For the carrots grown in Birlane-treated soils, the total carotene contents were on an average 18% higher in the carrots grown covered than in the uncovered; exceptions were the harvests of June 17, 1981, and June 24, 1981.

Carotene Stereoisomers. None of the cultural conditions used (the pesticide treatment or the covering with perforated plastic film) appeared to have significant effects on the formation of carotene stereoisomers (Tables II and III). The *all-trans*-α-carotene is known to have a lower provitamin A value than the *all-trans*-β-carotene; the cis stereoisomers of α- and β-carotenes are known to have lower provitamin A values than do the *all-trans*-carotenes (Zechmeister, 1962); the fact that the cultural conditions did not cause significant changes in the ratio of the *all-trans*-α-carotene to the *all-trans*-β-carotene and in the ratio of cis isomers to all-trans isomers is therefore important from the standpoint of the quality of the food.

There was no insect attack on the carrots from both the treated and the untreated soils; the marked difference in carotene in the insecticide-treated plots over untreated plots thus seems to be actually due to an interaction between carrot and pesticide; this chloroorganophosphate, or some of its metabolites, thus would directly influence the metabolism of the carrots.

The effect of the pesticide on the total carotene content was not the same when the carrots were covered or not covered. Several speculations may be proposed in order to interpret these differences. The enhanced yield (weight of root per square meter), obtained here by covering with a plastic film (whether there was a pesticide treatment or not), clearly indicated that covering significantly altered the physiology and the metabolism of the carrots, among others by increasing the soil temperature (Benoit and Ceustermans, 1977, 1978a,b, 1979); moreover, the degradation of the pesticide in the soil, the evaporation of the pesticide from the soil, and thus the influence of the pesticide and of its metabolites on the metabolism of the carrots all could also be influenced by soil covering and by the effect of this last factor at least on the soil temperature; thus, it is not astonishing that the effects of the pesticide treatment were different according to the covering or not of the carrots with a plastic film.

In any of the assays made here, the soil treatment with chlorfenvinphos was beneficial to the total carotene content of the carrots, without altering the distribution of the carotene isomers and thus the biopotency of the total of the carotenes.

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Automated Gel Permeation System for Rapid Separation of Industrial Chemicals and Organophosphate and Chlorinated Pesticides from Fats

Marvin L. Hopper

A gel permeation chromatography (GPC) system for the rapid separation of industrial chemicals and organophosphate and chlorinated pesticides from fats has been developed. The system uses Bio-Beads SX-3 with a methylene chloride–*n*-hexane (50:50 v/v) solvent system. This gives good recoveries for a wide range of industrial chemicals and pesticides. Less than 1% fat remained in the pesticide fraction.

Gel permeation chromatography (GPC) was introduced as a cleanup technique for pesticides by Stalling et al. (1972). They found that Bio-Beads SX-2 gel with a cyclohexane solvent system removed the lipids from fish extracts and gave good recoveries of pesticides. This GPC system was automated by Tindle and Stalling (1972). The automated method was evaluated by Griffitt and Craun (1974) as a cleanup procedure for the removal of fats and oils from foods during pesticide residue analysis. The automated system was found to be more efficient and faster in the analysis of pesticide residues in fats than the acetonitrile–petroleum ether partitioning cleanup procedure. The recoveries for the pesticides through the GPC system were better than the acetonitrile–petroleum ether partitioning and Florisil cleanup method. The dump fraction removed 98% of the lipds from the pesticide fraction.

Stalling (1974) introduced a different GPC system that used Bio-Beads SX-3 and toluene–ethyl acetate (25:75 v/v) as the solvent system. This system gave quantitative recoveries of nonionic chlorinated pesticides and PCB's. This system could clean up a wider range of sample types, including green plant lipids, animal feed extracts, human adipose tissue, and beef tallow. Johnson et al. (1976)

Total Diet Research Center, Food and Drug Administration, Kansas City, Missouri 64106.

