Occurrence of Butyltin Compounds in Human Blood

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Residues of butyltin compounds, including mono- (MBT), di- (DBT) and tributyltins (TBT), were measured in human blood collected from central Michigan, U.S.A. MBT, DBT, and TBT were detected in 53, 81, and 70% of the 32 blood samples examined. Concentrations of butyltins were in the order of MBT > DBT ≥ TBT, with total butyltin concentrations ranging from less than the limit of detection to 101 ng/mL. Exposure of humans to butyltin compounds used as stabilizers or as biocides in household articles has been regarded as a source in addition to the ingestion of contaminated foodstuffs. There was no significant difference in concentrations of butyltin compounds between sexes. Concentrations of butyltin compounds did not exhibit pronounced age-dependency, which is different from those observed for persistent pollutants such as polychlorinated biphenyls (PCBs). In general, concentrations of butyltins measured in blood were less than that affected human natural killer lymphocytes (a primary immune defense against tumor and virally infected cells). The toxicological significance of the concentrations of butyltins observed in this study is unknown. However, the potential for sporadic incidences of great exposure and possible synergistic effect on immune function when exposed in mixtures suggest a need for further investigations to evaluate sources and effects of butyltins in humans.

Introduction

Butyltin compounds including mono- (MBT), di- (DBT), and tri-butyltins (TBT) have been used as biocides or as stabilizers in a wide range of industrial and agricultural applications (*1*−*4*). Among the major uses of butyltins, heat stabilization of poly(vinyl chloride) (PVC) polymers by MBT and DBT represents a great proportion (>50%) of the global consumption (*5*). MBT and DBT are also used as catalysts in the production of polyurethane and silicone. On the other hand, TBT has been used primarily as an antifouling agent in paints applied on ships, boats, aquaculture nets, and docks and as a slimicide in cooling towers and in wood preservation (*4*, *6*).

While effects of TBT released from its use in antifouling paints has received considerable attention (*4, 7, 8*), contamination by MBT and DBT released from their use in commercial and household articles has received less attention. Particularly, the extent of exposure of humans to butyltins is relatively unknown. Occurrence of MBT and DBT in municipal wastewater and sewage sludge suggested the release of these compounds from a variety of household articles (*9*−*12*). Several household commodities consisting of polyurethane, plastic polymers, and silicones contained butyltin concentrations on the order of parts-per-million (μg/g) (*13, 14*). Butyltin compounds are also present in seafood such as fish and oysters collected from coastal areas (*15*−*19*). Despite the possibility of human exposure to butyltins from various sources, few studies have examined butyltins in human tissues. Concentrations of MBT, DBT, and TBT in the livers of Japanese were in the range of 14−22, 45−78, and <2 ng/g, wet wt, respectively (*14*). Total butyltin concentrations in human livers collected from Poland were in the range of 2.4−11 ng/g, wet wt (*20*). Blood is a good indicator of exposure to butyltins, which is exemplified by the binding of butyltin compounds to blood proteins in fish (*21*). In this study, we report for the first time concentrations of butyltin compounds in human blood.

Materials and Methods

Samples. Whole blood samples were collected from 17 male and 15 female volunteers during a blood drive organized by American Red Cross in central Michigan, U.S.A., in July 1998. Approximately 5 mL of blood drawn from each of the 32 volunteers (Table 1) was stored in vacutainers at 4 °C for 2 days and then at −20 °C until analysis.

Butyltin Analysis. The analytical method used for the determination of MBT, DBT, and TBT in whole blood has been described in detail elsewhere (*16, 22, 23*). Briefly, acidified blood samples (2−3 mL) were homogenized with 70 mL of 0.1% tropolone in acetone, and the solvent was transferred to 100 mL of 0.1% tropolone in benzene. Moisture in the organic extract was removed using 35 g of anhydrous sodium sulfate. The sample was concentrated to 5 mL using a rotary evaporator at 40 °C. The concentrated extract was propylated by a Grignard reaction with *n*-propylmagnesium bromide, ca. 2 mol/L in tetrahydrofuran (Tokyo Chemical Industries, Portland, OR). The derivatized extract was purified by passing it through a column packed with 6 g of wet Florisil. The eluant from the Florisil column was rotary evaporated to 0.5−3 mL.

