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CHROMATOGRAPHIC AND BIOLOGICAL ASPECTS OF THE PHTHALATE ESTERS

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1. INTRODUCTION

The aromatic dicarboxylic esters such as the phthalic acid esters are among the most important industrial chemicals employed. Although they have enjoyed extensive utility for three decades, primarily as plasticizers for a variety of films and plastics, it has only been within the last several years that their migration from plastics into human tissue as well as their increasing occurrence in the ecology have been reported. Hence, there is increasing concern regarding the consequences of chronic ingestion, adsorption and/or inhalation of low levels of a variety of phthalate esters to man.

The major objectives of this review are to delineate the biological properties, stability, episodes of occurrence in the environment as well as the salient chromatographic (*viz.* gas–liquid, thin-layer, and liquid–liquid) procedures that have been employed for the separation and identification of the phthalate esters.

2. PHYSICAL PROPERTIES AND UTILITY

Phthalates are made industrially by esterification of the requisite alcohol with phthalic anhydride, in the presence of a catalyst such as sulfuric acid or *p*-toluenesulfonic acid, or non-catalytically at high temperature. Phthalic anhydride itself is made by oxidizing naphthalene in air using vanadium pentoxide as a catalyst, and is purified by distillation. The phthalate esters are in most cases liquids with very high boiling points and very low vapor pressures. (The low vapor pressure is important in contributing to their general stability in plastics.) Table 1 lists the physical and chemical properties of the principal phthalate esters.

TABLE I

PHYSICAL AND CHEMICAL PROPERTIES OF PHTHALATE ESTERS

Unless otherwise specified, specific gravities are at 20/4, boiling points at 760 mm Hg, and solubilities at 20°C.

Compound	*Formula*	*Mol. wt.*	*Sp. gr.*	*M.p. (°C)*	*B.p. (°C)*	*Vapor pressure (mm Hg)*[a]	*Flash point (°F)*	*Solubility in water (g/100 g)*
Dimethyl phthalate	$C_6H_4(COOCH_3)_2$	194.18	1.189 (25/25)	0.0 (freezes)	282	<0.01 (20)	325	0.5
Diethyl phthalate	$C_6H_4(COOC_2H_5)_2$	222.23	1.123 (25/4)	−40.5	296.1	0.05 (70)	335	0.1
Dimethoxyethyl phthalate	$C_6H_4(COOC_2H_4OCH_3)_2$	282.0	1.171 (20)		190–210	0.01 (20) 0.25 (150)	379–4	0.85
Dibutoxyethyl phthalate	C_6H_4-1,2-$(COOH_2CH{:}CH_3)_2$	366.0	1.063		210	<0.01 (20)	208	<0.03
Diallyl phthalate		246.27	1.120 (20/20)	−77	290	2.4 (150)	166	<0.01
Dibutyl phthalate	$C_6H_4(COOC_4H_9)_2$	278.34	1.0465 (21)	−35	340	2.0 (150) 0.1 (115)	315	0.45 (25°C)
Diisobutyl phthalate		278.3	1.040	−50	327		322	0.01
Dihexyl phthalate	$C_6H_4[COOCH_2CH(C_2H_5)C_2H_5]_2$	334.0	0.990			5 (210)		Insoluble
Di-*n*-octyl phthalate		391.0	0.978	−25	220	<0.2 (150)	219	Insoluble
Di(2-ethylhexyl) phthalate (octyl phthalate)	$C_6H_4[COOCH_2CH(C_2H_5)C_4H_9]_2$	391.0	0.985 (20/20)	−50	(4 mm) 386.9 (5 mm)	<0.01 (20)	425	0.01
Di(2-ethylhexyl) isophthalate		391.0			400	1 (223)		Insoluble

Diisooctyl phthalate	$C_6H_4(COOC_8H_{17})_2$	391.0	0.981	− 4	239	1.0	(200)	410	Insoluble
Dinonyl phthalate		419.0	0.965		413	1	(205)	420	Insoluble
Butyl octyl phthalate	$C_6H_4[COO(CH_2)_3CH_3]$ $[COO(CH_2)_7CH_3]$	334.0		−50	340			370-4	
Dicyclohexyl phthalate	$C_6H_4(COOC_6H_{11})_2$	330.0	1.20 (25/25)	62–65 (pour p.)	220–228 (4 mm)	<0.06	(150)	440.6	Insoluble
Methylphthalyl ethyl glycolate		266.24	1.220 (25/25)	<−35	189 (5 mm)			193 (°C)	0.053
Ethylphthalyl ethyl glycolate		280.27	1.220 (25/25)	20	320			386	<0.08
Butylphthalyl butyl glycolate	$(C_4H_9OOCC_6H_4CO)OCH_2(COOC_4H_9)$	336.37	1.097 (25/25)	<−35	219 (5 mm)			199 (°C)	0.0012
Dimethyl terephthalate	$C_6H_4(COOCH_3)_2$	194.18	1.04	140	285 (subl.)	16 140 78	(100) (150) (200)	146 (°C)	0.3 (in hot water)
Di(2-ethylhexyl) tetra-hydrophthalate	$C_6H_8[COOCH_2CH(C_2H_5)(CH_2)_3CH_3]_2$	395	0.969	−50 (pour p.)	219 (5 mm)			350	

[a] The temperature (°C) is indicated between brackets.

In terms of quantity, use applications and concomittant environmental considerations, the area of plasticizers is by far the most important aspect of the phthalate esters. Plasticizer production of phthalate esters in the United States has grown from approx. 300 million pounds in 1960 to approx. 1.30 billion* pounds in 1970. (Approx. 250 million pounds of phthalate esters are produced annually in Western Europe.) Last year di(2-ethylhexyl) phthalate (DEHP) (DOP) production as a general-purpose-plasticizer for polyvinyl chloride (PVC) was 350 million pounds or a fourth of the total plasticizer production. The closely related phthalates, diisooctyl phthalate (DIOP) and diisodecyl phthalate (DIDP) accounted for another fourth of the market, *viz.* 85 and 123 million pounds, respectively. Other phthalates added another 296 million pounds or approx. 20%. These include 59 million pounds of *n*-octyl *n*-decyl, 23 million pounds of dibutyl, and 21 million pounds of diethyl phthalate, respectively. It is of interest to note that since PVC began to be commercialized in the early 1930's, by 1966 over 2 billion pounds had been produced with forecast of 6 billion by the mid 1970's.

Phthalate ester plasticizers are extensively used in PVC for food packaging, refrigerator gasketing, luggage, handbags, coated cloth, vinyl floor coverings (tile and sheet goods), waterproof boots and shoes, electrical insulation, industrial hose and tank liners. Besides PVC, the phthalates are used to plasticize polyvinyl acetate, polyvinylidene chloride, polystyrene, ethyl cellulose, cellulose nitrate, acetate and acetate butyrate, chlorinated rubber, high styrene–butadiene protein compounds, shellac, acrylic-type resins, polyamides, polyesters, epoxy alkyds, phenolic alkyds, polyurethan, nitrile and neoprene rubber, and chloroethylene resins.

Other phthalate esters of importance are the octyl decyl esters where low-temperature properties and low volatility are important. The lower members of the series, particularly the methyl, ethyl and butyl derivatives, find large volume application as plasticizers for polar polymers such as polyvinyl acetate and the cellulosics. The methyl and ethyl esters also serve as insect repellant[1] when mixed with indalone, and a variety of alkyl phthalates have been used extensively as stationary phases in gas chromatography. Other suggested areas of utility have included the use of dimethoxyethyl phthalate mixed with polypropylene-polymethyl acrylate, for cigarette filters[2], and in aerosol-pesticide formulations.

3. TOXICITY AND METABOLISM

A number of the phthalate esters have been studied both for their single acute lethal doses and in chronic feeding experiments, because of their use in food packaging. Many of the phthalates have high oral lethal dosages and could be tolerated in relatively high levels in the diet (in some cases this was apparently the result of poor adsorption). WILLIAMS[3] suggested that these esters are very likely hydrolyzed *in vivo* yielding phthalic acid and an alcohol. Since phthalic acid is of low toxicity and is believed to be excreted quantitatively, the toxicity of these materials was suggested to depend to a large extent on the nature of the alcohol released on hydrolysis. The pathology produced by the phthalate esters is usually non-specific unless the alcohol portion released on hydrolysis *in vivo* has activity[4].

* The American billion (10^9) is meant.

Table 2 summarizes the physiological response of laboratory animals to a number of phthalate esters. Di(2-ethylhexyl) phthalate (DEHP), the most widely used phthalate ester, has received extensive toxicological scrutiny. The acute and chronic toxicities in various species by various routes have been studied by SHAEFFER *et al.*[5], HODGE[6], CARPENTER *et al.*[7], and HARRIS[8]. The acute oral LD_{50} value of DEHP in rats is about 30–34 g/kg. The intraperitoneal LD_{50} in the rat is about 24–30 g/kg, and the principal finding was the presence of unadsorbed milky emulsion and some non-specific changes in the liver.

The metabolism of DEHP has been elaborated by SHAEFFER *et al.*[5]. In rats and rabbits there was no evidence of conjugation of the carboxy groups of the phthalate. However, in the case of rabbits, sufficient phthalate was excreted in the urine to account for 26–65% of the total dose, with considerable variation in different animals. In dogs, however, the excretion of phthalate accounted for only 2–4.5% of the total dose. When two human subjects were given single doses of 5 and 10 g of DEHP, only 4.5% of the total dose could be accounted for as phthalic acid in the urine in the succeeding 24-h period (the majority was excreted between a 5- and 7-h period after administration). No symptoms resulted from the 5-g dose, and only mild gastric disturbances and some loose stools were noted from the larger dose.