Table I. Elution Profiles of Fats

fats fractionated	% fat collected in each fraction from 1 g of fat placed on the column				
	20-30 mL	30-40 mL	40-50 mL	50-60 mL	60-140 mL
butterfat	2.6	27.0	58.0	12.0	0.4
Wesson Oil	2.3	28.4	60.0	9.0	0.3
cod-liver oil	3.5	30.0	51.0	15.0	0.5
composite 1 (dairy)	2.3	25.0	56.0	16.3	0.4
composite 2 (meats)	3.0	30.0	55.0	11.4	0.6
composite 10 (fats and oils)	1.4	27.6	57.0	13.3	0.7

further evaluated this system and expanded its capabilities while maintaining good recoveries of pesticides. The nonionic chlorinated pesticides and PCB's were found to elute quantitatively in the 100–150-mL fraction except for 18% of the *p,p'*-DDD. Organophosphate insecticides and chlorophenoxy ester herbicides eluted in the 90–150-mL fraction. The dump fraction from 0 to 100 mL removed 98% of the plant and animal lipids.

A GPC cleanup of vegetables, fruits, and crops for the analysis of organophosphate pesticide residues was introduced by Ault et al. (1979). This GPC system used Bio-Beads SX-3 with methylene chloride–cyclohexane (15:85 v/v) as the eluting solvent. The elution volumes of 33 organophosphate compounds were determined and ranged from 80 to 320 mL for complete elution of all compounds. The GPC system demonstrated flexibility for cleanup of different sample matrices with recoveries comparable to those of other methods reviewed to date. The above publications have demonstrated that gel permeation cleanup is efficient and quantitative for chlorinated and organophosphate pesticide residues from various sample matrices.

The Bio-Beads SX-3 and methylene chloride–*n*-hexane (50:50 v/v) system reported here exhibits improvements over the SX-3 GPC systems described above. The use of a solvent system that is more easily evaporated, a smaller dump fraction (0–60 mL) containing 99% of the fat, and a reduced total elution volume (60–140 mL) while maintaining quantitative recovery of the pesticides make this a more efficient GPC cleanup system for industrial chemicals and chlorinated and organophosphate pesticide residues in fat and lipid matrices.

EXPERIMENTAL SECTION

Apparatus. The gel permeation chromatograph (GPC) was an Auto-Prep 1001 (Analytical Biochemistry Laboratories, Inc., Columbia, MO) equipped with a 30 cm × 2.5 cm i.d. column (Kontes, Vineland, NJ), slurry packed with 33 g of Bio-Beads SX-3 resin (200–400 mesh, Bio-Rad Laboratories, Richmond, CA) and compressed to a bed length of approximately 20 cm. The eluting solvent was methylene chloride–hexane (50:50 v/v) pumped at a flow rate of 5.0 mL/min with an operating pressure range of 8–11 psig. The GPC system was set up with a 12-min dump, 16-min collect, and 0-min wash cycle.

The miniature concentrating system contained a distilling trap adapter (No. 5226, Ace Glass, Inc., Vineland, NJ) and a micro evaporative concentrator (No. 6709, Ace Glass, Inc.). The filter consisted of a Millipore Swinny stainless adaptor (Catalog No. IEAXX 3001200) with Millpore 5.0-μm LS-type filter material (Catalog No. LSW 01300, Millipore Corp., Bedford, MA).

Three gas chromatographs were used. One was a Tracor Model 560 gas–liquid chromatograph (GLC) equipped with a nickel-63 electron capture detector and a Model 702 N-P alkali flame ionization detector. A 6 ft × 4 mm i.d. glass column was packed with 2% diethylene glycol succinate (DEGS) on 100–120-mesh Chromosorb WHP, and the effluent was linked to the above detectors through a 1:1 glass-lined splitter (Scientific Glass Engineering, Austin, TX) with a flow rate of approximately 60 mL/min helium. The EC detector was set up so a purge gas of 10% methylene in argon flowed through the detector at a flow rate of 70 mL/min. The following temperature parameters were used: inlet, 216 °C; oven, 200 °C; EC detector, 348 °C; N/P detector, 258 °C.

