Bull. Environ. Contam. Toxicol. (1986) 37:41–46

Bulletin of Environmental Contamination and Toxicology

Levels of Triaryl/Alkyl Phosphates in Human Adipose Tissue from Eastern Ontario

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Trialkyl-, tri(haloalkyl)- and triaryl phosphate esters (TAAP's) are widely used as flame retardant plasticizers, fire retardant hydraulic fluids and as additives in lubricants, adhesives and coatings (Midwest Research Institute, 1979; Environmental Health Directorate, 1982). TAAP's have been found in Canadian drinking water supplies (LeBel and Williams, 1981; Williams and LeBel, 1981; Williams et al., 1982) and fish (Lombardo and Egry, 1979). Further, tris(1,3-dichloro-isopropyl) phosphate has also been found in human seminal fluid (Dougherty et al., 1981). Because of the potential for bioaccumulation of some TAAP's in fatty tissues (Saeger et al., 1979), we have developed a method for the determination of TAAP's in human adipose tissue (LeBel and Williams, 1983). Analysis of a small number of samples indicated the presence of some TAAP's in human adipose tissues.

As part of a Great Lakes Water Quality program to study pollution of the Great Lakes and its implication to human health, data on the levels 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. Human adipose tissues, obtained from two Eastern Ontario cities, Kingston and Ottawa, were analyzed for organic phosphate triesters. The tissues had been analyzed previously for halogenated pesticides and polychlorinated biphenyls (Williams et al., 1984) and also polychlorinated dibenzo-p-dioxins and furans (Ryan et al., 1985).

MATERIALS AND METHODS

Tissue samples, obtained from cadavers at autopsies, were taken from the greater omentum and placed in clean glass vials which were sealed with Teflon-lined screw caps and frozen (-20°C) until analyzed. Available patient data in most cases were restricted to age, sex, body weight and autopsy number.

The analytical procedure was similar to that described earlier (LeBel and Williams, 1983). Adipose tissue (3.0 g) was extracted with 15 mL of 15% acetone-hexane (v/v) in the presence of anhydrous sodium sulfate (ca 1 g) using an overhead homogenizer. After

rinsing of the homogenizer shaft, the extract was centrifuged, filtered through anhydrous sodium sulfate and evaporated just to dryness using a rotary evaporator. The extracted fat was dissolved in gel permeation chromatography (GPC) solvent (5% methylene chloride-cyclohexane) and made up to 10 mL. A 1 mL aliquot was taken and concentrated to dryness to determine the % fat in each sample. The TAAP's were separated from the fat by gel permeation chromatography. An aliquot was loaded into a Waters U6K injector modified with a 4.0 mL sample loop and injected onto a calibrated GPC column packed with 50 g of Bio-Beads S-X3 to a height of 26.7 cm and eluted with GPC solvent. The eluent fraction from 0 - 85 mL was discarded and the fractions from 85 - 120 mL (fraction I) and 120 - 355 mL (fraction II) were collected. Fraction I contained tributyl and tributoxyethyl phosphate (TBEP) while fraction II contained tris(2-chloroethyl), tris(1,3-dichloropropyl) (TRIS), triphenyl, o-isopropylphenyldiphenyl (o-IPDP), tri(o-tolyl), tri(m-tolyl), p-t-butylphenyldiphenyl (p-t-BPDP) and tri (2,4-xylyl) phosphates. Fractions I and II were concentrated to a small volume on a rotary evaporator, transferred to graduated centrifuge tubes and further concentrated under a gentle stream of pre-purified nitrogen to 0.3 mL for further Florisil clean-up. The Florisil clean-up procedures were as described previously (LeBel and Williams, 1983).

The extracts were analyzed on a gas chromatograph (Hewlett-Packard Model 5880A) equipped with nitrogen-phosphorus selective detector (NPD). The column was a 30 m x 0.25 mn id DB-5 (J&W) capillary column. The GC was operated under the following conditions: injector-250°C; detector-300°C; oven temperature- initial-60°C (0.5 min), then programmed at 30°C/min to 200°C and finally at 8°C/min to 275°C with a final hold time of 5 min. The carrier gas was nitrogen at 1.5 mL/min with detector make-up gas (nitrogen) flow of 25 mL/min. The detector response was adjusted to obtain full scale deflection for 50 pg injected of triphenyl phosphate. Quantitation was made with the aid of an electronic integrator. The phosphate esters were determined by comparison of peak areas with the corresponding peak area of the appropriate TAAP standard run under similar conditions. All the results were corrected for blank contribution.