CARPENTER *et al.*[7] and HARRIS *et al.*[9] described the chronic oral toxicity of DEHP in rats over a 2-year period. There was no apparent effect noted in either study on any of the usual criteria for chronic toxicity when the levels were below 0.13% in the diet. In both studies some retardation of growth and some statistically significant increases in weight of liver and kidneys were noted at levels of 0.4–0.5% in the diet (corresponding to 0.2–0.4 g/kg per day). (The increase in weight of these organs was not accompanied by any definite histopathology.) No definite effects were noted in dogs given 5 g/kg of DEHP in the diet for 14 weeks[9]. Studies by CARPENTER *et al.*[7] included a 1-year oral feeding to guinea pigs at levels up to 0.13% of DEHP, and except for a possible change in liver weight of the females, no deleterious effect was noted.

CALLEY *et al.*[10] evaluated a series of phthalic acid esters for parenteral toxicity, including LD_{50} values and hexobarbital narcosis. Experiments utilized included: i.p. injections into mice for acute toxicity profile, i.v. administration in rabbits for blood pressure and respiration effects, intradermal injections into rabbits for initiation effects, and repeated i.p. doses of the phthalates on mice over a period of time for effects over that period (examination of organs, weight gain, and the blood). Tissue culture experiments were also conducted to attempt to correlate certain of the toxicity manifestations.

Table 3 illustrates the estimated LD_{50} values for a series of phthalate esters, and indicates that the acute lethal i.p. toxicity was generally of a low order. LD_{50} values ranged from 1.58 g/kg for dimethyl phthalate to 14.19 g/kg for DEHP and dicapryl phthalate. A correlation appeared to exist between water solubility and degree of toxicity (although water solubility of all the phthalates was extremely low, ranging from 0.85 g/100 g to insolubility). The three esters with greatest solubility (*viz.* dimethyl, diethyl, and dimethoxyethyl phthalates) exhibited the greatest toxicity. An inverse relationship also existed between toxicity and molecular weight of the phthalates.

Table 4 illustrates the effect of phthalates on hexobarbital narcosis. Only two

TABLE 2

PHYSIOLOGICAL RESPONSE TO PHTHALATE ESTERS

Compound	LD_{50}			*Skin* LD_{50}	
	Species	*Route*	*Dose (g/kg)*	*Species*	*Dose (ml/kg)*
Dimethyl phthalate	mouse	oral	7.2	rabbit	>10
	mouse	i.p.	3.6		
	rat	oral	6.9		
	guinea pig	oral	2.4		
Diethyl phthalate	rabbit	oral	1.0		
	mouse	i.p.	2.8		
	guinea pig	s.c.	3.0		
Dimethoxyethyl phthalate	rat	oral	> 4.4	guinea pig	>10
	mouse	oral	3.2–6.4		
	guinea pig	oral	1.6–3.2		
Dibutoxyethyl phthalate	rat	oral	8.4		
Diallyl phthalate	rat	oral	1.7	rabbit	3.4
	rabbit	oral	1.7		
	mouse	i.p.	0.7		
Dibutyl phthalate	rat	oral	8.0	rabbit	>20
	rat	i.m.	> 8.0		
Diisobutyl phthalate	mouse	oral	>12.8	guinea pig	>10
	rat	oral	20–25		
Dihexyl phthalate	rat	oral	30	rabbit	>20
Di(2-ethylhexyl) phthalate	rat	oral	>26	guinea pig	>10
	rat	i.p.	30		
	rabbit	oral	34		
Di-*n*-octyl phthalate	mouse	oral	>13	guinea pig	> 5
Di(2-ethylisohexyl) phthalate	rat	oral	>26	guinea pig	>10
	mouse	oral	>26		
	rat	i.p.	>13		
Di(2-ethylhexyl) isophthalate	rat	oral	> 3.2	guinea pig	>20
Dinonyl phthalate	rat	oral	> 2		
Methylphthalyl ethyl glycolate	rat	oral	9.0	guinea pig	>10
	rat	oral	3.2–6.4		
Ethylphthalyl ethyl glycolate	rat	oral	10% in diet[a]		
Butylphthalyl butyl glycolate	rat	oral	14.6		
Dimethyl terephthalate	rat	oral	> 3.2	guinea pig	> 5 (g/kg)
	rat	i.p.	> 3.2		
Di(2-ethylhexyl) tetrahydrophthalate	rat	oral	114		

[a] All dead in 7–15 days.

Inhalation toxicity				*Irritant effect*		
Species	*Concn. (p.p.m.)*	*Time (h)*	*Mortality*	*Species*	*Skin*	*Rabbit eye*
cat	1213 (9.3 mg/l)	6.5	lothal	guinea pig	no sensi-tization	slight
cat	2 (mg/l)	6	nasal irritation			
rat	511 (air through liquid at 150°C)	6	(0/3) vasodila-tation of ears and foot	guinea pig	slight	slight
cat	1120 (10 mg/l) (mist)	5	nasal irritation	guinea pig		slight
rat	5500 (air through liquid at (200°C)	1.5	(3/3) nasal irritation	guinea pig	no irritation or sensi-tization	none
rat	1595	6	(3/3) nasal irritation	guinea pig		none
rat	770	6	(0/3) no symptoms	guinea pig		none
rat	145 (air through liquid at 100°C)	6/day for 62 days	(0/3) no symptoms	guinea pig		none
				rabbit	slight	slight
				human	irritant	slight
cat	1 (mg/l)	5.5	nasal irritation	guinea pig	no irritation	slight
				guinea pig	no irritation or sensi-tization	slight
rat	satd. vapor	8	no deaths	rabbit	slight	slight
rat	8 (100°C)	6	0/3	guinea pig	slight no sensitization	slight
				guinea pig	slight	slight slight
				guinea pig	slight	slight
rat	Satd. vapor (28°C)	6/day for 12 days	no effect	rat human	none none	
rat	2500 (320°C)	6	0/3			
rat	290	6/day for 62 days	no effect	guinea pig	no irritation or sensi-tization	none
				guinea pig	slight no sensitization	slight

TABLE 3

ESTIMATED LD_{50} VALUES FOR A SERIES OF PHTHALATE ESTERS

Phthalate ester	*i.p. LD_{50} in mice*	*Confidence limits*	*Mol. wt.*	*Solubility in water*
	Dose (g/kg)	*95%*		*(g/100 g)*
Dimethyl phthalate	1.58	0.98– 1.99	194	0.45
Diethyl phthalate	2.83	2.42– 3.29	222	0.1
Dibutyl phthalate	4.00	2.94– 5.45	278	0.45
Diisobutyl phthalate	4.50	3.36– 6.02	278	0.01
Dimethoxyethyl phthalate	2.51	1.82– 3.45	282	0.85
Butyl benzyl phthalate	3.16	2.51– 3.98	312	Insoluble
Di(2-ethylhexyl) phthalate[a]	14.19	12.62–15.78	390	0.01
Dicapryl phthalate	14.19	11.21–15.87	390	0.03

[a] Abbreviated to DEHP.

TABLE 4

EFFECT OF PHTHALATE ESTERS ON HEXOBARBITAL SLEEPING TIME IN MICE

Groups of 10 white mice weighing 14 to 20 g were administered 500 mg/kg doses of the phthalate esters and, after an interval of 30 min, injected i.p. with 60 mg/kg of sodium hexobarbital.

Phthalate ester	*No. of mice narcotized*	*Sleeping time ± S.E. (min)*	*CNS effect*	
			Depression	*Stimulation*
Control (0.5 ml) (3% acacia)	10	46 ± 1.66	—	—
Dimethyl phthalate	10	52 ± 1.15	+	—
Diethyl phthalate	10	88 ± 2.94[a]	+	—
Dibutyl phthalate	10	55 ± 2.18	+	—
Diisobutyl phthalate	10	72 ± 2.83[a]	+	—
Dimethoxyethyl phthalate	10	50 ± 1.71	+	—
Butyl benzyl phthalate	10	62 ± 2.45[b]	+	—
Di(2-ethylhexyl) phthalate (DEHP)	10	36 ± 1.14[b]	—	+
Dicapryl phthalate	10	36 ± 1.14[b]	—	+

[a] Indicates significant difference from control value ($p > 0.01$).
[b] Indicates significant difference from control value ($p > 0.05$).

compounds, DEHP and dicapryl phthalate, showed indications of CNS stimulation. All other phthalates appeared to demonstrate CNS depression.

The toxicity of phthalate esters to cultured cells is depicted in Table 5. None of the phthalates (at levels of 0.05 ml of a 50-mg/ml emulsion) demonstrated toxicity to chick embryo cells, but three of the phthalates did show toxicity to mouse fibroblast cells (L-cells) in these same amounts.

The toxicology of some alcohol mixtures, containing 7–9 and 9–11 carbon atoms, and the corresponding phthalate esters was described by BROWN *et al.*[11]. In single-dose acute and oral toxicity tests with rats and mice, di(2-ethylhexyl), di(2-octyl), and dinonyl phthalates were of a low order of toxicity (*e.g.*, $LD_{50} > 26$ g/kg). Repeated daily dosing of rats with 5 ml/kg per day of the phthalates (and alcohols) for seven consecutive days produced diarrhea and irritability with no overt signs of intoxication other than depression with the phthalates.