Butyltin compounds were quantified by capillary gas chromatograph with flame photometric detection (GC-FPD). Chromatographic separation was performed on a Hewlett-Packard 5890 series II gas chromatograph with a 30 m × 0.25 mm (i.d.) DB-1 capillary column coated at 0.25 μm film thickness. The flame photometer was operated using a hydrogen-air-nitrogen flame and was equipped with a 610 nm band-pass filter that is selective for tin-containing compounds.

One hundred nanograms each of butyltin trichloride, dibutyltin dichloride, and tributyltin chloride were spiked into the liver of a homogenized sample of lake trout (*Salvelinus namaycush*), containing butyltins that were less than the limit of detection, passed through the whole analytical procedure, and used as an external/matrix recovery standard. Freshly derivatized external standards prepared along with every set of eight samples were used to estimate concentrations. Concentrations were quantified by comparing peak heights of butyltins in samples with those in the external standards. Tributylhexyltin (synthesized by the reaction of *n*-hexylmagnesium bromide with tributyltin chloride) was added to each sample as an internal standard

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TABLE 1. Concentrations (ng/mL) of Butyltin Compounds in Human Blood

age	MBT	DBT	TBT	BTs[b]
		Male		
36	13	5.1	7.2	25.3
59	21	9.4	6.9	37.3
64	27	10	11	48
68	<7[a]	<2.5	2.8	2.8
60	<7	<2.5	<1	ND[c]
48	<7	<2.5	<1	ND
48	<7	<2.5	2.9	2.9
41	18	9	7.1	34.1
65	8	4	3	15
46	<7	4.9	6.9	11.8
51	<7	3.6	4.1	7.7
48	18	4.3	8.7	31
27	18	4.1	6.5	28.6
40	15	3.6	5.8	24.4
79	9.5	6.3	10	25.8
64	<7	3.9	<1	3.9
67	<7	4.8	4.5	9.3
mean	**8.68**	**4.29**	**5.14**	**18.1**
SD	**9.11**	**3.08**	**3.28**	**14.4**
		Female		
56	17	10	8.9	35.9
56	<7	3.8	<1	3.8
56	<7	<2.5	<1	ND
64	23	10	11	44
46	10	4.4	2.9	17.3
64	<7	4.4	2.4	6.8
47	8	<2.5	<1	8
55	14	4.9	<1	18.9
32	11	3	4.8	18.8
29	<7	4.6	4.8	9.4
70	<7	13	39	52
81	<7	16	85	101
63	<7	2.7	4.9	7.6
47	13	3.4	4.8	21.2
78	18	5	5.8	28.8
mean	**7.60**	**5.68**	**11.6**	**24.9**
SD	**7.9**	**4.4**	**21.7**	**25.0**
overall mean	**8.17**	**4.94**	**8.18**	**21.3**
SD	**8.56**	**3.83**	**15.4**	**20.4**

[a] Values below the detection limit were assigned zero for calculating arithmetic mean. [b] BTs = MBT + DBT + TBT. [c] ND = Not detected.

prior to extraction. A procedural blank was analyzed with every set of eight samples to check for interfering compounds and to correct sample values, if necessary. MBT, probably originating from commercial solvents or reagents, was found at trace levels in reagent blanks. The values obtained for MBT in samples were, therefore, corrected for blank concentrations. However, blanks never contained DBT or TBT. The detection limits of MBT, DBT, and TBT were 7.0, 2.5, and 1.0 ng/mL, respectively. The average recoveries of monobutyltin trichloride, dibutyltin dichloride, and tributyltin chloride dissolved in hexane, spiked into the sample matrix, and passed through the whole analytical procedure were between 90 and 110% for each compound. All concentrations reported in this study refer to butyltin species as the corresponding ion, and they were not corrected for recovery of the internal standard, which was 102 ± 32%. Vacutainers filled with distilled water were also analyzed to check for butyltin contamination from containers used to store blood samples. Butyltin compounds were not found in vacutainers. Representative GC-FPD chromatograms of butyltin standard and blood samples are shown (Figure 1). Mean butyltin concentrations were compared by paired *t*-test ($\alpha = 0.05$).

