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Note

Background contamination by phthalates commonly encountered in the chromatographic analysis of lipid samples

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The pollution of the environment with phthalates is becoming a serious problem in analytical biochemistry, because contamination by phthalates from commercial reagents and equipment commonly used in laboratories often leads to errors in the analytical results. It has been reported that the contamination by phthalates from these sources, of grass flavours¹, carbohydrates², ocean biota³ and egg shell lipids⁴ gave interfering peaks in the gas-liquid chromatograms. Asakawa and Genjida⁵ detected phthalates in the plastic apparatus used in their laboratory, and in four organic solvents purchased as extra-pure grade materials (benzene, chloroform, *n*-hexane and light petroleum ether).

As phthalates are fat-soluble, they can generally easily migrate into lipid samples during the extraction of the latter. This paper reports the contamination by phthalates of the commercial reagents and equipment used for the extraction and the chromatographic analysis of lipids, and a method for the removal of these phthalates is discussed.

EXPERIMENTAL

All glassware was washed with water, heated at 250° for 10 h in a stainless-steel oven and/or rinsed with acetone four times and *n*-hexane twice to remove phthalates. Extra-pure grade solvents used for the analysis of phthalates were purchased from Wako (Tokyo, Japan) and distilled in all-glass apparatus. It was confirmed that a 200-fold concentration (100 ml-concentrated to 0.5 ml) did not give any interfering peaks under the conditions used for gas-liquid chromatography (GLC).

GLC analysis

A Hitachi Model 063 gas chromatograph, equipped with a flame-ionization detector and a 2 m × 3 mm I.D. stainless-steel column packed with 1.5% SE-52 on 60-80 mesh Chromosorb W or 1.5% OV-17 on 80-100 mesh Shimalite W, was used. The carrier gas was nitrogen at a flow-rate of 30 ml/min. The oven temperatures were 230° with SE-52 and 240° with OV-17. The injection and detector temperatures were 300°. Individual phthalates were identified on the basis of their retention times compared with those of standards. Dinonyl phthalate was used as an internal standard.

Water. After a water sample (5 l) had been washed with *n*-hexane (200 ml) three times, the combined *n*-hexane extracts were evaporated to dryness. The residue was dissolved in 1 ml of diethyl ether and an aliquot was injected for GLC analysis.

Organic solvents. The phthalate concentration in eleven organic solvents (Wako), which were of guaranteed reagent (GR) grade or chemicals for the analysis of pesticide residues (PR), were analysed. Each organic solvent (100 ml) was evaporated to dryness under reduced pressure, the residue was dissolved in 0.5 ml of diethyl ether and an aliquot was injected for GLC analysis.

Solid reagents. Silicic acid for thin-layer chromatography (TLC) (Type 60, E. Merck, Darmstadt, G.F.R.) and for column chromatography (Mallinckrodt, St. Louis, Mo., U.S.A.), Florisil, ion-exchange resin, anhydrous sodium sulphate and sodium chloride were analysed. Each powdered reagent (20–100 g) was immersed in 100–300 ml of chloroform–methanol (1:1) overnight, filtered through phthalate-free filter-paper and rinsed three times with 100 ml of the same solvent mixture. The combined solvents were evaporated to dryness under reduced pressure and the residue was subjected to GLC.

Aluminium foil and other materials. Aluminium foil and materials such as rubber tubing, rubber stoppers and filter-papers were cut into small pieces, immersed in chloroform–methanol (1:1) overnight and the extracts were analysed by GLC as described above.

RESULTS AND DISCUSSION

Water

Phthalates were detected in all of the water samples analysed (Table I). It is noteworthy that the phthalates were not removed by distillation.

TABLE I

CONCENTRATIONS OF DI-*n*-BUTYL PHTHALATE (DBP) AND DI-2-ETHYLHEXYL PHTHALATE (DEHP) IN ORGANIC SOLVENTS AND WATERS

The organic solvents were purchased as GR materials from Wako.

| Sample | DBP | DEHP | Other unknown peaks |
|-------------------------|----------|----------|---------------------|
| City water* | 2.04 ppb | — | — |
| Well water* | 2.49 ppb | 4.82 ppb | — |
| Tapp water (well water) | 1.93 ppb | 3.85 ppb | — |
| Ion-exchanged water | 0.83 ppb | 1.31 ppb | + |
| Benzene | 0.17 ppm | 1.96 ppm | + |
| Acetone | Trace** | — | — |
| <i>n</i> -Hexane | — | — | — |
| Chloroform | — | — | — |
| Diethyl ether | — | 43.6 ppb | + |
| Methanol | — | 78.7 ppb | + |
| Ethanol | 69.3 ppb | 61.7 ppb | — |
| Light petroleum | — | — | — |
| Dichloromethane | — | — | — |
| Ethyl acetate | — | — | — |
| Acetonitrile | — | 0.18 ppm | — |

* From Tohoku University.