The subacute and chronic effects of dioctyl and dibutyl phthalates in rats has been described by PIEKACZ[12]. The phthalates were given to male and female Wistar

TABLE 5

TOXICITY OF PHTHALATE ESTERS TO CULTURED CELLS

Dose in both cases, 0.05 ml of a 50-mg/ml emulsion. — no cell toxicity; + cell death.

Phthalate ester	*Chick embryo cells*	*Mouse fibroblast cells*
Dimethyl phthalate	—	+
Diethyl phthalate	—	+
Dibutyl phthalate	—	—
Diisobutyl phthalate	—	—
Dimethoxyethyl phthalate	—	+
Butyl benzyl phthalate	—	—
Di(2-ethylhexyl) phthalate (DEHP)	—	—
Dicapryl phthalate	—	—

rats, orally or by stomach tube in doses of 1, 5, and 10% of LD_{50} in studies of subacute toxicity, or with the diet containing 1% of LD_{50} in studies of subacute and chronic toxicity. Dioctyl phthalate in a dose of 1% of LD_{50} and dibutyl phthalate in concentrations of 1 and 10% of LD_{50} caused a statistically significant enlargement of the liver. Moreover, dioctyl phthalate in concentrations of 1 and 5% of LD_{50} caused a statistically significant rise in the activity of glutamic–pyruvic transaminase. Dioctyl phthalate in amounts of 3.5 g/kg of food caused in males a statistically significant fall in body weight, in females an increase in the weight of the liver and kidneys, and a rise in the activity of glutamic–pyruvic and glutamic–oxalacetic transaminases. These changes had no correlation with the histological results of the viscera of the experimental animals.

BOWER *et al.*[13] investigated eight phthalate esters to determine their effects on the developing chick embryo. Dibutoxyethyl phthalate acted as a teratogenic agent when injected into the yolk sac of the chick embryo by the third day of its development. Congenital malformations such as crania bifida and anophthalmia, were observed as well as marked exophthalmia resulting from an absence of bone tissue of the skull forming the orbit of the eye, and blindness due to failure of the cornea to develop. It was also found that dibutoxyethyl, di(2-methoxyethyl), and octyl isodecyl phthalates were capable of causing damage to the central nervous system of the developing chick embryo. This was manifested after hatching, by grossly abnormal behavior of chicks, such as tremor, non-purposeful bodily movement and a total incapability of either standing or walking normally. Table 6 shows the toxigenicity and teratogenicity of eight phthalate esters for chick embryos. Di(2-methoxyethyl) phthalate was the most toxic of the compounds tested for the chick embryo. Forty-six of fifty chick embryos (*i.e.*, 92%) died during incubation after injecting only 0.025 ml into the yolk sac, while from control chick embryos 31% died uninoculated, and 45 and 53% after 0.10-ml injections of sesame oil and crisco oil, respectively. CALLEY *et al.*[10] found di(2-methoxyethyl) phthalate as one of the most toxic of the phthalate esters following i.p. administration in mice.

GUESS and coworkers[10,14–17] have stressed the "subtle toxicities" of plasticizers and stabilizers used in the manufacture of polyvinyl plastics.

Table 7 lists the effects of certain plastic additives on selected biological systems. Certain of the heat stabilizers, especially those containing heavy metals such as tin, cadmium and zinc, showed a marked incompatibility with the biological systems

TABLE 6

TOXIGENICITY AND TERATOGENICITY OF PHTHALATE ESTERS FOR THE CHICK EMBRYO

Phthalate ester	*Dose injected (ml)*	*No. of eggs injected*	*No. of chick embryos dying*	*No. of chicks hatched*			
				Total	*Normal*	*Showing adverse effects*[a]	*Having congenital malformations*
Butyl benzyl phthalate[b]	0.05	32	15 (46.8)[c]	17	17	0	0
Dibutyl phthalate[b]	0.10	24	19 (79.1)	5	5	0	0
	0.05	24	16 (66.6)	8	8	0	0
Diethyl phthalate[b]	0.025	32	22 (68.7)	10	10	0	0
Dioctyl phthalate[b]	0.10	20	5 (25.0)	15	15	0	0
Di(2-methoxyethyl) phthalate	0.025	50	46 (92.0)	4	1	3	0
Dibutoxyethyl phthalate[b]	0.10	118	75 (63.5)	43	29	10	4
	0.05	82	41 (50.0)	41	35	4	2
Octyl isodecyl phthalate	0.05	13	12 (92.3)	1	1	0	0
	0.025	64	32 (50.0)	32	31	1	0
n-Octyl-*n*-decyl phthalate	0.10	21	10 (47.6)	11	10	1	0
	0.05	21	13 (61.9)	8	5	3	0
Controls							
Sesame oil	0.10	107	48 (44.8)	59	59	0	0
Crisco oil	0.10	43	23 (53.4)	20	19[d]	0	0
Uninoculated	None	154	48 (31.1)	106	106	0	0

[a] Neuromuscular effects: tremor, swaying, unsteadiness, and an impairment in the ability of the chick to either stand or walk.

[b] Esters of phthalic acid approved by the Food and Drug Administration as components for adhesives. Amendment 21, Code of Federal Regulations, paragraph 121.2520 (1969).

[c] Values in parentheses indicate percentages of chick embryos dying.

[d] One of the control chicks receiving crisco oil appeared malnourished and ill after hatching. However, this bird demonstrated neither any congenital malformations nor neuromuscular adverse effects.

TABLE 7

EFFECTS OF CERTAIN PLASTIC ADDITIVES ON SELECTED BIOLOGICAL SYSTEMS

+ intradermal reaction; — no change; H = hemolysis; K = killed; F = cells fixed; IN = interferes with antibody agglutination; GE = growth enhanced.

Material tested	*Effect on RBC*	*Effect on antibodies*		*Effect on cell culture*					*Rabbit skin*	*Mouse L.P.*
		Natural	*Immune*	*L.*	*Ch. E.*	*K–B*	*Amnion*	*Green monk, kid*		
Plasticizers										
Butyl octyl phthalate	—	—	—	—	—	GE	GE	—	—	—
Butyl decyl phthalate	—	—	—	—	—	—	—	—	—	—
Dibutyl ethyl sebacate	—	—	—	—	—	GE	GE	—	—	—
Dimethyl sebacate	—	—	—	K	—	K	K	K	—	—
Dioctyl adipate	—	—	—	—	—	GE	GE	GE	—	—
Heat stabilizers										
Cd–Zn organic inhibitor	H	IN	IN	K	K	K–F	K–F	K–F	+	K
Ca–Zn organic inhibitor	H	IN	IN	K	K	K–F	K–F	K	+	K
Dibutyltin diisooctyl maleate	H	IN	IN	K	K	K–F	K–F	K–F	+	K

TABLE 8

TOXICITY OF TWO POLYVINYL CHLORIDE PLASTICIZERS INOCULATED INTO THE CHORIO-ALLANTOIC CAVITY OF CHICK EMBRYOS

Compound	*Total No. of eggs inoculated on 9th day*	*No. and % of chicks hatched*	*No. of chicks dying in days*[a]		*No. of chicks showing*		*No. and % of chicks apparently normal from hatch on*
			0–6	*7–10*	*Visible deformity of feet*	*Toxicity of GI tract or nervous system*[b]	
Butyl octyl phthalate	30	21 (70%)	5	3	2	8	13 (62%)
Butyl decyl phthalate	28	21 (75%)	4	0	2	5	13 (62%)
Control	44	31 (70%)	5	0	0	0	26 (84%)
Total	102	73 (73%)	14	3	4	13	52 (74%)

[a] The inoculating day is day 0.
[b] The cases of feet deformity are included.

used. Destructive effects such as those observed in cell cultures were seen even when the compounds were diluted to less than 0.5%. The reactions observed with the five plasticizers were considered as "subtle toxicities". Butyl octyl phthalate, dibutyl-ethyl sebacate, and dioctyl adipate stimulated the growth of human amnion (Wish strain) cells and the K–B (Eagle-human cancer line) tissue culture. One of the possibilities considered was, that these agents complement the growth of human cell culture on a cell surface or metabolic basis. However, the observation of increased growth (especially with the human cancer line) might also be considered as a potential subtle change that might be undesirable.

Table 8 illustrates the toxicity of butyl octyl and butyl decyl phthalates when injected into the allantoic cavities of nine-day-old chick embryos. The butyl octyl phthalate, which was innocuous by some test parameters (Table 8), produced changes in the developing embryo of the kind not observable in tissue cultures or in adult animals. The loss of chicks within the first two weeks was greatest with this group (8 of 21 chicks). The toxicity, as shown by difficulty in maintaining balance and lethargy, was somewhat greater with the butyl octyl phthalate (8 chicks) as compared to butyl decyl phthalate (5 chicks).

4. STABILITY AND ENVIRONMENTAL SOURCES

Stability

Polyvinyl chloride (PVC) is unique in its acceptance of large amounts of plasticizers with gradual change in physical properties from rigid soft to soft gel to viscous liquid. It was suggested[18], that PVC has a helical structure with repeating units of $C_{28}H_{42}Cl_{14}$, and that there must be one molecule of plasticizer to block each polar group on the polymer chain to the helical picture of PVC, and hence the need of approx. 45 parts of DOP to 100 parts of resin to complete plasticization.

Although PVC itself, because of its high chlorine content, does not burn, the flammability of plasticized PVC depends primarily on the kind and amount of plasticizer present. Plasticizers, such as phthalates, adipates and polyesters, yield PVC films that burn.

