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Identification of Aryl/Alkyl Phosphate Residues in Foods

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During our screening assays for chemical contaminants in food samples from FDA's Total Diet program (PROGRAM GUIDANCE MANUAL 1981), we found phosphorous-containing residues with retention times longer than pesticides normally encountered. These residues are associated with non-pesticidal esters of phosphoric acid which are used widely for flame retardation. Foods sometimes become contaminated with these chemicals by diffusion from phosphate-treated packaging materials. Food samples also become contaminated during analysis through laboratory reagents that contain these esters, eg. bulk alcohols and organic solvents.

The retention times (t_R) for most industrial phosphates fall into a 12-40 min range at our prescribed gas chromatographic conditions (PESTICIDES ANALYTICAL MANUAL I 1982). Several of them have complex elution patterns. This paper deals with the ability to identify these late-eluting compounds rapidly. By using known analytical characteristics such as Florisil recoveries (ERGY & LOMBARDO 1978) and tabulated retention data, phosphate esters can be identified as soon as they appear on food sample chromatograms.

We have compiled retention tables for 15 industrial phosphates using four general purpose column packings at 200°C. These tables permit easy comparison of retention data. Confirmations can be accomplished with single injections of the appropriate working standards.

MATERIALS AND METHODS

Total Diet samples (market baskets) were collected in grocery stores from each of 30 different sections of the United States. Each market basket was composited into 12 food categories, eg. meats, dairy products, fruits and root vegetables, and subjected to screening analysis. Phosphate reference standards were obtained from chemical supply outlets via the Bureau of Foods, Washington, D.C.

The gas chromatographs (GC) were Tracor 222's or 560's equipped with 1.8 m X 2 or 4 mm id glass columns and flame photometric (FPD) or nitrogen/phosphorous (NPD) detectors (phosphorous selective), and operated at the following parameters: injector, 225°C; oven, 200°C; detectors, FPD - 190°C, NPD - 250°C; carrier flows, 30 & 65 mL/min respectively for 2 & 4 mm id columns; chart speeds, 10 & 12.7 mm/min; sensitivity, ½ full scale deflection (FSD) for 2 ng of para-

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TABLE I. Retentions relative to parathion for 15 phosphate esters on the methyl silicone and mixed phase column packings.

No.	Concn ug/mL	Phosphate name	DC-200, OV-101 & SP-2100 (t_R) Parathion, 4.8 min; EHDP, 20.0 min	MIXED (t_R) Parathion, 6.4 min; EHDP, 21.0 min
1.	0.3	tris(2-chloroethyl)	0.50	0.52
2.	0.4	tris(2-chloropropyl)	0.55	0.52, 0.56
3.	0.6	methyl diphenyl	0.79, 3.6	0.69, 2.9
4.	2.0	tris(1,3-dichloroisopropyl)	1.6, 2.9, 3.4	1.7, 2.9
5.	2.0	triphenyl	3.6	2.9
6.	20.0	isodecyl diphenyl	3.6, 4.2, 6.0, 6.8, 7.5, 7.8, 8.4, 9.2, 9.8	2.9, 3.3, 4.5, 5.1, 5.7, 6.1, 6.7, 7.2, 7.9
7.	6.0	cresyl diphenyl	3.6, 4.7, 5.2, 5.6, 6.3, 6.8, 7.3	2.9, 3.9, 4.2, 5.0, 5.5, 6.0
8.	3.0	2-ethylhexyl diphenyl (EHDP)	3.6, 4.2, 5.0	2.9, 3.3, 3.6
9.	3.0	tris(2-butoxyethyl)	4.7	3.6
10.	3.0	tris(2-ethylhexyl)	5.8	3.9
11.	15.0	tricresyl	3.6, 4.7, 5.2, 6.3, 6.9, 8.2, 9.0, 9.8, 10.4, 11.5	2.9, 3.9, 4.2, 5.0, 5.5, 6.5, 7.2, 7.9, 9.8, 9.7
12.	4.0	tri-p-cresyl	11.0	8.5
13.	12.0	isopropylphenyl diphenyl	3.6, 5.6, 7.1, 8.6, 10.9, 13.2, 15.8	2.9, 4.2, 5.4, 5.9, 6.6, 7.7, 9.0, 9.4, 10.7
14.	50.0	trixylenyl	3.6, 4.7, 5.2, 5.6, 6.3, 6.8, 8.2, 9.0, 9.5, 10.5, 12.0, 13.6, 15.4, 17.2, 18.8, 21.2	2.0, 3.9, 4.2, 5.0, 5.5, 6.3, 6.7, 7.3, 8.2 9.1, 10.2, 11.4, 12.8, 14.2, 15.2
15.	100.0	tris(2,3-dibromopropyl)	3.4, 4.1, 5.0, 9.2, 23.7	1.9, 2.9, 3.7, 7.9, 22.3

NOTE: All retention ratios greater than 10% FSD are underscored.