Results and Discussion

MBT, DBT, and TBT were observed at concentrations greater than the method detection limits in 53, 81, and 70%, respectively, of the blood samples analyzed (Table 1). Mean (± SD) concentrations of MBT, DBT, and TBT for those samples with detectable concentrations were 15.4 ± 5.2, 6.08 ± 3.33, and 10.5 ± 16.7 ng/mL, respectively. When butyltin values for samples containing concentrations less than the limit of detection were assigned a value of zero, MBT concentrations declined significantly ($p < 0.01$) (8.17 ± 8.56 ng/mL), while the concentrations of DBT and TBT did not differ significantly ($p > 0.05$) (4.94 ± 3.83 and 8.18 ± 15.4 ng/mL, respectively). Total butyltin (BTs = MBT + DBT + TBT) concentrations (21.3 ± 20.4 ng/mL) were not significantly different when a value of zero was assigned for nondetectable observations ($p > 0.05$). Median, mode, and geometric mean of butyltin concentrations in human blood are reported in Table 2. For further discussions, concentrations of butyltin compounds for nondetectable observations were assigned a value of zero, unless specified otherwise.

The greatest total butyltin and TBT concentrations of 101 and 85 ng/mL, respectively, found in the blood of an 81 year old female donor (Table 1), were greater than 4.5 times the standard deviation of mean concentrations. Similarly, TBT concentration of 39 ng/mL, found in blood that had the second greatest concentration, was 4.5 times the standard deviation of mean. Therefore, these values appear to qualify as outliers. When these two greatest concentrations were removed from the data set, the mean (± SD) concentrations of MBT, DBT, and TBT were 8.72 ± 8.57, 4.31± 3, and 4.59 ± 3.37 ng/mL, respectively. Nevertheless, the samples with the greatest TBT and total butyltin concentrations were analyzed twice and the results were confirmed (Figure 1). Even though the two greatest values represent valid results, because these values greatly increased the mean concentrations and were represented by only a few observations, for further discussions, the two samples with the greatest butyltin concentrations were excluded from the means.

Occurrence of butyltin compounds in human blood suggests possible widespread exposure of humans to these compounds. Two major categories of butyltin sources to which humans can be exposed are suggested; indirect exposure from household items that contain butyltin compounds and direct exposure through the ingestion of contaminated foodstuffs. While DBT was observed to occur most frequently, concentrations of MBT were the greatest in blood. This suggests exposure to MBT and DBT, which are used as stabilizers or as catalysts in a variety of household commodities. For example, diaper covers made up of polyester fabrics and sanitary napkins made up of nylon and polyurethane contained up to 33.7 and 5.5 μg/g DBT, respectively (*13*). Baking parchments that are used in preparing bakery items contained MBT, DBT, and TBT concentrations of up to 130, 140, and 0.8 μg/g, respectively (*14*). MBT and DBT in these parchments originate from silicones with which they are impregnated. The source of MBT and DBT in silicones is from their use as catalysts in silicone production. Cookies, that were prepared using the siliconized baking parchment, contained MBT, DBT, and TBT concentrations of up to 260, 720, and 15 ng/g, respectively (*14*). Similarly, certain brands of gloves, cellophane wrap, and sponges have been reported to contain MBT and DBT (*14*).

Foodstuffs stored in containers made up of PVC polymers have been reported to contain butyltin compounds (*24, 25*). Several brands of wine collected across Canada contained MBT (1.7−20 μg/L), DBT (0.3−160 μg/L), and TBT (0.8−1.6 μg/L) (*24*). Similarly, low (<0.06−0.2 and <0.08−0.3 ng/mL for MBT and TBT, respectively) but detectable concentrations of butyltins have been measured in fruit juices that were