The use of plasticized vinyl on the interior of cars, as in seat covers, crash pads and door covers, is accompanied by "fogging", partly due to the plasticizer, distilling from the PVC and condensing on the windshields when cars are parked in the sun.

Degradation of PVC articles after manufacture and during storage, appears as mottling or darkening of the plastic, frequently with the development of a bad odor. Oxidation of the plasticizer during processing forms peroxides, which later decompose with development of color and odor bodies. SEARS[19] determined the rates of reaction and energies of activation for peroxide formation and decay, in five plasticizers, *e.g.*, di(2-ethylhexyl) phthalate (DEHP) and adipate, diisodecyl phthalate and adipate, and HB-40. The 2-ethylhexyl group was more resistant to oxidation than the isodecyl group, while the phthalate radical appeared to offer some stability lacking in the adipates. The alkyl aromatic hydrocarbon (HB-40) was slightly easier to oxidize than the esters. All of the plasticizers produced two or more peroxides except DEHP. Bisphenol A as an antioxidant inhibited peroxide formation and latent color development, both in plasticizers *per se* and in vinyl formulations.

The influence of acids, PVC and water on the thermal decomposition of DEHP was studied utilizing gas–liquid chromatography (GLC) to identify the decomposition products[20]. The decomposition products identified from 1 mole of DEHP were: phthalic anhydride (1), 3-methyleneheptane (6.97), mixture of 3-methyl-2-heptene and 3-methyl-3-heptene (0.05), 2-ethylhexane (0.78), and water (0.07 mole, respectively). Heating of DEHP for 40 h at 170° in the presence of sulfuric acid or PVC, yielded in all cases 3-methylheptene and its isomerization products. Under these conditions, no alcoholic component was formed. Acids, water and PVC catalyzed the thermal decomposition of both di(2-ethylhexyl) phthalate and di(2-ethylhexyl) sebacate.

HAGEN[21] investigated the pyrolysis of a variety of plasticizers at 730°, by using a glowing wire and analyzing the resultant products by GLC. In the pyrolysis of dibutyl phthalate, isobutene, butene and propylene were the main products.

The pyrolysis of dicyclohexyl phthalate and dibutyl phthalate gave approximately equal mixtures of olefin and alcohol, consistent with a mechanism involving the initial normal ester pyrolysis of dialkyl phthalate to olefin and alkyl hydrogen phthalate, followed by a displacement of alcohol by internal attack of the carboxyl group[22]. Air in contact with PVC-118 polymer membrane (1 m^3/m^2 surface) was found to contain after 30 h at 20°: dibutyl phthalate, 6.4; CO_2, 3520; CO, 30; and hydrocarbons, 552 mg/m^3, respectively. At 100°, the polymer was oxidized and depolymerized, and after 1 h the following components were present along with traces of fatty acids: aldehydes, 0.68; dibutyl phthalate, 31.2; CO_2, 38; hydrocarbons, 96; CH_2CHCl, 152; and HCl, 8 mg/m^3, respectively[23].

Environmental sources

An increasing number of reports have been cited concerning the appearance of phthalate esters in the environment (including in the food chain as well as in human tissue). The widespread occurrence of the phthalates in aquatic ecosystems was recently announced by the US Bureau of Sport Fisheries and Wildlife[24]. The compounds identified in various species of fish were di-*n*-butyl phthalate and DEHP in concentrations ranging from 0.2 to 3.2 p.p.m. These two plasticizers are quite stable, are stored in fish tissue, and are suggested to be probably concentrated in food chains. Phthalate esters have been found in water, sediments, and in fish and other aquatic organisms in industrial and heavily populated areas in the United States[25,26]. The presence of dioctyl phthalate[27] and DEHP[28] in various crude oils and petroleum has been reported. (The detection of phthalic anhydride in crude oils from North Africa has also been cited[29].)

Phthalic acid as well as its short-chain alkyl esters have been found in lipid extracts of plant materials, microorganisms, and in tobacco smoke[30]. Diheptyl phthalate has been produced by *Alternaria kikachiana* Tanaka fungus which produces a black spot on pears[30]. Phthalic acid can be synthesized in rat liver and some bacteria can make diesters of phthalic acid[31].

It has been increasingly apparent that the phthalate esters which are lipid-soluble substances can migrate from plastic packaging into foods, particularly into fatty foods[32]. FEOFANOV *et al.*[33] has reported up to 150 mg of dibutyl phthalate per kg of cheese with 15% fat content. Phthalate esters have also been identified in fat used in deep-fryers[34].

There has been evidence reported of the presence of phthalate esters in animal tissues such as beef pineal gland[35] and heart[36]. Perhaps the most dramatic recent finding has been that of JAEGER AND RUBIN[37] who reported that DEHP and butylglycolyl butyl phthalate (BGBP), often used in the PVC used in bags to store human blood and also in tubing through which blood is passed in heart lung machines and kidney machines, are leached out of the plastic and into the blood. It was found that at the end of the maximum storage period of 21 days, blood stored in PVC bags, contained 5–7 mg of DEHP per 100 ml of blood (50–70 p.p.m.). It was calculated by the authors[38], that under these conditions an average-size man, requiring a total of 14 pints (not uncommon in the treatment of severe hemorrhage) could receive intravenously as much as 350 mg of plasticizer.

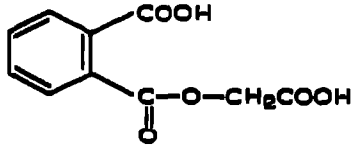
NEERGUARD[39] reported the leaching of diethyl phthalate from PVC tubing: 10–20 mg of plasticizer was leaching into every liter of dialysis fluid. However, despite this evidence, no cause and effect relationship between patients' hepatitis symptoms and plasticizer was shown. It has also been reported in some preliminary work at the National Institutes of Health in the United States that phthalate esters may be present in concentrations of 20 p.p.m. and higher in blood taken from human patients[40].

Physicians in the United States are reporting an increasing incidence of shock lung, a condition characterized by an impeded circulation of blood in the lungs. Although the cause of shock lung (a sometimes fatal disorder) is not definitively known, one theory suggests, that it is related to the presence in the blood of microemboli consisting of aggregates of blood platelets. RUBIN[41] has suggested that the mounting incidence of shock lung may be caused by the increasing medical use of pasticized PVC.

NAZIR *et al.*[42] have recently reported the specific localization of DEHP in bovine, dog, rabbit, and rat heart mitochondria, respectively. It was noted that the mitochondrial compound differed from commercial DEHP, in showing some evidence of optical activity and being crystallizable from hexane at low temperatures. It is not yet clear, whether (and to what extent) the phthalate ester occurs naturally in heart mitochondria or is ingested by the animals as an environmental pollutant[31]. The specific localization of DEHP in heart muscle mitochondria was considered to be significant, in that this substance may possibly influence the bioenergetics of the myocardial cell.

5. ISOLATION FROM BIOLOGICAL SOURCES AND FOOD

JAEGER AND RUBIN[37] described the extraction, metabolism and accumulation by some biological systems of plasticizers from plastic devices. One such plasticizer, butylglycolyl butyl phthalate (BGBP), was metabolized by the isolated perfused rat liver to glycolyl phthalate (GP) while another plasticizer di(2-ethylhexyl) phthalate (DEHP)



Glycolyl phthalate (GP)

was found to be accumulated in the liver unchanged. DEHP, in addition, was identified in samples of human tissues taken from patients who had received transfusions of blood stored in plastic bags.

In Fig. 1 are shown the elution patterns from the chromatographic fractionation of acid-soluble extracts of perfusion plasma, which had either perfused a liver, or had circulated in the apparatus in the absence of a liver. The first peak (I) was common to both experimental conditions and was identified as uric acid (based on its UV spectrum and elution pattern). Peak II, a compound formed only when a liver was present, has not yet been identified. Peak III, which also appeared only when a functioning rat liver was present, was subsequently identified as GP (based on comparison of its IR spectrum of the methyl ester with an analogous synthetic GP derivative).

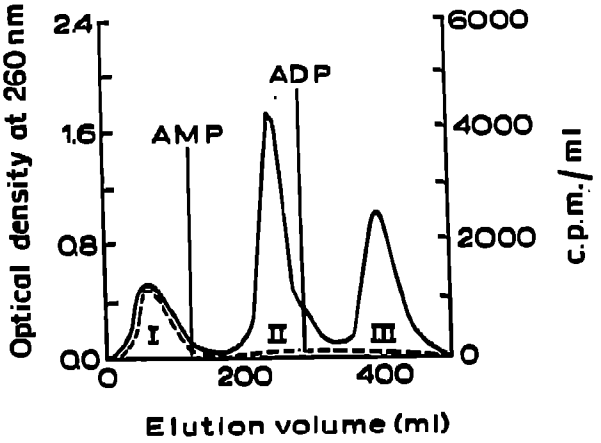


Fig. 1. Circulation of perfusion fluid in the presence (———), and in the absence (— — —) of a rat liver. An amount of [^{14}C]adenosine diphosphate (ADP) and [^{14}C]adenosine monophosphate (AMP) was added to a neutralized extract of plasma, following perfusion, to act as a marker during further chromatographic fractionation. The total extract was applied to a 0.7 cm × 10 cm column of Dowex-1 (formate form) anion-exchange resin. Elution of the column was with a non-linear gradient of ammonium formate (0–2 *N*, pH 5.5), and the absorbance at 260 nm was monitored continuously in a Gilford spectrophotometer. Portions of each fraction were counted in a Packard TriCarb liquid scintillation counter. In order to simplify this figure, only the peak-radioactive fractions are displayed.