Confirmation of the TAAP's was effected by analysis of the extract by GC/EIMS (Finnigan 4500/INCOS system) on a 30 m x 0.25 mm id DB-5 (J&W) fused silica capillary column. The GC oven was temperature programmed at 20°C/min from 40°C-150°C, then at 6°C/min from 150°C-275°C where it was held for 10 minutes. Positive identification was done by selected ion monitoring of at least four characteristic ions which had to be in the correct ratio.

RESULTS AND DISCUSSION

Fifty-eight adipose tissue samples obtained from a Kingston hospital and fifty-seven adipose tissue samples obtained from an Ottawa hospital were analyzed for phosphate triesters. Sixteen samples (eight from each site) had been analyzed by one laboratory

in an earlier study (LeBel and Williams, 1983) and ninety-nine were analyzed by a second laboratory.

The extracted fat was determined for all the samples and the level of TAAP's calculated on an extracted fat basis. The mean % extracted fat for all the tissue samples was 71% with a range of 4.8 - 94.4%. The distribution of % extracted fat for both sites is illustrated in Figure 1.

Preliminary analysis on sixteen samples had shown mainly the presence of tributoxyethyl phosphate (TBEP) and tris(1,3-dichloropropyl) phosphate (TRIS) in the tissue samples (LeBel and Williams, 1983). The analysis of a larger number of samples confirmed that these two phosphates were the main TAAP's found in human adipose tissue from the two cities.

The levels of TBEP and TRIS in the adipose tissue are listed in Tables 1 and 2 respectively and show the mean, range and occurrence according to site and sex. The sensitivity of the GC/MS was not adequate for very low levels of phosphate esters but the presence of the TAAP's was confirmed by GC/MS on a selected number of samples where the concentration was greater than 10 ng/g.

Some fat samples were found to contain high concentrations of both TBEP and TRIS as seen in the range column of Tables 1 and 2. The distribution of TBEP and TRIS in the human adipose tissue is illustrated in Table 3, showing the relatively small number of samples with high levels of TBEP and TRIS. When the TBEP and TRIS values were compared statistically, no significant differences (t-test, 95% confidence limit) were found between the various site/sex combinations (Table 1 and 2).

Because of the ubiquitous presence of TAAP's in various chemicals and equipment (LeBel and Williams, 1981), trace level analysis of TAAP's is difficult and the analyst must be aware of potential analytical problems. Further, interference may differ depending on sources of chemical, apparatus, laboratory, etc. Method blanks in the preliminary analyses from the first lab showed the presence of tributyl phosphate and TBEP as interferences. However, in the second laboratory, the interferences were mainly tributyl and triphenyl phosphates and the method blanks produced during the course of the analyses contained 1.8 ± 1.0 ng (mean ± standard deviation, eight determinations) tributyl phosphate and 3.3 ± 4.2 ng triphenyl phosphate per extract. Due to the presence and variability of tributyl and triphenyl phosphates in the method blanks, no conclusive data can be presented for trace levels of these two TAAP's although tributyl phosphate was detected in three samples (9.0, 10.0 and 103.8 ng/g) and triphenyl phosphate detected in two samples (13.6 and 11.2 ng/g) at levels significantly above

Table 1. Tributoxyethyl phosphate (ng/g)[1] in human adipose tissue

City[2]	Sex	Mean[3]	Range	Frequency
K	M	3.6 ± 3.9	0.7 - 12.9	8/30
	F	8.7 ± 7.7	1.5 - 26.8	12/28
	M+F	6.6 ± 6.8	0.7 - 26 8	20/58
O	M	7.9 ± 6.4	1.0 - 21.7	13/38
	F	28.6 ± 50.0	0.9 - 142.2	8/19
	M+F	15.8 ± 31.7	0.9 - 142.2	21/57
K+O	M	6.3 ± 5.9	0.7 - 21.7	21/68
	F	16.6 ± 32.2	0.9 - 142.2	20/47
	M+F	11.3 ± 23.3	0.7 - 142.2	41/115