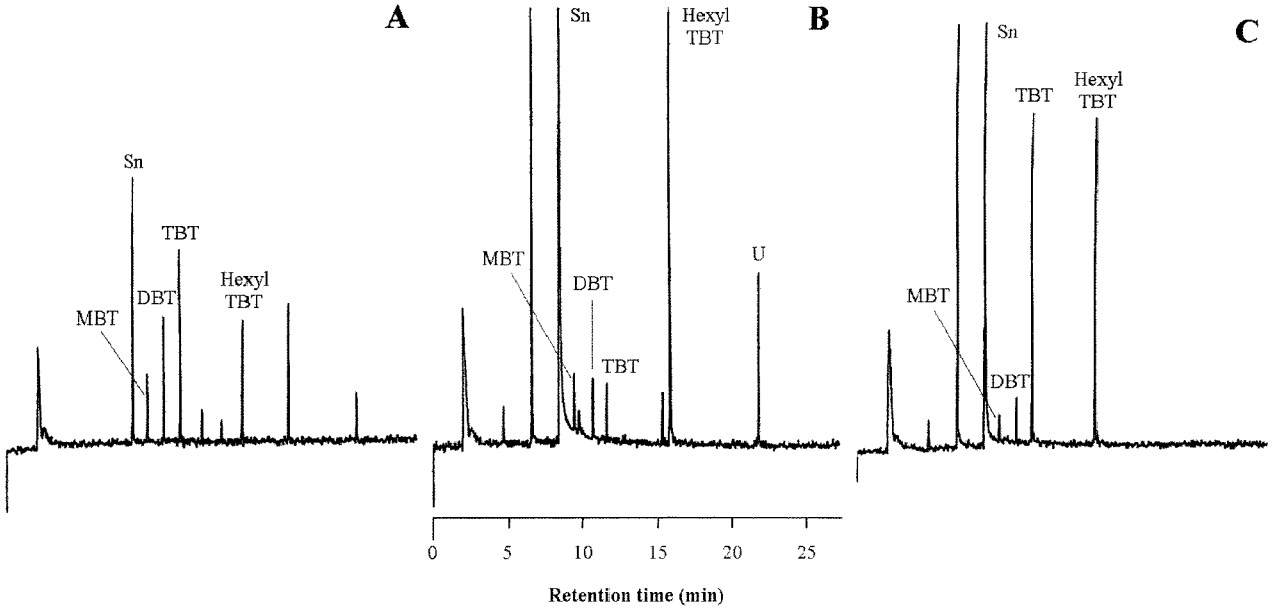

FIGURE 1. GC-FPD chromatograms of butyltin standard (A) and human blood extracts (B and C). B: Blood sample showing typical pattern of butyltin contamination (17, 10, and 8.9 ng/mL of MBT, DBT, and TBT, respectively). C: Sample with the greatest TBT concentration (<7, 16, and 85 ng/mL of MBT, DBT, and TBT, respectively). Hexyl TBT is internal standard. U: unknown peak. Final volume of the sample extract varied (0.5−3 mL) depending on the peak resolution.

TABLE 2. Median, Mode, and Geometric Mean (ng/mL) of Butyltin Concentrations in Human Blood

function	MBT	DBT	TBT	BTs[a]
median	15.0	4.70	5.80	18.1
mode	18.0	10.0	4.80	NA[b]
geometric mean	14.5	5.40	6.58	16.4

[a] BTs = MBT + DBT + TBT. [b] Not available.

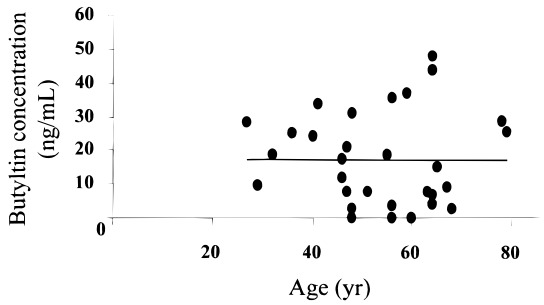

FIGURE 2. Relationship of total butyltin concentrations in human blood with age (outliers excluded).

stored with acidic/alcoholic preservatives (*25*). MBT has been detected (4.6 ng/L) in tap water that passed through PVC pipes (*26*). Although these studies have reported a low incidence of butyltin occurrence, the potential for human exposures from the above sources cannot be ignored. Aquatic food products such as fish, bivalves (oysters), and crab are another source of human exposure to butyltins (*15, 16, 20, 22, 27−29*). Dibutyltin dilaurate has been used as an anthelmintic and coccidiostat in turkey and poultry production. The livers of turkeys contained DBT concentrations in the range of <0.2−6 μg/g, wet wt, whereas those in muscle were less than the method detection limit of <0.2 μg/g, wet wt (*30*). Studies have reported that cooking was not effective in eliminating butyltin compounds from foods (*14, 29, 31*). The estimated daily intake of TBT (from cooked food) based on a market basket survey conducted in 1992 in Shiga, Japan, was 6.7 μg/person (*31*). The daily intake estimates for total butyltins in contaminated fish from certain Asian countries were in the range of <0.027−2.1 μg/person (*16*). These observations support the conclusion that humans are exposed to butyltin compounds via foods.