It was also found that DEHP, unlike BGBP, was not de-esterified by the isolated rat liver, but rather was accumulated by that organ, primarily in unmetabolized form. JAEGER AND RUBIN[43] also reported, that although simple saline solutions were unable to extract DEHP from PVC tubing, even after 6 h of recirculation in the tubing, a 4% bovine serum albumin solution was found to extract approx. 40% of the amount of plasticizer extractable by whole blood. Fractionation of the blood indicated, that the plasticizer was located almost entirely in the plasma fraction and was specifically associated with the lipoprotein fraction of plasma. Preliminary experiments with tissue obtained from two patients who had received blood transfusions (from DEHP-plasticized blood bags) indicated that spleen, liver, lung, and abdominal fat all contained significant quantities of DEHP ranging from 0.025 mg/g (dry weight) in spleen to 0.270 mg/g (dry weight) in abdominal fat[38].

The contamination of blood stored in plastic packs was also studied by MARCEL AND NOEL[44], who identified the presence of dihexyl phthalate in lipid extracts of

plasma from the transfusion packs. The dihexyl phthalate content of the lipid extracts (obtained via extraction of plasma with chloroform–methanol mixtures according to FOLCH *et al.*[45]) were enriched by chromatography on thick layers of Silica Gel H[46], and final purification was achieved by TLC on the same medium with hexane–diethyl ether–acetic acid (90:10:1). The concentrations of dihexyl phthalate (mg/100 ml of plasma) were 0, 4, 7, 11.5, and 11.5 for storage periods in plastic packs of 0, 4, 8, 15, and 21 days, respectively. The identity of the plasma-extracted phthalate ester was confirmed by IR spectrophotometry and GLC as dihexyl phthalate.

The isolation, identification and specific localization of DEHP in bovine heart muscle mitochondria was described by NAZIR *et al.*[42]. Lipids were separated by a modified silicic acid procedure[47].

The composition of methylated fatty acids was determined by GLC using argon beta-ionization detectors containing 20 mCi of ^{90}Sr-sources (Barber-Colman Model 10 and Electronic Instruments for Research Model AU-8). Siliconized, U-shaped glass columns, 8 ft. × 4 mm I.D. and 6 ft. × 5 mm I.D., were used containing Gas-Chrom P, 80–100 mesh, previously acid washed and deactivated with 2% dimethyl dichlorosilane[48], and coated with either diethylene glycol succinate (200 ml of an 8% solution in acetone per 15 g of support) or Apiezon L (AEI, Manchester) (200 ml of a 4% solution in chloroform per 15 g of support). The diethylene glycol succinate and Apiezon L columns were operated at temperatures of 180 and 200° and at outlet flow rates of 75 and 300 ml/min, respectively, with a detector polarizing voltage of 1000 V.

DEHP was quantitatively determined following its initial isolation from mitochondrial fractions, on a modified silicic acid column[40]. (The fraction eluted with 4% ether in hexane was evaporated to a small volume under partial vacuum, and aliquots of 5–10 μl were analyzed by GLC on a two-component SE-52–XE-60 column as described by NAIR *et al.*[49] at a temperature of 200° and outlet flow rate of 250 ml/min.) Peak areas obtained by triangulation were compared with those obtained from authentic DEHP.

The retention values characteristic of the isolated compound from heart muscle mitochondrial triglyceride, relative to methyl octadecanoate and compared with DEHP, are shown in Table 9. (The retention values were obtained by GLC on diethylene glycol succinate, Apiezon L, and SE-52–XE-60 columns, as described above.)

The isolated compound was subsequently isolated in pure form by preparative GLC on diethylene glycol succinate (10 mm × 8 mm column operated at 200° and at

TABLE 9

GLC RELATIVE RETENTION TIME DATA[a] OF THE UNIDENTIFIED COMPONENT FROM BOVINE HEART MUSCLE MITOCHONDRIAL TRIGLYCERIDE COMPARED WITH DEHP

GLC conditions: Diethylene glycol succinate column temperature, 180°; outlet flow-rate, 75 ml/min. Apiezon L column temperature, 200°; outlet flow-rate, 300 ml/min. SE-52–XE-60 (2:1, v/v) column temperature, 200°; outlet flow-rate, 250 ml/min.

Stationary phase	*Unknown compound*	*DEHP*
Diethylene glycol succinate	13.6	13.5
Apiezon L	4.4	4.4
SE-52–XE-60	4.6	4.6

[a] Relative to methyl octadecanoate = 1.00.

an outlet flow-rate of 750 ml/min). Microhydrogenation[50] and microozonization[51] of the DEHP isolated by preparative GLC was carried out and showed it to be unaltered, indicating the absence of aliphatic double bonds, thus indicating that the isolated compound could not be an unsaturated fatty acid.

Fig. 2 shows a diagramatic representation of structural subunits derived from DEHP upon carbon-skeleton chromatography. In this technique[50], multiple bonds are saturated and functional groups containing oxygen are stripped from compounds, giving as products the parent hydrocarbon and/or the next lower homolog, which are identified by their retention times. The unknown compound subjected to carbon-skeleton chromatography gave peaks with retention times corresponding to *n*-heptane (11.9 min), a branched-chain hydrocarbon 3-methylheptane, which is 2-ethylhexane (20.0 min), and a weak peak for benzene (9.5 min).

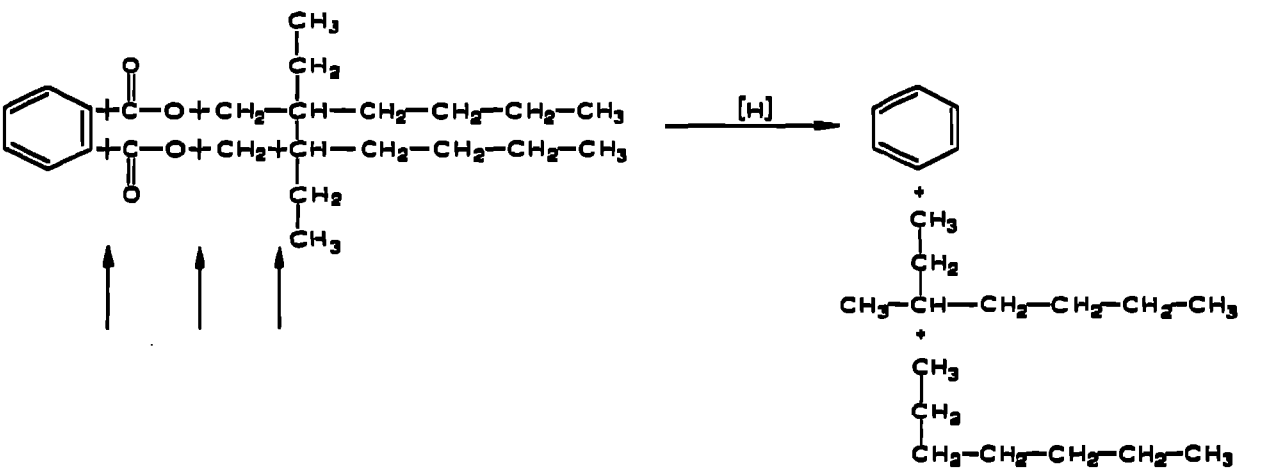


Fig. 2. Diagramatic representation of structural subunits derived from DEHP upon carbon-skeleton chromatography.

This breakdown pattern (Fig. 2) and relative proportions in which each of the hydrocarbons were obtained, suggested a disubstituted benzene ring with a 2-ethylhexyl side-chain. (Identical results were obtained when authentic DEHP was subjected to carbon-skeleton chromatography.) Following drastic alkaline hydrolysis, the alcohol portion upon GLC on diethylene glycol succinate, Apiezon L, and SE-52–XE-60 columns revealed the presence of a single component having retention times identical with those of authentic 2-ethyl-1-hexanol (Table 10). When the acidic portion was methylated and subjected to GLC, only one component having the same retention characteristics as those of dimethyl phthalate *(e.g., ortho)* was detected (Table 11). The separation of several diesters of phthalic acid was achieved on a 6 ft. × 6 mm O.D. stainless-steel column containing 5% SE-30 on Chromosorb W at a column temperature of 225° with a nitrogen flow-rate of 30 ml/min (Fig. 3).

The mass spectrum of the isolated compound was identical with that of an authentic sample of DEHP when determined on a Consolidated Electrodynamics Corp. Model 21-110B mass spectrometer. The molecular formula established by peak matching the molecular ion $C_{24}H_{38}O_4^+$, was found to be 390.2765 compared to a theoretical value of 390.2770. The *m/e* values of fragments with highest relative abundance corresponded to rearrangement ions of phthalic anhydride (149; M—241),

TABLE 10

GLC RELATIVE RETENTION TIME DATA[a] FOR SEVERAL CLOSELY RELATED ALCOHOLS AND AN UNKNOWN SAMPLE ON THREE DIFFERENT STATIONARY PHASES

Unknown sample represents the alcohol moiety of the compound isolated from bovine heart muscle mitochondria. Experimental conditions are described in the text.

Stationary phase	*1-Butanol*	*Isobutyl alcohol*	*Isoamyl alcohol*	*4-Heptanol*	*2-Ethyl-1-hexanol*	*1-Octanol*	*2-Octanol*	*Unknown*
Diethylene glycol succinate	0.73	0.51	1.00[b]	1.58	4.77	7.15	3.42	4.77
Apiezon L			1.00[c]	5.49	14.82	20.75	12.60	14.82
SE-52–XE-60			1.00[d]	3.19	8.72	12.45	6.82	8.72

[a] Relative to isoamyl alcohol = 1.00.
[b] Isoamyl alcohol = 4.3 min.
[c] Isoamyl alcohol = 1.7 min.
[d] Isoamyl alcohol = 2.0 min.