TABLE 2. Relative retentions (RR_p) on the OV-225 and DEGS column packings.

No.	Phosphate name	OV-225 (t_R) Parathion, 5.7 min; EHDP, 16.0 min	DEGS (t_R) Parathion, 4.9 min; EHDP, 12.4 min
1.	tris(2-chloroethyl)	0.72	1.0
2.	tris(2-chloropropyl)	0.47, 0.51	0.47, 0.52
3.	methyl diphenyl	0.71, 3.9	0.88, 4.6
4.	tris(1,3-dichloroisopropyl)	5.7	6.5
5.	triphenyl	3.9	4.6
6.	isodecyl diphenyl	2.8, 3.5, 3.9, 4.2, 4.8, 5.0, 5.6, 6.4, 6.9	2.5, 3.5, 4.0, 4.6, 5.0, 5.3, 5.8, 6.3
7.	cresyl diphenyl	3.9, 5.0, 5.6, 6.5, 7.2	4.6, 5.5, 6.3, 6.8, 7.7, 8.7
8.	2-ethylhexyl diphenyl (EHDP)	2.0, 2.8, 3.9	2.0, 2.5, 4.6
9.	tris(2-butoxyethyl)	2.7	3.6
10.	tris(2-ethylhexyl)	1.4	2.2
11.	tricresyl	3.9, 6.5, 7.3, 8.4, 9.3, 10.4	3.6, 4.6, 5.5, 6.3, 6.8, 7.7, 8.3, 9.4, 10.7, 12.1
12.	tri-p-cresyl	11.7	12.0
13.	isopropylphenyl diphenyl	3.9, 4.8, 5.7, 6.6, 8.3, 9.3, 9.9	4.6, 5.1, 6.0, 6.6, 7.5, 8.7, 9.3, 10.9
14.	trixylenyl	3.9, 5.0, 5.6, 6.5, 7.2, 8.5, 9.3, 9.8 10.3, 11.8, 12.1, 13.9, 16.2	4.6, 5.5, 6.3, 6.8, 7.4, 7.7, 8.9, 10.7 11.9, 12.7, 14.2, 15.5
15.	tris(2,3-dibromylpropyl)	0.67, 1.3, 1.8, 4.9, 5.7, 7.5, 20.0	1.4, 2.1, 3.6, 5.9, 9.2, 26.5

NOTE: All retention ratios greater than 10% FSD are underscored.

thion; chromatograph rate, the overall retention times for parathion and 2-ethylhexyl diphenyl phosphate (EHDP) are 5.5 & 17.0 min respectively. Based on 10% FSD, the average limit of detection for the 15 phosphates is 0.03 ug.

Column packings were prepared on Chromosorb W(HP) solid support, 80 - 100 mesh, and conditioned at 200-240°C for 36 hr. Liquid phases were: a) methyl silicone which includes 5% DC-200, 5% OV-101, & 3% SP-2100; b) 1:1 phase mixtures of 5% OV-101 & 7½% QF-1 (BURKE & HOLSWADE 1966); c) 3% OV-225; and d) 2% stabilized diethylene glycol succinate (DEGS).

Standard solutions were made to the concentrations in Table I with 2,2,4-Trimethylpentane. Five ul of each working standard were injected with interspersed 2 ng injections of parathion on all column packings. Retention ratios relative to parathion were calculated, rounding to one decimal place. These data were correlated, averaged, and tabulated according to column packings, Tables 1 & 2.

RESULTS AND DISCUSSION

General. In our final steps for cleaning up food samples, the extract is passed through a Florisil column (PESTICIDES ANALYTICAL MANUAL I, 1982) with three fractions of mixed ethers (6, 15, & 50% ethyl/petr. ethers). Residual phosphates usually elute in the 50% mixed ether fraction. Occasionally, in samples of high fat content, a phosphate will elute in the 15% fraction. If an ester elutes in the 6% fraction, it will most likely be an artifact of a contaminated solvent, in which case, this residue may partially elute in all three Florisil fractions as well as those of the reagent blank.