1 extracted fat basis
2 K = Kingston, O = Ottawa
3 mean ± standard deviation

Table 2. Tris(1,3-dichloropropyl) phosphate (ng/g)[1] in human adipose tissue

City[2]	Sex	Mean[3]	Range	Frequency
K	M	30.7 ± 79.7	0.5 - 257.1	10/30
	F	19.7 ± 29.2	0.6 - 70.4	11/28
	M+F	24.9 ± 57.6	0.5 - 257.1	21/58
O	M	24.8 ± 31.4	3.9 - 80.0	5/38
	F	48.7 ± 101.1	0.8 - 229.5	5/19
	M+F	36.8 ± 71.7	0.8 - 229.5	10/57
K+O	M	28.7 ± 66.1	0.5 - 257.1	15/68
	F	28.7 ± 59.0	0.6 - 229.5	16/47
	M+F	28.7 ± 28.7	0.5 - 257.1	31/115

1 extracted fat basis
2 K = Kingston, O = Ottawa
3 mean ± standard deviation

the blank values (t-test, 95% confidence limit). None of the other TAAP's, i.e., tris(2-chloroethyl), tri(o-tolyl), tri(m-tolyl), o-IPDP, p-t-BPDP and tri(2,4-xylyl) phosphates were found in the samples at a detection limit of 1 ng/g.

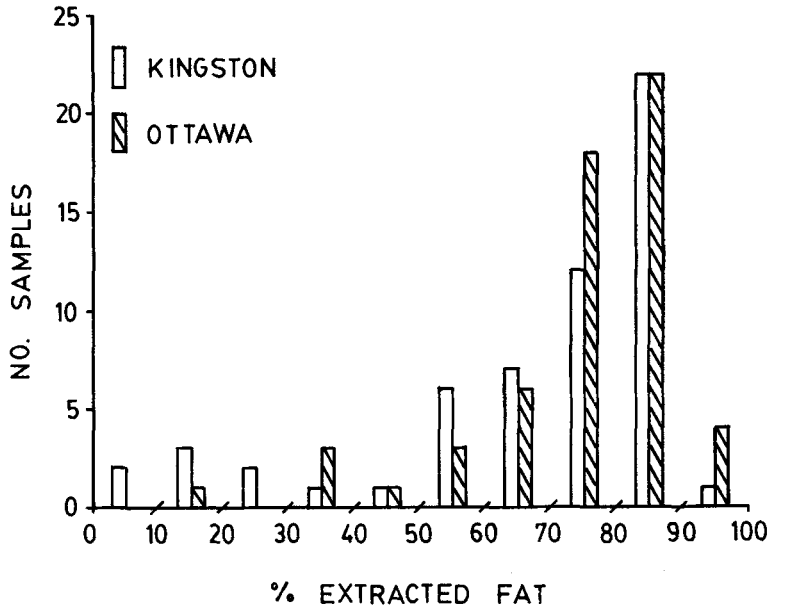

Figure 1. Distribution of extracted fat found in adipose tissue samples

Table 3. Distribution of TBEP and TRIS in human adipose tissue

Level (ng/g)[1]	TBEP		TRIS	
	Kingston	Ottawa	Kingston	Ottawa
>50	0[2]	2	4	2
<50; >10	5	3	3	1
<10	15	16	14	7

1 extracted fat basis
2 number of samples

The results substantiate the data obtained in a limited earlier survey suggesting that TBEP and TRIS were the main TAAP found in adipose tissues. The range and frequency of occurrence suggest that their presence is common, if not ubiquitous. Further monitoring of TAAP's in human adipose tissue over a wider time frame and wider areas is required.

Acknowledgement. Those TAAP analyses done under contract were conducted by Mann Testing Laboratories Limited, Mississauga, Ontario.

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Received October 8, 1985; Accepted October 21, 1985.