TABLE 11

GLC RELATIVE RETENTION TIME DATA[a] FOR *o*-, *m*-, AND *p*-DIMETHYL ESTERS OF BENZENE DICARBOXYLIC ACIDS ON THREE DIFFERENT STATIONARY PHASES

Unknown sample represents the methylated acid moiety of the compound isolated from bovine heart muscle mitochondria.

Stationary phase	*Ortho*	*Meta*	*Para*	*Unknown*
Diethylene glycol succinate	1.21	1.10	1.00[b]	1.21
Apiezon L	0.63	1.00	1.00[c]	0.63
SE-52–XE-60	0.92	1.07	1.00[d]	0.92

[a] Relative to *p*-dimethyl terephthalate = 1.00.
[b] *p*-Dimethyl terephthalate = 11.7 min.
[c] *p*-Dimethyl terephthalate = 3.8 min.
[d] *p*-Dimethyl terephthalate = 2.5 min.

the diacid fragment (167; M—223), and the one derived by the loss of a 2-ethylhexyl group from the parent molecular ion (279; M—111).

WANDEL AND TENGLER[52] described the GLC determination of DEHP and tributyl-O-acetyl citrate (citroflexa 4) in foods in contact with plastic film. Dry or non-fatty foods such as rice, meal, herbs, instant coffee, or cocoa, were analyzed by a direct method. For example, a 50-g sample of food was mixed with sand, and extracted in a Soxhlet extraction apparatus with methylene chloride. The methylene chloride extract was concentrated, the residue dissolved in methanol, and DEHP was determined on a 50 cm × 3 mm column, containing either 5% SE-52 on Celite 545 or 10% Resoflex LAC-2-R-446 on Chromosorb, at 230° and with a helium flow of 42 ml/min, using dioctyl adipate as internal standard and flame ionization for detection. Tributyl-O-acetyl citrate was determined on the same column at 200° with dibutyl phthalate as internal standard. The foods examined by the above method contained 4–16 p.p.m. of plasticizer or 0.06–0.29 mg/dm^2 of packaging surfaces. The relative error was about 10%. As little as 0.1 mg of DEHP or tributyl-O-acetyl citrate in the 50-g sample (corresponding to 2 p.p.m.) could be detected.

Samples of cheese or sausage were analyzed by an indirect method. The evaporated residues from the methylene chloride extraction were shaken fifteen times with

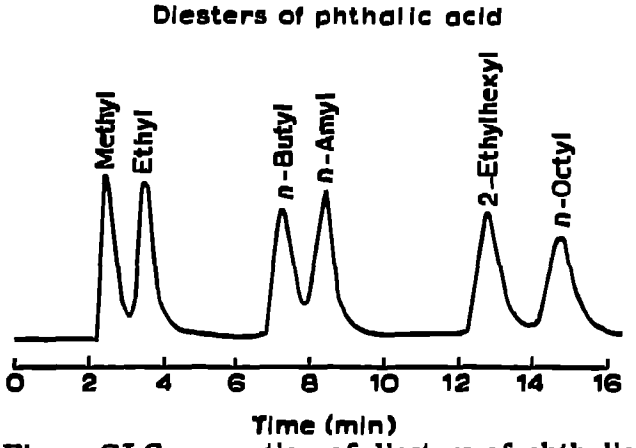


Fig. 3. GLC separation of diesters of phthalic acid on a 5% SE-30 column.

10-ml portions of methanol, the combined methanol extracts were concentrated to 3–5 ml and quantitatively saponified with *p*-toluenesulfonic acid for 3 h at 130°. The saponified mixture was analyzed for alcohols using a flame ionization detector and a 2 m × 4.65 mm I.D. copper column, packed with 15% polyethylene glycol P1500 on Celite 545, or 10% Resoflex LAC-2-R-446 on Chromosorb. 2-Ethylhexanol from DEHP was determined at a column temperature of 100° and a helium flow of 92 ml/min, using heptanol as an internal standard. Butanol from tributyl-O-acetyl citrate was determined at a column temperature of 90° and a helium flow of 45 ml/min, using propanol as an internal standard.

A method for the isolation and detection of DEHP from milk lipids was described by CERBULIS AND ARD[53]. Milk was dialyzed and evaporated to dryness, and the residue extracted with petroleum ether. The petroleum ether extract was chromatographed on an alumina column as described by HANAHAN[54], and the fraction containing free fatty acids and DEHP was then separated by TLC on Silica Gel G. DEHP was distinguished from other phthalates by TLC and IR spectroscopy. The solvent systems used for the alumina (activated, chromatographic grade, from Matheson, Coleman and Bell) column were (1) chloroform, (2) chloroform–methanol (1:1), (3) and (4) ethanol–chloroform–water (5:2:1) and (5:2:2), respectively, (5) methanol; and for TLC: (6) petroleum ether (b.p. 30–60°)–diethyl ether–acetic acid (90:10:1), (7) chloroform–methanol–water (65:25:4), and (8) benzene.

Table 12 shows the results of chromatography of milk lipids on the alumina column. The residue of the system 4-fraction, containing free fatty acids and DEHP, was chromatographed on Silica Gel G, and Fig. 4 shows a TLC chromatogram of this phthalate-containing fraction, developed with solvent system 6. Fig. 5 shows a TLC chromatogram of phthalates (*viz.* phthalic acid, dimethyl, diethyl, dibutyl, and dioctyl phthalate) as well as of DEHP isolated from milk and Fig. 6 shows a TLC chromatogram of the phthalate-containing system 4-fraction when developed with solvent system 7. Fig. 7 illustrates the IR spectra of authentic DEHP compared with an isolated sample from milk.

TABLE 12

CHROMATOGRAPHY OF MILK LIPIDS ON AN ALUMINA COLUMN

No.	*Solvent system*	*Volume of solvent (ml)*	*Yield (g)*	*Lipid components*
1	Chloroform	2000	53.0	glycerides, cholesterol, pigments
2	Chloroform–methanol (1:1)	500	traces	lecithin, sphingomyelin
3	Ethanol–chloroform–water (5:2:1)	800	traces	inositide (?)
4	Ethanol–chloroform–water (5:2:2)	900	0.8	free fatty acids, DEHP
5	Methanol	900	no residue	

The migration of DEHP and Mesamoll (ethane sulfonate of phenol and cresol) from nitrile rubber into milk was studied by GLC[55]. Potassium oxalate was added to the milk to prevent coagulation, after which the plasticizers were extracted with a methanol–diethyl ether mixture. The extract was then concentrated and the products saponified to form 2-ethylhexanol, phenol, and cresol (from Mesamoll). The saponified

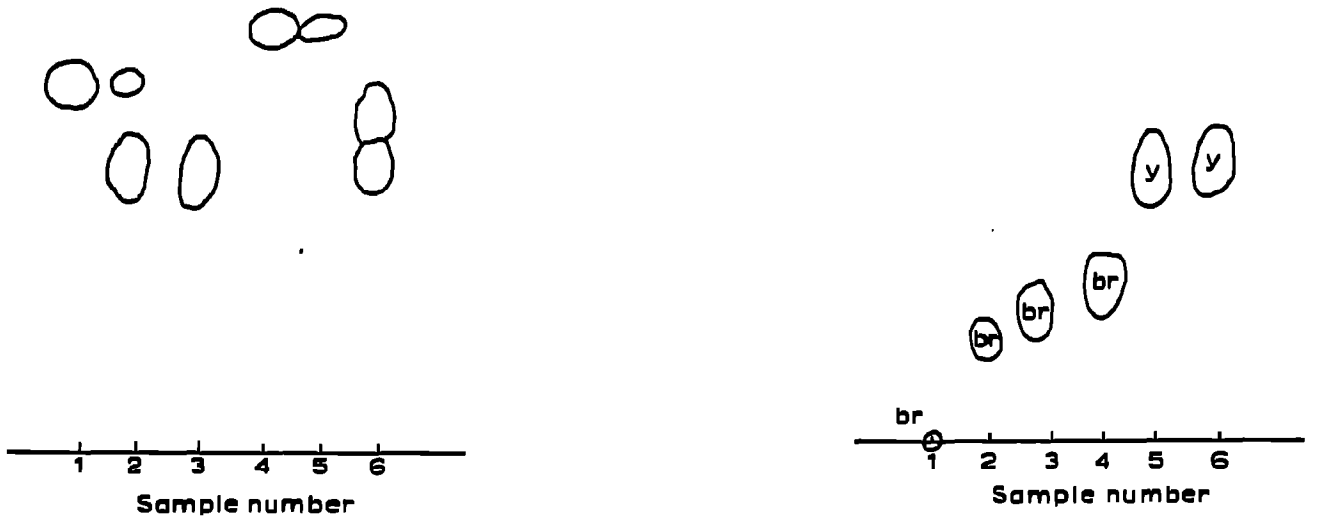


Fig. 4. TLC diagram of the phthalate-containing fraction. Solvent system: petroleum ether–diethyl ether–acetic acid (90:10:1). (1) DEHP, isolated from milk; (2) system 4-fraction; (3) oleic acid; (4) methyl esters of system 4-fraction acids; (5) methyl oleate; (6) triglycerides of whole milk.