A second Tracor Model 560 GLC was equipped with a flame photometric detector (FPD) utilizing a phosphorus-specific filter (526 nm). A 6 ft × 4 mm i.d. glass column was packed with mixed 5% OV-101 and 7.5% OV-210 (1:1 w/w) on a 80–100-mesh Chromosorb WHP support. Nitrogen was used as the carrier gas with a flow rate of approximately 60 mL/min nitrogen. The following temperature parameters were used: inlet, 225 °C; FPD detector, 190 °C; oven, 204 °C.

A Model 5880 Hewlett-Packard GLC was equipped with a nickel-63 electron capture detector. A 6 ft × 2 mm i.d. glass column was packed with 3% SP-2100 on 80–100-mesh Supelcoport with a flow rate of approximately 30 mL/min 10% methane in argon. The following temperature parameters were used: inlet, 220 °C; EC detector, 300 °C; oven, 180 °C.

Test Materials. Chlorinated and organophosphate pesticide standards were diluted from 1 mg/mL stock solution in methylene chloride–*n*-hexane (50:50 v/v). Butterfat, corn oil, cod liver oil, and fat extracts from the FDA Adult Total Diet Market Basket [see Johnson and Manske (1981) for a description] were used in the elution profiles. The fats of composite 10 (fats and oils), composite 2 (meats), and composite 1 (dairy) were extracted according to sections 211.13c and 211.13f of McMahon et al. (1978).

Reagents. Methylene chloride, *n*-hexane, diethyl ether, petroleum ether, acetonitrile, acetone, and 95% ethanol (USP) were all pesticide grade (such as Burdick & Jackson, Muskegon, MI).

Procedure. The elution profiles of butterfat and several representative compounds were used to calibrate the Auto-Prep 1001 gel permeation chromatograph (GPC) system. The Auto-Prep 1001 was set up to fractionate each sample into 23 10-mL fractions by collecting the column effluent for 2 min at a flow rate of 5.0 mL/min. A 5-mL holding loop was filled with sample by using a Millipore Swinny filter adapter and filter. If cloudy, sample solutions were centrifuged prior to loading. The elution profile of butterfat was determined with no more than 1 g of fat loaded on the GPC column. The weight of butterfat in each fraction was determined after solvent evaporation. The elution profile of the butterfat was used to determine column efficiency. Good column efficiency is obtained when the fat band elutes in a tight band without skewing on the column. The elution profiles of diazinon (0.2 μg/mL), parathion (0.4 μg/mL), ethion (0.6 μg/mL), lindane (0.1 μg/mL), and pentachloroaniline (0.05 μg/mL) in methylene chloride–*n*-hexane (50:50 v/v) were then determined on the GPC system by fractionation. Sample

fractions were concentrated in a 10-mL graduated tube to a small volume (<3 mL) with a distilling trap adapter and micro evaporative concentrator. *n*-Hexane was added and the eluate reconcentrated to ensure the complete removal of the methylene chloride. The final volume of 5 mL was obtained by the addition of acetone to the eluate. The eluates were then analyzed by GLC.

The elution profiles of butterfat and organophosphate pesticides are used to determine the dump and collect fractions. The dump fraction for this system was 0–60 mL with a 60–140-mL collect fraction. Repacking the column is required if the fat band becomes skewed or if more than 15 mg of fat is contained in the collect fraction.

The milk extraction procedure, of McMahon et al. (1978, sectin 211.13 h) was used to extract 100 g each of unfortified and fortified aliquots of Infant Formula with iron. A standard solution containing three organophosphate and eight chlorinated pesticides was added to the sample. The samples were extracted with a mixture of ethyl ether and petroleum ether and dried by passing through a anhydrous sodium sulfate column. The effluents were collected in a Kuderna-Danish evaporator (500 mL) with a 10-mL concentrator tube (Ace Glass, Inc.). The samples were concentrated to a small volume. Methylene chloride (100 mL) was then added and the eluate reconcentrated. The samples were transferred to a calibrated 25-mL graduated cylinder and diluted first to a 12.5-mL with methylene chloride and then to 25 mL with *n*-hexane. One milliliter of each sample solution was pipetted into a tared beaker and the weight determined after the solvent was evaporated. If the determined weight is greater than 0.2 g, a known aliquot of the sample is adjusted to a concentration of 0.2 g/mL or less with methylene chloride–*n*-hexane (50:50 v/v). The samples were loaded on the automated GPC as described earlier and the samples collected.