Organic solvents are primary sources for phosphate background occurrences. During a one year period, random ckecks were made on pesticide grade solvents such as hexane, acetonitrile, methylene chloride, ethyl & petr. ethers. The following three esters were detected: triphenyl; 2-ethylhexyl diphenyl; & tris(2-ethylhexyl) phosphates. One or more of these residues were found intermittently in amounts from 0.01 to 0.1 ppm. The reagent blanks from our food assays usually have contained at least one of these background residues. Bulk ethanol, used for rinsing glassware, has contained phosphate esters. Phosphate has been found in analytical grade filter papers also, and in the airborne debris that occurs right after replacing the air filters in our building air handling system. Our glassware cleaning soaps and bottled distilled water have checked

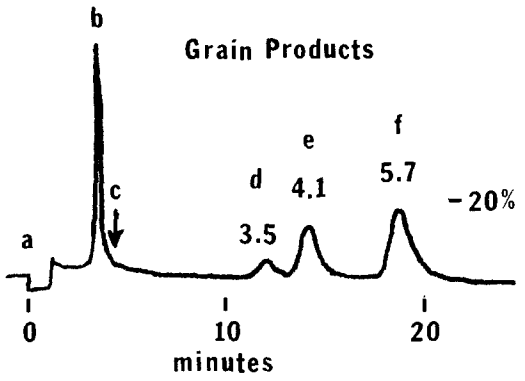

FIGURE 1. Chromatogram of a grain products composite (100 mg) on a DC-200 column: a) injection/vent; b) malathion; c) t_R of parathion; d), e), & f) triphenyl, 2-ethylhexyl diphenyl, & tris (2-ethylhexyl) phosphates.

negative for these residues.

Traces (0.01-0.1 ppm) of typically detected phosphates are pointed out in the grain products chromatogram in Figure 1 (flours, breads, cereals). Peaks "d" and "f" came from the solvents used in the analysis. Peak "e", an incurred residue, came from the sample composite. Attempts to quanify incurred residues at these levels requires strict handling of reagent blanks.

Although laboratory solvents contain these residues, our organophosphate pesticide assays have not been adversely affected because the retentions of these esters are generally beyond the range of normally found pesticides. On the OV-225 column packing, which interchanges elution sequences (MCCULLOUGH 1980), the ester, tris-(2-ethylhexyl) phosphate elutes more quickly (RR_p = 1.4), but has not interfered with known pesticides. Another ester, 2-ethylhexyl diphenyl phosphate has eluted towards the end of all our chromatograms (15-25 min). For that reason, its t_R has been given in the tables as an indicator of chromatograph rate.

Our instrumental parameters are stable, but repeated injections of food sample extracts over an eight hour day can sometimes slow down our GC elution rates. Phosphate retentions may be lengthened by 5-10 min towards the end of that period. This rate change is caused by coextractive buildup in the column packings. The columns recover in a few hours, usually overnight. We use a relatively early-eluting reference like parathion (RR_p = 1.0) for measuring retention ratios. Its t_R of about 5 min allows analysts to make on-the-spot injections that represent the chromatograph rate at the time phosphates emerge on recorder charts. Identifications made in this lab two years after the initial compilation of the tables have had the same retention ratios.

Chromatographic patterns. Several phosphate reference solutions yeild multiple-peaked chromatograms as illustrated in Figure 2. A problem arising in this kind of pattern is determining which peaks are indigenous to a mixture of isomers, and which are caused by

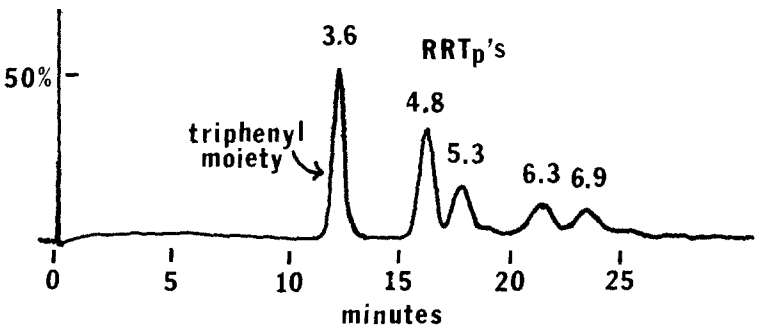

FIGURE 2. The multi-peaked elution pattern of cresyl diphenyl phosphate (25 ng) on a SP-2100 column packing with the major peaks shown.

