Di-n-butyl-and Di-2-Ethylhexyl Phthalate in Human Adipose Tissue

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Introduction

The toxicity and analysis of phthalate esters and their presence in the environment have recently been reviewed by several investigators (ZITKO 1972, HUGOS 1972a, HUGOS 1972b). Human blood stored in plastic transfusion packs was found to be contaminated with di-2-ethylhexyl phthalate (DEHP) (MARCEL 1973). The same ester was also found in other human tissues, including one case of adipose tissue, as a possible result of the use of medical devices (JAEGER and RUBIN 1973). The absence of information on the levels of di-n-butyl phthalate (DBP) and di-2-ethylhexyl phthalate in humans warranted a limited survey of these esters in human adipose tissue. Analytical data presented in this paper were obtained from the fatty tissue of the abdominal region only.

Sampling

Human adipose tissue was collected during autopsies on accident victims. The fat samples were deposited in glass jars, previously acid washed and rinsed with residue free acetone and hexane. The caps were supplied with aluminum foil liners. Samples were immediately frozen and kept frozen until analysed.

Except for 2 samples from Vancouver and one from Montreal, all samples came from the Toronto area.

Analytical Methods

All solvents were of a glass-distilled, residue free grade and checked for the absence of DBP and DEHP. Di-n-butyl phthalate and di-2-ethylhexyl phthalate were supplied by Gulf Oil Canada Ltd. Extraction: The fatty tissue in each jar was cut up into small pieces and thoroughly mixed. A 5 gm sample was extracted for 3 min in a 100 ml mixture of benzene and acetone (1:1 v/v) with a Silverson High Speed homogenizer (Silverson Machines Ltd., Chesham, England). The extract was filtered through anhydrous Na_2SO_4, prewashed with the extraction solvent and the filtrate concentrated on an all-glass rotatory evaporator (<25°C) and made up to 50 ml. A 1 ml aliquot was evaporated in a preweighed aluminum dish to determine lipid content. Cleanup and separation: The fat was removed by low temperature precipitation (MCLEOD and WALES 1972) and

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the fat free extract thus obtained carefully evaporated to dryness at room temperature under a gentle stream of N_2. The residue was redissolved in 1-2 ml of hexane and applied to a 2% Florisil column (deactivated with 2% water after heating for 8 hrs at 300°C) (MCLEOD and RITCEY 1973). The polychlorinated biphenyls and organochlorine pesticides were eluted in 250 ml of 30% methylene chloride in hexane (v/v) and DBP and DEHP were then eluted with 100 ml of 10% ethylacetate in hexane (v/v). The phthalate ester fraction was first concentrated and then evaporated to dryness as above. The residue was redissolved in 5 ml of hexane.

Identification and quantification: A 5 µl aliquot was injected into a Varian Aerograph Series 1200 gas chromatograph with an electron capture detector (Tritium foil) under the following conditions:

Column: $\frac{1}{4}$"x6' glass, packed with 6% OV-210 + 4% SE-30 on Chromosorb W(AW) 60/80 (0.6 gm OV-210 + 0.4 gm SE-30 + 10 gm solid support).

Temperatures: Injector 206°C
Column 202°C
Detector 228°C

To give a retention time of 22 min for DEHP an approximate flow rate of 40 ml N_2/min was used. A standard solution contained 4×10^{-4} µg/µl and 5×10^{-4} µg/µl of DBP and DEHP respectively. A 5 µl injection of standard solution was made before and after each two sample injections. Phthalate esters were quantitated from the chromatograms by using peak heights.

Confirmation: Several phthalate ester fractions were pooled to give 3 samples of approximately 10 µg of estimated phthalates per sample. Thin layer chromatography (TLC) was carried out on a precoated aluminum oxide (type E) F_{254} 20x20 cm plate (Brinkman Instruments (Canada) Ltd.), activated at 110°C for 1 hr. The pooled samples were spotted in duplicate to give 5 µg/spot along with two reference spots at each end of the line of origin, consisting of 16 µg DBP plus 20 µg DEHP per spot. The TLC plate was developed in 10% ethylacetate in hexane until the solvent front reached the top of the plate (~1 hr). The spots were visualized under U.V. light. The area of adsorbent containing sample or blank and corresponding to DBP and DEHP reference spots was removed and the phthalate esters eluted with 3-5 ml of 10% ethylacetate in hexane. The phthalates collected in this manner were rechromatographed under the same GC conditions as above. The cleanest of these rechromatographed samples was subjected to a GLC-MS analysis using low resolution mass spectrometry (Hitachi-Perkin Elmer, RMS4).

Controls: Samples were spiked at the 1 and 5 ppm levels and carried through the entire procedure together with appropriate solvent blanks.

