. C., Ali, N., Neels, H. & Covaci, A. Talanta 89, 292–300 (2012).
16. Butt, C. M., Miranda, M. L. & Stapleton, H. M. Anal. Bioanal. Chem. 408, 2449–59 (2016).
17. Cooper, E. M., Covaci, A., van Nuijs, A. L. N., Webster, T. F. & Stapleton, H. M. Anal. Bioanal. Chem. 401, 2123–2132 (2011).
18. Stapleton, H. M. et al. Environ. Sci. Technol. 43, 7490–7495 (2009).
19. Butt, C. M., Congleton, J., Hoffman, K., Fang, M. & Stapleton, H. M. Environ. Sci. Technol. 48, 10432–8 (2014).
20. Lyche, J. L., Skaare, J. U., Larsen, H. J. S. & Ropstad, E. Chemosphere 55, 621–9 (2004).
21. You, L. et al.Toxicol. Appl. Pharmacol. 157, 134–144 (1999).
22. Becker, M. M. & Gamble, W. J. Toxicol. Environ. Health 9, 225–34 (1982).
23. Spindler-Vomachka, M. & Vodicnik, M. J. J. Pharmacol. Exp. Ther. 230, 263–8 (1984).
24. Parker, C. R., Deahl, T., Drewry, P. & Hankins, G. Early Hum. Dev. 8, 289–95 (1983).
25. Cao, J. et al. Reprod. Toxicol. 58, 282–294 (2015).
26. Schecter, A., Colacino, J., Sjodin, A., Needham, L. & Birnbaum, L.. Chemosphere 78, 1279–1284 (2010).

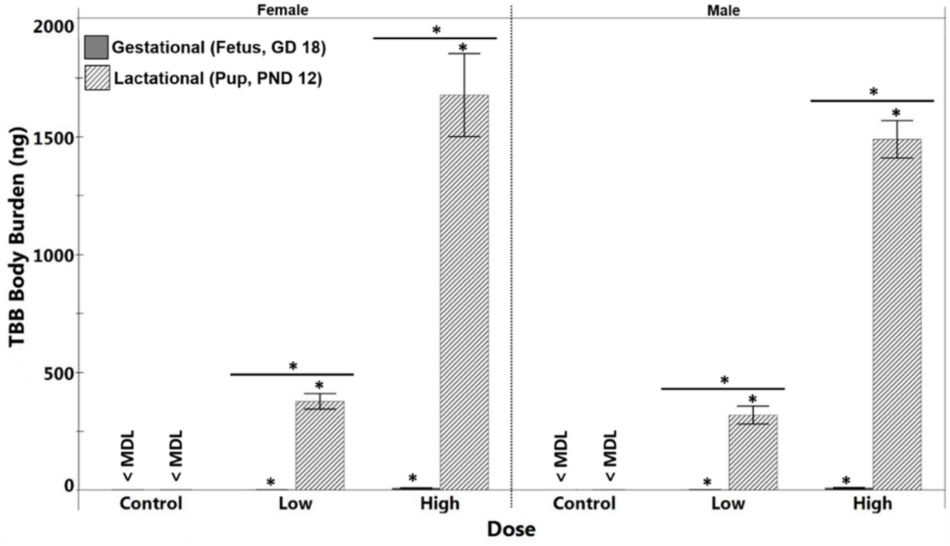

Figure 1. TBB body burdens in pups resulting from lactational transfer are 200-300 times higher than body burdens in fetuses resulting from gestational transfer.