Fig. 5. TLC diagram of phthalates. Solvent system: petroleum ether–diethyl ether–acetic acid (90:10:1). br, Brown; y, yellow; (1) phthalic acid; (2) dimethyl phthalate; (3) diethyl phthalate; (4) dibutyl phthalate; (5) dioctyl phthalate; (6) DEHP isolated from milk.

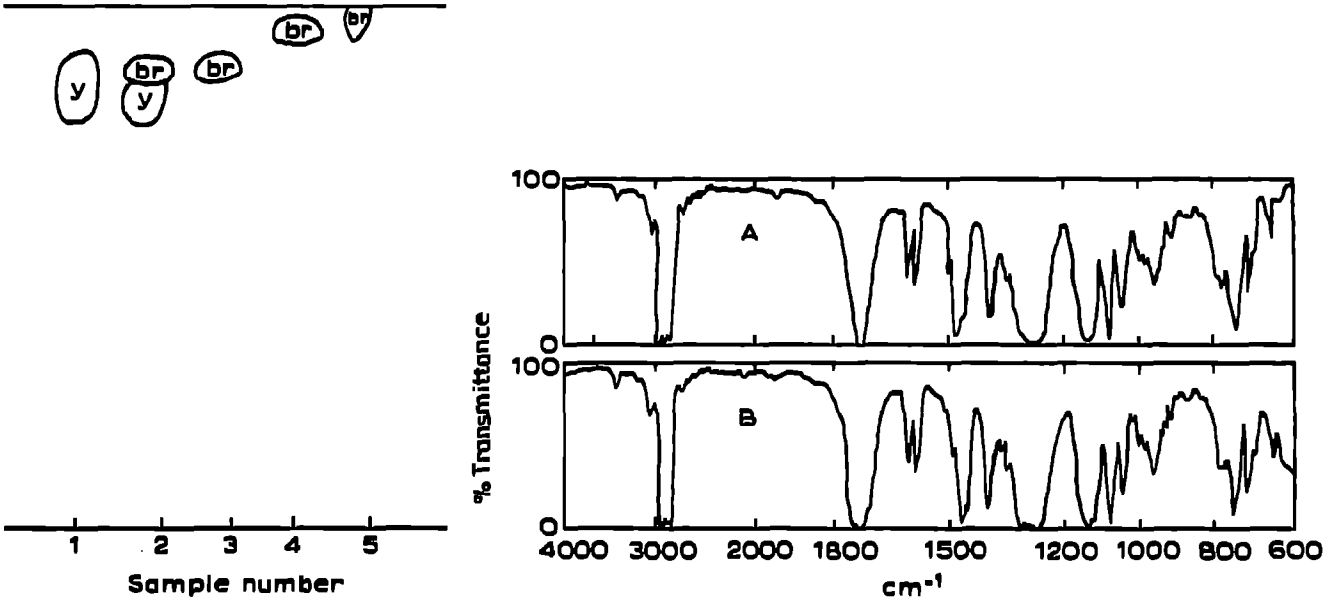


Fig. 6. TLC diagram of the phthalate-containing fraction. Solvent system: chloroform–methanol–water (65:25:4). br, Brown; y, yellow; (1) DEHP; (2) system 4-fraction; (3) oleic acid; (4) methyl oleate; (5) whole milk triglycerides.

Fig. 7. IR spectra. (A) Authentic DEHP (Flexol Plasticizer DEHP from Union Carbide Chemicals Co.). (B) matching microsample from milk.

mixture was steam-distilled and the 2-ethylhexanol, phenol, and cresol in the distillate were extracted with diethyl ether and quantitatively determined by GLC using a P-2000 column and adding triethylphenol as an internal standard. The relative error for the range 2–40 p.p.m. was 20–30%.

PARODI AND DUNSTAN[56] identified dibutyl phthalate by GLC as the compound isolated from an initial TLC separation from the unsaponifiable fraction of butterfat. A source of the contaminant was believed to be the plasticizer used in tubing in the dairy or laboratory.

SCHETTINO AND LA ROTONDO[57] studied the release of additives from surgical instruments and pharmaceutical containers made from PVC. Plasticizers such as DEHP and acetylated tributyl citrate were detected chromatographically in ether, to a lesser degree in physiological saline or acetic acid, and to a very slight degree in urine in which plasticized PVC had been incubated. The significance of exposure time on the leaching of these plasticizers from PVC, is highly important, since the plastic is commonly used in in-dwelling surgical devices such as catheters as well as in tubing used in heart-lung and kidney machines.

6. THIN-LAYER CHROMATOGRAPHY

The TLC analysis of plasticizers has been described by PEEREBOOM[58,59]. The plasticizer or the residue obtained by extraction of a food-packing material was dissolved in 5% diethyl ether, concentrated, and applied on Silica Gel G plates containing 0.005% of the water-soluble fluorescence indicator Ultraphor (Badische Anilin & Soda Fabrik). On irradiation with UV light (365 nm) all plasticizers became visible as fluorescent spots or in the case of phthalates as dark spots. The following solvent mixtures were preferably used: isooctane–10% ethyl acetate; benzene–5% ethyl acetate; and dibutyl ether–20% hexane.

The R_F values of the plasticizers were calculated with reference to dibutyl sebacate (Table 13). Only the placticizer mixtures 5–6, 5–6–7, and 7–8–9 were not sufficiently separated in any of the systems, and had to be identified by means of more-or-less specific color reactions. Table 14 contains a survey of the obtained spot colors. Reaction procedures were: (1) Spray with a solution of phosphomolybdic acid (10% in ethanol); heat approx. 20 min at 100°. (2) Spray with a solution of resorcinol (20% in ethanol, add some $ZnCl_2$); heat 10 min at 150°; spray with 4 *N* H_2SO_4; heat approx. 20 min at 120°; spray with 40% KOH. (3) Spray with a solution of thymol (20% in ethanol); heat 10 min at 90°; spray with 4 *N* H_2SO_4; heat approx. 10–15 min at 120°. (4) Spray with 1 *N* ethanolic KOH solution; heat 15 min at 60°. (Only the citrates become visible as yellow spots.) Spray with 50% urea solution. The citrates are visible in UV light (365 nm) as strong fluorescent spots. (5) Spray with a solution of vanillin (20% in ethanol); heat 10 min at 80°; spray with 4 *N* H_2SO_4; heat approx. 30 min at 110°. (6) Spray with a solution of 2,6-dichloroquinone chloroimide (2% in ethanol); spray after 2–3 h with 2% borax. (7) Spray with alkaline $KMnO_4$ solution (1% $KMnO_4$–2% Na_2CO_3 (1:1)). (The colors shown after 1–2 h are noted.) (8) Spray with acetic anhydride–50% H_2SO_4 (1:2); heat approx. 10 min at 80°. (9) Spray with 0.5 *N* ethanolic KOH solution; heat 15 min at 60°; spray with diazonium reagent (preparation: 0.8 g of *p*-nitroaniline in 250 ml of water and 20 ml of 25% HCl, 5% $NaNO_2$ solution is added till the reagent mixture is entirely colorless).

TABLE 13

RELATIVE R_F VALUES[a] OF SOME PLASTICIZERS OBTAINED BY MICRO-TLC IN THREE SOLVENT MIXTURES

Identification with 0.005% Ultraphor in UV light.
Developing solvents: (1) isooctane–10% ethyl acetate, (2) benzene–5% ethyl acetate, (3) dibutyl ether–20% hexane.

No.	*Plasticizer*	*Solvent 1*	*Solvent 2*	*Solvent 3*
1	Triacetin	0.18	0.34	0.17
2	Ethylphthalyl ethyl glycolate	0.22	0.66	0.30
3	Acetyl triethyl citrate	0.26	0.51	0.29
4	Triphenyl phosphate	0.33	0.80	0.50
5	Tricresyl phosphate	0.42	0.86	0.69
6	Butylphthalyl butyl glycolate	0.43	0.90	0.65
7	2-Ethylhexyl diphenyl phosphate	0.46	0.77	0.58
8	Diethyl phthalate	0.51	0.79	0.60
9	Acetyl tributyl citrate	0.53	0.85	0.70
10	Di-*n*-butyl phthalate	0.74	1.03	0.84
11	Diisobutyl adipate	0.83	0.86	0.85
12	Dibutyl sebacate	1.00	1.00	1.00
13	Dinonyl phthalate	1.01	1.18	1.14
14	Di(2-ethylhexyl) phthalate	1.14	1.16	1.15
15	Butyl stearate	1.61	1.23	1.28
16	Paraflex G 62 (epoxidized natural glycerides)	0.09	0.06	0.06
		0.15	0.13	0.14
		0.25	0.20	0.28
		0.33	0.26	0.42
		0.60	0.40	
		0.68	0.47	
		0.73	0.65	
		0.80	0.72	
		0.92	1.04	

[a] Relative to dibutyl sebacate = 1.00.

TLC of dimethyl and dibutyl phthalates, and of tricresyl phosphate on Aluminum Oxide D (VEB) was described by PIERSCH AND MAYER[60]. Benzene–chloroform (50:2.5) was used for development (10 cm, *ca.* 20 min) with detection of phthalate esters accomplished with a spray reagent consisting of 20% resorcinol in ethanol, 40% $ZnCl_2$ and 3% H_2SO_4. Heating the plates at 150°, revealed the phthalate esters as yellow spots. Detection of tricresyl phosphate was achieved via sequential use of: (a) 15% NaOH; (b) 0.1 g of *p*-nitroaniline in 5 ml of 25% HCl, diluted to 100 ml; and (c) 5% $NaNO_2$ solution. The plates were first sprayed with solution a, and kept in an oven at 110° for 30 min; 10 ml of solution b and 1 ml of solution c were mixed and sprayed on the cooled plate, revealing tricresyl phosphate as a violet spot. The R_F values of dimethyl and dibutyl phthalates were 0.21 and 0.33, respectively, and of tricresyl phosphate, 0.23. Any antioxidants added to the plastic material as stabilizers, appeared as blue or brown spots when sprayed with phosphomolybdic acid in ethanol.