Sample eluates were transferred to a Kuderna-Danish concentrator (500 mL) with a 5-mL graduated concentrator tube and concentrated to a small volume (<3 mL). Eluates were further concentrated (<1 mL) with *n*-hexane as previously described and diluted to a final volume of 2 mL. The organophosphate pesticides were quantitated by GLC without further cleanup, but the chlorinated pesticides needed further cleanup before being quantitated.

Florisil (McMahon et al., 1978, sections 252.11–252.13), was used for further cleanup of the chlorinated pesticides. The Florisil column was eluted with 200 mL of 50% methylene chloride–0.35% acetonitrile–49.65% hexane (v/v/v) in order to remove all pesticides of interest. The chlorinated pesticides were quantitated by GLC.

RESULTS AND DISCUSSION

The GPC system was used to fractionate 6 fats and 66 compounds. Table I shows single determinations for the elution profiles of the six different fats and demonstrates that less than 1% of each remains in the pesticide fraction. Table II shows the elution volumes and percent recoveries of the different compounds including industrial chemicals and chlorinated and organophosphate pesticides eluted once through the GPC system. The elution volumes for the compounds fractionated ranged from 60 to 140 mL, with recoveries ranging from 91% to 107%. The recovery of 1,2,3-trichlorobenzene was not determined due to an interference. The concentration of the compounds fractionated ranged from 0.02 μg/mL for 2,3,5,6-tetrachloroanisole to 6.0 μg/mL for systox.

The percent recoveries of selected compounds in fortified butterfat were determined once through the system. Table III shows the percent recoveries of five organophosphate pesticides and eight chlorinated pesticides in separate butterfat fortifications. Each compound was quantitated on GLC without further cleanup, and the recoveries ranged from 96% to 106%. Several pesticides were fortified at or near twice their action level.

Table II. Compounds Fractionated

compound (common name)	elution vol, mL	% recovered through the column in 60–140-mL fraction
fonofos (Dyfonate)	70–100	107
malathion	60–90	102
parathion	70–100	104
methidathion (Supracide)	80–100	102
ethion	60–90	102
diazinon	60–90	98
chloropyrifos (Dursban)	60–100	100
dimethoate	80–110	100
chlorpropham (CIPC)	60–100	108
phorate	70–90	98
methamidophos (Monitor)	80–110	102
acephate (Orthene)	70–100	107
fenthion	70–100	100
malaoxon	60–90	98
phenthoate	60–90	105
monocrotophos (Azodrin)	60–90	101
carbophenthion (Trithion)	60–100	104
leptophos	70–90	98
phosalon	70–100	103
systox	60–90	98
imidan	60–100	102
chlorothiophos	60–90	101
guthion	70–100	103
terbufos (Counter)	60–90	100
methyl parathion	70–100	103
α-mevinphos	60–90	106
β-mevinphos	60–90	102
vinclozolin	60–100	106
dimethoate, O analogue	70–90	106
paraoxon	60–90	101
phorate sulfone	60–90	99
triazophos	70–90	99
α-BHC	90–130	96
lindane	90–130	96
heptachlor	70–110	101
aldrin	70–110	102
heptachlor epoxide	70–110	101
β-chlordane (cis)	80–120	102
dieldrin	80–120	101
endrin	80–120	103
hexachlorobenzene (HCB)	80–120	99
kelthane	80–130	100
α-chlordane (trans)	80–120	102
trans-nonachlor	70–110	100
p,p'-DDT	70–120	102
methoxychlor	70–110	104
pentachlorobenzene	90–110	91
pentachloroanisole	80–110	95
pentachloronitrobenzene (PCNB)	80–120	93
pentachloroaniline (PCA)	90–140	95
pentachlorothioanisole (PCTA)	80–120	96
octachlor epoxide	70–100	95
p,p'-DDE	70–110	95
p,p'-TDE	80–120	95
tetrachloronitrobenzene (TCNB)	80–100	96
DCNA (Botran)	70–100	102
DPCA (Dacthal)	70–110	103
endosulfan sulfate	70–120	105
endosulfan I	70–110	102
endosulfan II	80–120	105
1,2,3-trichlorobenzene	90–120	ND[a]
1,2,3,5-tetrachlorobenzene	80–110	97
1,2,3,4-tetrachlorobenzene	80–120	96
hexachloronorbornadiene	80–110	104
2,3,5,6-tetrachloroanisole	100–130	104
Aroclor 1254	70–130	99

[a] ND = not determined.

