Bull. Environ. Contam. Toxicol. (1989) 43:225–230

Bulletin of Environmental Contamination and Toxicology

Triaryl/Alkyl Phosphate Residues in Human Adipose Autopsy Samples from Six Ontario Municipalities

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Trialkyl-, tri(haloalkyl)- and triaryl phosphate esters (TAAP) are widely used as flame retardant plasticisers, fire retardant hydraulic fluids and as additives in lubricants, adhesives and coatings (Midwest Research Institute 1979; Environmental Health Directorate 1982). TAAP have been found in Canadian drinking water supplies (LeBel et al. 1981; Williams and LeBel 1981; Williams et al. 1982) and fish (Lombardo and Egry 1979). Tris(1,3-dichloropropyl) phosphate has also been found in human seminal fluid (Dougherty et al. 1981).

As part of a Canada-United States Great Lakes Agreement negotiated to address perceived and emerging problems related to pollution in the Great Lakes Basin, data on the level of potentially toxic chemicals present in human tissue samples were required to provide an insight into human exposure to toxic chemicals and to aid in the assessment of the total intake of contaminants from all sources. A method was developed for the determination of TAAP in human adipose tissue (LeBel and Williams 1983) and some TAAP were found in a limited number of samples analysed. A further study (LeBel and Williams 1986) was conducted to investigate the levels of TAAP in human adipose tissues obtained from two municipalities, Ottawa and Kingston, located at the eastern end of the Great Lakes Basin. The present study was designed to extend the investigation to compare TAAP levels in adipose tissue samples from municipalities in other areas of the Great Lakes Basin.

MATERIALS AND METHODS

Tissue samples, obtained from cadavers at autopsies, were taken from the greater omentum, placed in clean glass vials which were sealed with teflon-lined screw caps and frozen (-20°C) pending analysis. The entire sample, accurately weighed, was extracted with 15%

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acetone/hexane mixture (5 mL/g of tissue) as described in LeBel and Williams (1983). The extract was evaporated to dryness and the residual fat was dissolved in 50% dichloromethane/cyclohexane to obtain approximately 0.2 g fat per mL of solution. An accurate extracted fat concentration was then determined on a 1 mL aliquot. Six mL aliquots were sealed in 10 mL ampules for storage (-20^{o}C) in a bank of tissue extracts established for the analysis of halogenated contaminants (Williams et al. 1988). Selected tissue extracts were subsequently analysed for TAAP. For these TAAP analyses, the solvent was exchanged to 5% dichloromethane/cyclohexane for optimisation of the gel permeation chromatography (GPC) separation step. The GPC separation of the fat/TAAP and the Florisil clean-up of the TAAP extracts were performed as previously described (LeBel and Williams 1983). The GPC and Florisil were calibrated prior to use to ensure proper collection of appropriate fractions.

The gas chromatography (GC) analyses of the extracts were conducted essentially as described previously (LeBel and Williams 1983). However, some modifications were made to improve the GC quantitative analysis: the final extract was concentrated to 0.2 mL in acetone and two internal standards (IS), tri-n-pentyl phosphate and m-cresyl diphenyl phosphate were added to the extracts to give a concentration of 100 pg/uL of each IS. The internal standards were added to correct for possible detector response variation as well as for variations in extract and injection volumes. The extracts were analysed by gas-chromatography using a Perkin-Elmer GC, Model 910, equipped with a nitrogen-phosphorus selective detector (NPD). The GC-NPD operating conditions were as previously reported (LeBel and Williams 1983) except that a 30 m x 0.32 mm id DB-1 column (J&W) was used with a temperature program rate of 6^{o}C/min. Peak integration was carried out using a Spectra-Physics, Model 4000, electronic integrator. The TAAP were quantitated using an internal standard technique involving comparison of sample peak areas, normalised to the IS, with the corresponding normalised peak areas from an appropriate TAAP standard run under similar conditions. Tri-n-pentyl phosphate was used as the IS for the early eluting TAAP (RT < 700 sec) and m-cresyl diphenyl phosphate was used for the TAAP eluting after 700 sec.

Quality assurance/quality control was ensured by carrying out recovery studies on clean fat fortified at 50 and 250 ng TAAP/g fat. Also, a method blank was run with each set of 7 samples and 10 % of the samples were analysed in duplicate. In a selected number of samples the identity of TAAP detected above 10 ng/g were confirmed by GC-MS as previously described (LeBel and

Williams 1986).

RESULTS AND DISCUSSION

Adipose tissue samples were obtained from six Ontario municipalities; Cornwall, London, St. Catharines, Toronto, Welland and Windsor, located on the Canadian side of the Great Lakes Basin. For each site, up to eight tissue extracts of each sex were randomly selected from the tissue bank. Each tissue extract was analysed for the presence of the TAAP's listed in Table 1. The recovery data for the fortified fat samples are also shown in Table 1 and are in agreement with recovery data published previously (LeBel and Williams 1983).