Results and Discussion

The data presented in Figure 1 for DBP were corrected for solvent blanks, while those for DEHP needed no correction. The low levels reported should not be considered as absolute, since spiked samples at the 1 ppm level gave only 45 and 38% of DBP and DEHP respectively, although at the 5 ppm level recovery was 85-100%.

As shown in Fig. 1, the level of DBP and DEHP in most samples was in the 0.10-0.30 and 0.30-1.00 ppm range respectively.

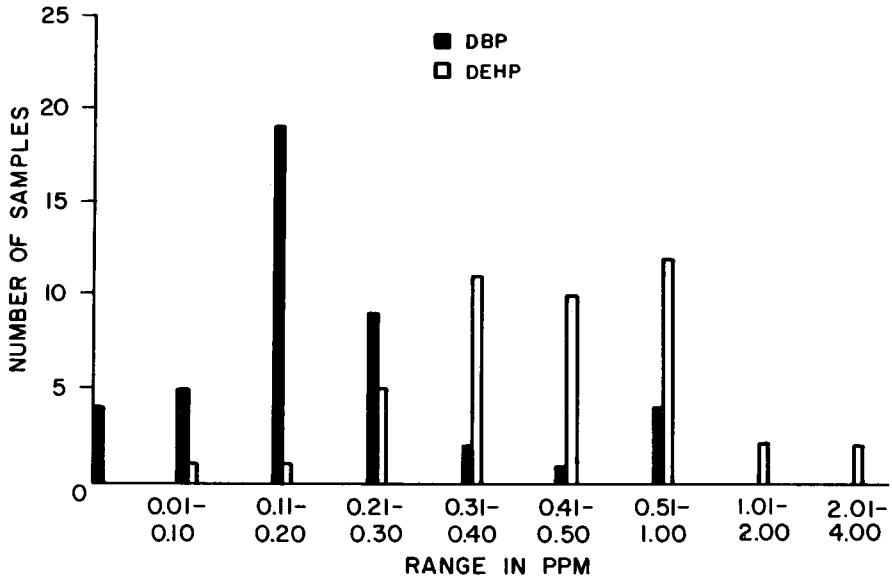

FIG. 1. Histogram of DBP and DEHP in human adipose tissue.

A typical GC elution pattern of DBP and DEHP in human adipose tissue is presented in Fig. 2, together with that of a standard mixture and solvent blank. The height of the peak corresponding to DBP in chromatogram B of Fig. 2 was reasonably constant from blank to blank. An unidentified peak (x) observed in both blank and sample preparations varied in magnitude from sample to sample and was occasionally part of a more complex fingerprint pattern.

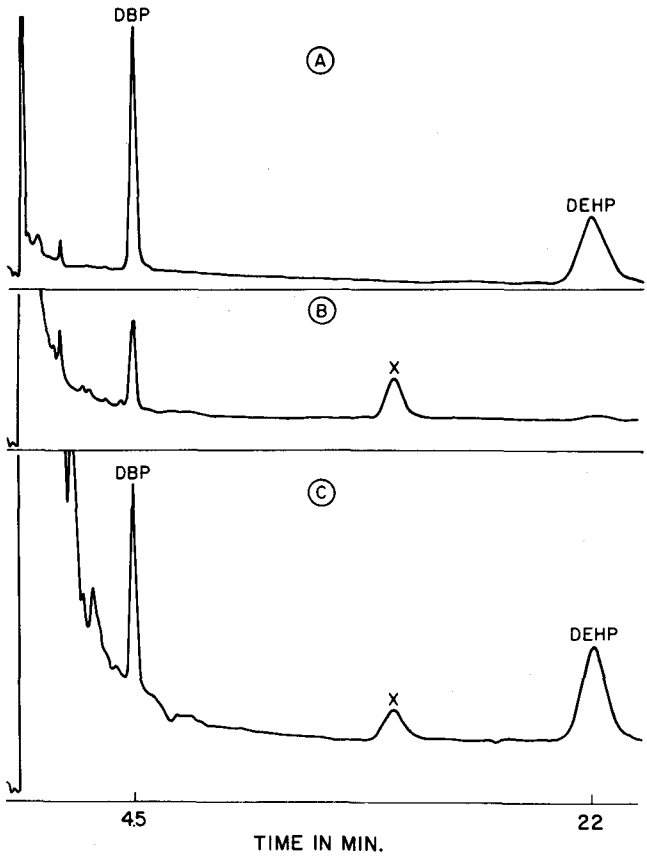

FIG. 2. Chromatogram A represents the GC elution pattern for a standard mixture of phthalate esters. Chromatogram B shows the GC results of a solvent blank. Chromatogram C is that of a typical phthalate ester GC pattern in human adipose tissue.

TLC did not remove the unidentified peak(s), but did confirm the presence of DBP and DEHP. The TLC fraction containing the phthalate esters gave a mass-spectrum similar to that of a standard mixture of DBP and DEHP.

The average lipid content of the human adipose tissue samples was 83.9%±9.2.

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