Our results have established for the first time that butyltins occur in human blood and suggest that human exposure to butyltin compounds can be widespread due to their presence in a variety of sources. Concentrations of butyltin compounds between sexes were not significantly different ($p > 0.05$). Also, when the outliers were excluded, concentrations of butyltins in human blood were not age-dependent for either sex ($r = 0.0002$) (Figure 2). This is different from those generally observed for persistent pollutants such as polychlorinated biphenyls, which tend to increase with age (*32*). Lack of an age-dependent increase in butyltin concentrations suggests that humans may excrete butyltins relatively rapidly or that butyltins are cleared from the blood by binding to other tissues. Although TBT is presumed to be metabolized in humans, the presence of greater proportions of TBT in two of the 32 blood samples suggests possible recent exposure (Figure 1). Exposure to TBT used as a disinfectant in waxes, polishes, sprays, and in laundry washes (*2*) to which these individuals may have been exposed could explain the relatively greater concentrations in the two samples.

The toxicological implications of the concentrations of butyltins measured in human blood are currently unknown, but because they were measured in blood they could have direct effects on haematological parameters and have the potential to be carried to other body tissues. DBT and TBT caused immune suppression in rodents (*33, 34*). Viability of human thymocytes, which play a central role in immunity, was decreased by approximately 50% in 24 h following exposure in vitro to 500 ng of DBT/mL (*35*). MBT, DBT, and TBT affected natural killer (NK) lymphocytes (a primary immune defense against tumor and virally infected cells) in human blood at concentrations of 5 μM, 1.5 μM, and 200 nM, respectively, after 24 h of exposure in vitro (*23*). Although the measured concentrations of MBT, DBT, and TBT in blood were approximately 5−50 times less than those affected NK lymphocytes, studies have reported synergistic effect of MBT, DBT, and TBT when exposed in combination (*23*). Similarly, butyltin compounds in blood can interact with other classes of contaminants such as organochlorines, which would lead

to adverse effects (*36*). Moreover, concentrations of 39 and 85 ng of TBT/mL measured in whole blood of two individuals are close to concentrations known to cause adverse effects on NK lymphocytes. Further studies are needed to evaluate the sources and long-term toxic effects of butyltin compounds in humans.