decomposition, or by manufacturing byproducts in the original solution. The technical grade of these chemicals may account for the presence of impurities. For example, we noticed and confirmed by mass spectometry a moiety of (#5) triphenyl phosphate in seven of the reference solutions. In four of these standards, (#3) methyl diphenyl; (#8) 2-ethylhexyl diphenyl; (#11) tricresyl; and (#14) trixylenyl phosphates, this triphenyl peak was about 5% FSD. In the other three solutions, (#6) isodecyl diphenyl; (#7) cresyl diphenyl; and (#13) iospropylphenyl diphenyl phosphates, this peak was about 45% FSD. We think this particular peak is a manufacturing byproduct, since we have found it at varying levels with incurred (#13) isopropylphenyl diphenyl phosphate. Most of the incurred residues we find in food however, have matched their respective reference standards as shown in Figure 3. Another tendency is for each phosphate ester to elute in a recognizably characteristic pattern on all of the column packings.

Residues in Food. Phosphate ester diffusion appears to happen in foods that are moist or lipoid in nature or that have made long term contact with a contaminated wrapping. We have not found phosphate in dry food products. The finding of 2-ethylhexyl diphenyl phosphate in the fats/oils composite of Figure 3 (salad oils, peanut butters, margarines) was caused by a margarine sample. The assay of a combined ¼ lb stick and its tissue-lined wrapper from this composite quantitated at 50 ppm. The margarine stick alone contained 20 ppm. We have found this ester in plastic bread bags up to 400 ppm levels, but the amounts in bread itself have remained below ½ ppm. In both of these cases, the incurred ester appears to have migrated from the wrapper to the food product. This assumption is supported by the fact that flame retardants are used in the manufacture of adhesives, paper goods, and plastics, all found in packaging materials.

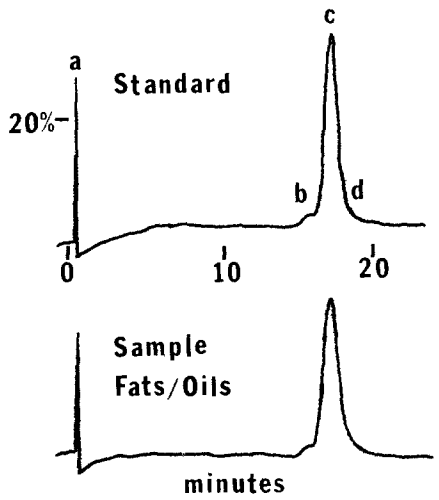

FIGURE 3. Chromatograms which demonstrate the similar elution characteristics between a reference standard of 2-ethylhexyl diphenyl phosphate (15 ng) and the 10 ppm found in 6 mg of the fats/oils composite on a mixed column packing: a) solvent front; b) triphenyl phosphate moiety/ impurity; c) major peak; d) small shoulder.

We have tested wrapping materials by immersing portions of them in aqueous solution under a layer of ether in g.s. flasks and letting them sit overnight with occasional swirling. Injections from the ether layer were made at random intervals. When we tested the above wrappers, the phosphate diffused from the margarine wrapper immediately. (½ ppm or higher with-

in 15 min). For the plastic bread bags, however, the migration was gradual. Significant levels did not occur until 6-12 hr after immersion, which may account for the low levels of phosphate in the bread even though the bags contained large amounts. We also have found phosphate in cap/lid liners of thick style salad dressings and sandwich spreads, but not in the ingredients themselves which had no prior contact with the liners. For the most part, paper and plastic wrapping materials are free of phosphates. We have tested about 50 food containers, lid or package liners, and individual-product wrappings and have found only traces (0.01-0.1 ppm) of phosphate residues.

Other phosphate esters have been reported in food items, notably in fish (LOMBARDO & ERGY 1979), and in drinking water (LEBEL et al. 1981, WILLIAMS & LEBEL 1981). Four flame retardants have been reported to have toxicities similar to that of organophosphate insecticides in test systems with fish (SASAKI et al. 1981), but little is known about the human toxicity of these substances. Knowing the chromatographic traits of industrial phosphate esters, however, facilitates their identification during the analysis of foods.

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