Table 1. Trialkyl/aryl Phosphate Recovery from Fortified Adipose Tissue

Phosphate	RT (sec)	% recoveries (± std. dev.)	
		50 ng/g	250 ng/g
Tri-n-butyl	352	110±14	92± 6
Tris(2-chloroethyl)	420	77± 9	65±12
Di-n-butylphenyl	538	100± 2	97± 4
Tri-n-pentyl (IS)*	575		
Tris(1,3-dichloropropyl)	880	93± 3	88± 7
Triphenyl	914	95± 4	95± 2
Tributoxyethyl	927	90±14	89± 6
m-cresyldiphenyl (IS)*	989		
o-Isopropylphenyldiphenyl	1031	98± 3	95± 1
Tri-o-tolyl	1072	92± 3	89± 2
Tri-m-tolyl	1114	102± 2	99± 1
p-t-butylphenyldiphenyl	1196	99± 2	94± 2
Tri(2,4-xylyl)	1384	69± 9	51± 3

* Internal standards

The TAAP concentrations in the actual samples were calculated on an extracted fat basis. The average percentage extracted fat for all the analysed samples was 71% [female: 70%, range: 33-96%; male: 72%, range: 29-86%].

The results of the study of TAAP in tissue samples from Kingston and Ottawa indicated mainly the presence of two phosphates, tributoxyethyl phosphate (TBEP) and tris (1,3-dichloropropyl) phosphate (TDCP). The results of the present survey also indicate the presence of these two TAAP. No other TAAP were detected above a detection limit, when applicable, of 1 ng/g. The concentrations of TDCP and TBEP in adipose tissue from the six municipalities are shown in Tables 2 and 3. The mean

contaminant levels reported are based on blank corrected values. All method blanks contained significant amounts of tri-n-butyl and tributoxyethyl phosphate which could not be eliminated even with the use of ultrapure solvents and cleaning of all adsorbents and glassware. In order to assess the contamination level and assign detection limits, a procedure blank was run with every set of 7 samples. The mean contamination concentration found in the blanks was 5±3 ng/g for n-butyl phosphate and 10±6 ng/g for tributoxyethyl phosphate. Therefore, using a value of 3 standard deviations above background contamination (Keith et al, 1983) as the detection limit, for n-butyl and butoxyethyl phosphates the detection limits were set at 10 and 20 ng/g respectively.

Differences in TDCP and TBEP levels between the sites and between the sexes were examined by analysis of variance (ANOVA). No statistical differences between sexes were found for either TDCP or TBEP.

Table 2. TriButoxyethyl Phosphate (ng/g) in Human Adipose Tissue

City	Sex	n	Mean[a]	Range	Frequency[b]
Windsor	m	8	ND[c]		
	f	8	ND		
London	m	8	ND		
	f	8	25[d]		1/8
Welland	m	8	ND		
	f	8	ND		
St.Catharines	m	8	ND		
	f	6	ND		
Toronto	m	4	425 ± 39	400 - 483	4/4
	f	3	357 ± 57	320 - 424	3/3
	m+f	7	396 ± 56	320 - 483	7/7
Cornwall	m	8	165 ± 33	ND - 202	3/8
	f	5	198[d]		1/5
	m+f	13	173 ± 32	ND - 202	4/13

[a] Mean ± standard deviation
[b] Based on detection limit of 20 ng/g
[c] Not detected
[d] Single value

Table 3. Tris(1,3-dichloropropyl) Phosphate (ng/g) in Human Adipose Tissue

City	Sex	n	Mean[a]	Range	Frequency[b]
Windsor	m	8	ND[c]		
	f	8	ND		
London	m	8	ND		
	f	8	1.5[d]		1/8
	m+f	16	1.5		1/16
Welland	m	8	14 ± 12	ND - 31	4/8
	f	8	22 ± 10	ND - 32	3/8
	m+f	16	17 ± 11	ND - 32	7/16
St. Catharines	m	8	ND		
	f	6	ND		
Toronto	m	4	ND		
	f	3	ND		
Cornwall	m	8	ND		
	f	5	ND		

[a] Mean ± standard deviation
[b] Based on detection limit of 1 ng/g
[c] Not detected
[d] Single value

TDCP was found in samples from two sites with one site at levels near the detection limit. TBEP was found in samples from three sites. Neither of TDCP and TBEP was detected in samples from St. Catharines or Windsor.

The results of this study are consistent with those from our previous study (LeBel and Williams 1986) which indicated that tris(1,3-dichloropropyl) phosphate and tributoxyethyl phosphate are the main TAAP contaminants in human adipose tissue samples and that there was no significant differences in TAAP levels in males and females. The apparent differences in TAAP levels between sites requires further study.

Acknowledgement. The authors thank R. O'Grady for GC-MS analyses, M. Goddard for assistance in statistical calculations and F.Benoit and I.Chu for their helpful criticism of the manuscript.

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Received March 11, 1988; accepted August 1, 1988.