** Less than 10 ppb (10²).

TABLE II
CONCENTRATIONS OF DBP AND DEHP IN SOLID REAGENTS

| Reagent | DBP | DEHP | Other unknown peaks | Company |
|--------------------------------------|----------|----------|---------------------|---------------|
| Silicic acid* | 0.59 ppm | 2.36 ppm | + | E. Merck |
| Silicic acid** | 0.23 ppm | 0.97 ppm | + | Mallinkrodt |
| Wakogel S-1 | 1.61 ppm | 0.84 ppm | — | Wako |
| Floridil (PR)*** | 23.8 ppb | 63.5 ppb | — | Floridin |
| Floridil (EP)† | 91.9 ppb | 87.5 ppb | + | Wako |
| Alumina powder | 41.8 ppb | 44.8 ppb | + | Wako |
| Celite (GR)‡ | 0.12 ppm | Trace*** | — | Wako |
| Activated charcoal powder | 95.8 ppb | 47.9 ppb | — | Wako |
| Na ₂ SO ₄ (EP) | 0.09 ppm | 0.04 ppm | + | Wako |
| Na ₂ SO ₄ (GR) | 24.3 ppb | 16.9 ppb | + | Wako |
| Na ₂ SO ₄ (PR) | 24.3 ppb | 14.5 ppb | + | Wako |
| Drierite | 16.7 ppb | Trace | + | W. A. Hammond |
| CaCO ₃ (GR) | 18.7 ppb | 21.6 ppb | + | Wako |
| NaCl (EP) | Trace | 0.11 ppm | + | Wako |
| NaCl (GR) | Trace | 11.8 ppb | + | Wako |
| DEAE-cellulose | 0.25 ppm | 0.41 ppm | + | Pharmacia |
| CM-cellulose | 9.89 ppm | 4.12 ppm | + | Pharmacia |
| Sephadex G-100 | 4.32 ppm | Trace | — | Pharmacia |
| Amberlite IR-45 | 2.27 ppm | 0.25 ppm | + | Rohm and Haas |
| Dowex L-X8 | 9.76 ppm | 0.19 ppm | + | Dow Chemical |

* For TLC.

** For column chromatography.

*** Chemical for analysis of pesticide residues.

† Extra-pure reagent.

‡ Guaranteed reagent.

*** Less than 10 ppb.

Organic solvents

The phthalates were detected in six GR solvents (benzene, acetone, diethyl ether, acetonitrile, methanol and ethanol) (Table I) and were removed completely by distillation. No detectable amounts of phthalate were present in the PR solvents.

Solid reagents

A yellow, oily material containing DBP and DEHP was extracted from most of the solid reagents (Table II). When the silicic acids were heated at 300° for 12 h the phthalates were almost removed. A similar effect was obtained by washing with an appropriate volume of chloroform-methanol (1:1) three times or more. The phthalates in the other powdered reagents were also removed by the methods described above.

Aluminium foil

Three aluminum foils of different brands were examined. All aluminum foils contained phthalates (Table III), though the levels were different. When the aluminum foils were heated at 350–400° for 14 h, all phthalates were removed.

TABLE III
CONCENTRATIONS OF DBP AND DEHP IN VARIOUS MATERIALS

| <i>Material</i> | <i>DBP</i> | <i>DEHP</i> | <i>Other unknown peaks</i> | <i>Company</i> |
|-------------------------------------|------------|-------------|----------------------------|------------------|
| Heavy-walled tubing (elicon tubing) | — | 67.2% | — | Seimi |
| Polyvinyl tubing | 23.3% | 11.6% | — | Plastec |
| Black rubber tubing | — | 0.2% | + | Seimi |
| PTFE tubing | — | — | — | Niplon Products |
| Black rubber stopper | Trace** | 0.3% | + | Seimi |
| Red rubber stopper | — | — | + | Seimi |
| Injection packing* | Trace | 0.25 ppm | + | Hitachi |
| Vial cap | 25.5 ppm | 0.65 ppm | — | Seimi |
| Wash bottle | — | — | + | Seimi |
| Cork stopper | — | — | + | Seimi |
| Tumble filter-paper | 0.05% | 1.34 ppm | + | Toyo Roshi |
| Filter-paper No. 2 | 5.28 ppm | 0.62 ppm | + | Toyo Roshi |
| Chromatography paper | 0.01% | 2.28 ppm | + | Whatmann |
| Glass-wool | 0.83 ppm | 4.15 ppm | + | Wako |
| Kimwipe | 41.9 ppm | — | + | Kimberly-Clark |
| Parafilm | — | — | + | American Can |
| Salanwrap | 8.28 ppm | 8.35 ppm | + | Asahidow |
| Aluminium foil A | 0.13 ppm | 4.85 ppm | + | Toyo Alumi |
| Aluminium foil B | 1.62 ppm | 1.01 ppm | + | Showa Alumi |
| Aluminium foil C | 0.97 ppm | 0.60 ppm | + | Mitsubishi Alumi |

* Injection packing for gas-liquid chromatograph.

** Less than 10 ppb.

Other materials

Heavy-walled tubing (Elicon tubing), polyvinyl tubing, rubber stoppers and vial caps contained phthalates (Table III). The heavy-walled tubing contained over 60% (w/w) of DEHP. Kimwipe, glass-wool and filter-paper also contained phthalates (Table III).

CONCLUSION

Most of the solvents, reagents and materials examined contained DBP and/or DEHP, and these phthalates are possible sources of background contamination. When lipids are analysed for phthalates, a suitable elimination method such as heating or washing with organic solvents must be applied as a pre-treatment.

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