Table III. Recovery of Pesticides from Butterfat

compound	μg of standard added/g of fat	% recovered through the column from butterfat (spike)
α-BHC	0.12	102
lindane	0.2	101
heptachlor	0.2	97
aldrin	0.2	101
heptachlor epoxide	0.4	99
β-chlordane (cis)	0.4	98
dieldrin	0.6	102
endrin	0.6	96
diazinon	0.2	97
malathion	0.6	104
malaoxon	1.0	103
azodrin	1.2	106
methidathion (Supracide)	1.0	106

Table IV. Recovery of Pesticides through Cleanup Procedures

compound	μg of standard added/g of sample	% recovered from Infant Formula with iron
α-BHC	0.0024	98
lindane	0.004	100
heptachlor	0.004	82
aldrin	0.004	82
heptachlor epoxide	0.008	103
β-chlordane (cis)	0.008	86
dieldrin	0.012	92
endrin	0.012	83
diazinon	0.008	87
malathion	0.024	88
methidathion (Supracide)	0.040	95

A sample of Infant Formula with iron was fortified with 11 pesticides, and the recoveries of these were determined after extraction and cleanup. Table IV shows the percent recoveries of the chlorinated and organophosphate pesticides once through the above procedure with the fortification level of several pesticides at or near their action level. The recoveries ranging from 82% to 103% of the pesticides through the extraction and cleanup steps are comparable with recoveries previously encountered. The organophosphate pesticides used in fortifying the Infant Formula with iron were chosen because they are recovered through the section 211.13h extraction of McMahon et al. (1978). The Foods and Feeds Extraction and Cleanup Table 201-A of McMahon et al. (1978) should be referenced when analyzing for organophosphate and chlorinated pesticides in fatty or nonfatty samples to ensure that the pesticides of question are completely extracted.

The development of this system showed that there can be differences between batches of SX-3 beads even though the manufactuer declares 3% cross-linkage. The original system in this work was developed on a specific batch of SX-3 beads with a methylene chloride–*n*-hexane (60:40 v/v) solvent system. The 60:40 (v/v) system was tried on a different batch of SX-3 beads in the process of validating the system and found not to work. A methylene chloride–*n*-hexane (50:50 v/v) system was then used on the second batch of SX-3 beads and found to reproduce the results of the 60:40 (v/v) system on the first batch of beads. When a good fat separation cannot be achieved on a specific batch of SX-3 beads with methylene chloride–*n*-hexane (50:50 v/v), the eluting solvent composition ratio must be adjusted if it has been demonstrated that the column does not need to be repacked. The SX-3 with methylene chloride–*n*-hexane (50:50 v/v) system gave good fat separations on three of the four different batches of SX-3 beads in our laboratory.

The different solvents used in fat extractions can be a problem in the GPC cleanup step if great care is not taken to make sure the final mixture contains only the methylene chloride–*n*-hexane solvent. Other solvents contained in the final mixture can cause the fat to be retained on the column and thus more fat will be eluted in the collect fraction. Isooctane is one solvent that will cause the sample band to be retained on the column and should not be used because it can never be eliminated from the mixture due to its high boiling point.

The SX-3 Bio-Beads with methylene chloride–*n*-hexane (50:50 v/v) solvent system has demonstrated that it gives good recoveries of industrial chemicals and chlorinated and organophosphate pesticides from fats with less than 1% of the fat in the pesticide fraction. The savings in time and solvents coupled with the automated GPC instrument makes this system a valuable tool in the analysis of pesticide residues in fat extracts. The SX-3 GPC system is being evaluated further with other compounds and solvent modifications that will be reported on in the future.

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