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Literature Cited

(1) Evans, C. J.; Karpel, S. *Organotin Compounds in Modern Technology;* Elsevier: Amsterdam, 1985; 279 pp.
(2) Maguire, R. J. *Water Pollut. Res. J. Canada* **1991**, *26*, 243−360.
(3) Ainsworth, S. J. *Chem. Eng. News* **1992**, *70*, 34−52.
(4) Fent, K. *Crit. Rev. Toxicol.* **1996**, *26*, 1−117.
(5) Maguire, R. J.; Long, G.; Meek, M. E.; Savard, S. *Canadian Environmental Protection Act Prority Substances List Assessment Report − Nonpesticidal Organotin Compounds*; Department of the Environment, Commercial Chemicals Evaluation Branch, Ottawa, Ontario K1A 0H3, Canada, 1993; 32 pp.
(6) Luijten, J. G. A. In *Organotin Compounds*; Sawyer, A. K., Ed.; Dekker: New York, 1972; Vol. 3, p 931.
(7) Huggett, R. J.; Unger, M. A.; Seligman, P. F.; Valkirs, A. O. *Environ. Sci. Technol.* **1992**, *26*, 232−237.
(8) Kannan, K.; Guruge, K. S.; Thomas, N. J.; Tanabe, S.; Giesy, J. P. *Environ. Sci. Technol.* **1998**, *32*, 1169−1175.
(9) Fent, K.; Müller, M. D. *Environ. Sci. Technol.* **1991**, *25*, 489−493.
(10) Chau, Y. K.; Zhang, S.; Maguire, R. J. *Sci. Total Environ.* **1992**, *121*, 271−281.
(11) Donard, O. F. X.; Quevauviller, P.; Bruchet, A. *Water Res.* **1993**, *27*, 1085−1089.
(12) Kannan, K.; Senthilkumar, K.; Sinha, R. K. *Appl. Organomet. Chem.* **1997**, *11*, 223−230.
(13) Yamada, S.; Fujii, Y.; Mikami, E.; Kawamura, N.; Hayakawa, J.; Aoki, K.; Fukaya, M.; Terao, C. *J. AOAC Int.* 1993, *76*, 436−441.
(14) Takahashi, S.; Mukai, H.; Tanabe, S.; Sakayama, K.; Miyazaki, T.; Masuno, H. *Environ. Pollut.* **1999**, in press.
(15) Suzuki, T.; Matsuda, R.; Saito, Y. *J. Agric. Food Chem.* **1992**, *40*, 1437−1443.
(16) Kannan, K.; Tanabe, S.; Iwata, H.; Tatsukawa, R. *Environ. Pollut.* **1995**, *90*, 279−290.
(17) Senthilkumar, K.; Duda, C.; Villeneuve, D. L.; Kannan, K.; Falandysz, J.; Giesy, J. P. *Environ. Sci. Pollut. Res.* **1999** In press.
(18) Garcia-Romero, B.; Wade, T. L.; Salata, G. G.; Brooks, J. M. *Environ. Pollut.* **1993**, *81*, 103−111.
(19) Alzieu, C.; Sanjuan, J.; Deltreil, J. P.; Borel, M. *Mar. Pollut. Bull.* **1986**, *17*, 494−498.
(20) Kannan, K.; Falandysz, J. *Mar. Pollut. Bull.* **1997**, *34*, 203−207.
(21) Oshima, Y.; Nirmala, K.; Go, J.; Yokota, Y.; Koyama, J.; Imada, N.; Honjo, T.; Kobayashi, K. *Environ. Toxicol. Chem.* **1997**, *16*, 1515−1517.
(22) Kannan, K.; Yasunaga, Y.; Iwata, H.; Ichihashi, H.; Tanabe, S.; Tatsukawa, R. *Arch. Environ. Contam. Toxicol.* **1995**, *28*, 40−47.
(23) Whalen, M. M.; Loganathan, B. G.; Kannan, K. *Environ. Res.* **1999** In press.
(24) Forsyth, D. S.; Weber, D.; Dalglish, K. *J. AOAC Int.* **1992**, *75*, 964−973.
(25) Forsyth, D. S.; Weber, D.; Barlow, L. *Appl. Organomet. Chem.* **1992**, *6*, 579−585.
(26) Sadiki, A.-I.; Williams, D. T.; Carrier, R.; Thomas, B. *Chemosphere* **1996**, *32*, 2389−2398.
(27) Espourteille, F. A.; Greaves, J.; Huggett, R. J. *Environ. Toxicol. Chem.* **1993**, *12*, 305−314.
(28) Kannan, K.; Tanabe, S.; Tatsukawa, R. *Bull. Environ. Contam. Toxicol.* **1995**, *55*, 510−516.
(29) Short, J. W.; Thrower, F. P. *Mar. Polut. Bull.* **1986**, *17*, 542−545.
(30) Epstein, R. L.; Phillippo, E. T.; Harr, R.; Koscinski, W.; Vasco, G. *J. Agric. Food Chem.* **1991**, *39*, 917−921.
(31) Tsuda, T.; Inoue, T.; Kojima, M.; Aoki, S. *J. AOAC Int.* **1995**, *78*, 941−943.
(32) Grimvall, E.; Rylander, L.; NilssonEhle, P.; Nilsson, U.; Stromberg, U.; Hagmar, L.; Ostman, C. *Arch. Environ. Contam. Toxicol.* **1997**, *32*, 329−336.
(33) Snoeij, N. J.; Penninks, A. H.; Seinen, W. *Int. J. Immunopharmacol.* **1988**, *10*, 891−899.
(34) Penninks, A. H. *Food Addit. Contam.* **1993**, *10*, 351−361.
(35) Seinen, W.; Vis, J. G.; Spanje, I. V.; Snoek, M.; Brands, R.; Hooykaas, H. *Toxicol. Appl. Pharmacol.* **1977**, *42*, 197−212.
(36) Kannan, K.; Villeneuve, D. L.; Blankenship, A. L.; Giesy, J. P. *J. Toxicol. Environ. Health* **1998**, *55*, 373−384.

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