BRAUN[61,62] separated a variety of plasticizers on Silica Gel G with methylene chloride, after they had been extracted from the plastic material with benzene or ether (provided the polymer itself was not soluble). Detection was accomplished with $SbCl_5$–chloroform or carbon tetrachloride (1:4) (freshly prepared before use), which yielded brown spots with most of the plasticizers, after the plate was heated to 210°. Phthalate esters were also detected using resorcinol–$ZnCl_2$–H_2SO_4 reagent[58]. Table 15 lists the R_F values of twenty-six common plasticizers on Silica Gel G developed with methylene chloride.

TABLE 14

SPOT COLORS OF SOME PLASTICIZERS ON CHROMATOPLATES

500 μg of plasticizer on TLC plates were detected by means of reaction procedures 1–9 mentioned in text. ± Only a faint color is obtained; + visible in UV light (365 nm).

No.	*Plasticizer*	*Spot color*								
		Proc. 1	*Proc. 2*	*Proc. 3*	*Proc. 4*	*Proc. 5*	*Proc. 6*	*Proc. 7*	*Proc. 8*	*Proc. 9*
1	Triacetin	yellow	purple	yellow-green	—	blue-black	—	purple	—	—
2	Ethylphthalyl ethyl glycolate	—	yellow	purple	—	—	—	—	—	—
3	Acetyl triethyl citrate	—	—	—	+	blue	—	±yellow	—	yellow
4	Triphenyl phosphate	orange-yellow	—	—	—	red	blue	yellow	—	orange-purple
5	Tricresyl phosphate	red	—	—	—	red	blue	yellow	—	orange-purple
6	Butylphthalyl butyl glycolate	yellow	yellow	purple	—	—	—	—	—	—
7	2-Ethylhexyl diphenyl phosphate	blue	purple	green	—	blue	blue	yellow	purple-brown	orange-purple
8	Diethyl phthalate	yellow	yellow	—	—	—	—	purple-red	—	—
9	Acetyl tributyl citrate	±yellow	—	—	+	blue	—	±	±pink	yellow
10	Di-*n*-butyl phthalate	±yellow	yellow	blue	—	—	—	±yellow	—	—
11	Diisobutyl adipate	±yellow	—	±yellow	—	green-blue	—	±yellow	±pink	—
12	Dibutyl sebacate	±yellow	—	±blue	—	—	—	±yellow	±	—
13	Dinonyl phthalate	blue	±orange	brown-red	—	blue	—	—	purple-brown	—
14	Di(2-ethylhexyl) phthalate	blue	±orange	green	—	black	—	±yellow	purple-brown	—
15	Butyl stearate	blue	—	—	—	—	—	yellow	brown-red	—
16	Paraflex G 62[a]	blue	±brown	brown	+	black	—	yellow	brown	—

[a] R_F = *ca.* 0.00.

TABLE 15

$R_F \times 100$ VALUES OF IMPORTANT PLASTICIZERS ON SILICA GEL G

Solvent: methylene chloride.

Compound	*$R_F \times 100$ value*	*Compound*	*$R_F \times 100$ value*
Dimethyl phthalate	51	Di(2-ethylhexyl) adipate	44
Dibutyl phthalate	69	Dinonyl adipate	44
Dihexyl phthalate	80	Adipic acid polyester	2
Dioctyl phthalate	86	Dibutyl sebacate	41
Di(2-ethylhexyl) phthalate	85	Dioctyl sebacate	61
Didecyl phthalate	85	Di(2-ethylhexyl) sebacate	61
Diisodecyl phthalate	84	Sebacic acid polyester	2
Trioctyl phosphate	23	Triethyl citrate	12
Diphenyl octyl phosphate	42	Tributyl citrate	14
Triphenyl phosphate	47	Triethyl acetyl citrate	15
Diphenyl cresyl phosphate	51	Tributyl acetyl citrate	24
Tricresyl phosphate	53	Tri(2-ethylhexyl) acetyl citrate	46
Dioctyl adipate	42	Glycerol triacetate	13

The identification of a variety of plasticizers in plastics by TLC was described by SWIATECKA AND ZOWALL[63]. Samples of the plastics were first extracted with ether and the extracts concentrated, chromatographed on Silica Gel G, and developed with ethyl acetate–cyclohexane for all plasticizers with the exception of the phosphates, which were chromatographed with methylene chloride. Following development, the plates were exposed to iodine vapor and then sprayed with 20% recorcinol solution in ethanol containing traces of $ZnCl_2$, and (or) with 25% $SbCl_5$ solution in carbon tetrachloride. The R_F values found for the phthalate esters were: dimethyl, 0.22; dibutyl, 0.54; dicyclohexyl, 0.60; di-*n*-octyl, 0.79; diisooctyl, 0.79; di(2-ethylhexyl), 0.79; dinonyl, 0.78; didecyl, 0.79; diisodecyl, 0.8; for the phosphate esters: tricresyl, 0.36; triphenyl, 0.04; dicresyl phenyl, 0.18, tri-*n*-octyl, 0.36; for the sebacic acid esters: dimethyl, 0.41; diethyl, 0.53; di-*n*-octyl, 0.67; di(2-ethylhexyl), 0.81; and for the adipates: diethyl, 0.39; dibutyl, 0.56; dicyclohexyl, 0.63; and di-*n*-octyl, 0.71.

Phthalate ester plasticizers have been separated on silica gel plates and identified by spraying with resorcinol and H_2SO_4, as yellow spots on a light background[64]. Very good results were obtained by mixing the Silica Gel G with fluorescent dyes such as Blankophor, DCB. When observed under UV light, the lower-member phthalate esters appeared as dark stains. Higher phthalate esters ($\geqq$ octyl) appeared as light-fluorescing spots. A mixture of dimethyl and diethyl phthalates with triphenyl phosphate was separated on Silica Gel G plates (with incorporated Blankophor, DCB) using isopropyl ether–petroleum ether (70:30). Higher-boiling phthalate esters were separated by TLC with isopropyl ether–petroleum ether (10:90) as developing solvent.

For GLC, columns of 10% Reoplex 400 on silica gel were generally successful. In isothermal operation, a column temperature of 190° was used for the separation of dimethyl, diethyl, and dibutyl phthalates and at 230° for the higher-boiling phthalates except for diisodecyl phthalate. For a positive identification, 10–100 mg of plasticizer were sealed in a small tube with approx. 1 ml of dry methanol, containing 3% by weight of *p*-toluenesulfonic acid, and heated for 3 h at 130°. The alcohol was identified by GLC using a column containing 10% Reoplex 400 on 80–100 mesh silica gel, with temperature programmed from 80 to 160° at 1.25°/min, and a carrier gas flow-rate of 42 ml/min.

Both TLC and GLC methods were examined by DIEMAIR AND PFEILSTICKER[65] for the rapid isolation and concentration of monomeric ester plasticizers. The extraction with nitromethane as solvent, and TLC on Silica Gel G with nitromethane at 50°, proved successful for the separation and analysis of plasticizers from food products. The plasticizers studied were: dioctyl, dibutylglycol, didecyl, and dinonyl phthalate, tricresyl phosphate, tributyl citrate, tri(2-ethylhexyl)acetyl citrate, and dioctyl adipate. GLC was carried out with a Perkin-Elmer Fraktometer F6 gas chromatograph equipped with a flame ionization detector. A 30 cm × 6.35 mm U-column was packed with 3% Silopren-R (Bayer) on Chromosorb R, 50–60 mesh. The injection port and detector temperatures were 350 and 400°, respectively, and the column was run under temperature programming from an initial temperature of 160° at 10°/min. Helium was the carrier gas at 120 ml/min.

HELMSTEDT *et al.*[66] described the analysis of components of plastics by TLC on silica gel. Separation of plasticizers, antioxidants and most stabilizers (R_F, 0.5–0.8) was accomplished using carbon tetrachloride–chloroform (4:1) and chloroform–ether (1:1). The compounds separated were: dipropyl and dibutyl phthalates, dioctyl adipate, tributyl citrate, dicresyl phosphate, dioctyl sebacate, Leuna ML, barium, cadmium, and lead stearates, Advastab OM18, Advastab OM17, Advastab 405, Advastab 406, Ionol, Nonox WSP, 2,2'-dihydroxy-4,4'-dimethoxy benzophenone, dicyandiamide, phenylurea, and propyl gallate.

JAMINET[67] utilized TLC on 0.3-mm layers of Silica Gel G for the determination of citric and phthalate ester plasticizers. For phthalates, the developing solvent systems were: (1) petroleum ether (b.p. 40–60°)–ethyl acetate (9:1); (2) isooctane–ethyl acetate (9:1); or (3) benzene–ethyl acetate (19:1). The plates were sprayed with a mixture of equal volumes of 4 *N* H_2SO_4 and 20% ethanolic resorcinol, and then heated in an oven at 120° for 10 min, and allowed to cool. Brown spots, which turned orange in ammonia vapor, indicated the presence of phthalate esters with a sensitivity of 20 μg (citrate esters were not detected). The R_F values of various phthalate esters using solvent system 3 were: di(2-methoxyethoxy), 0.18–0.21; di(2-butoxyethoxy), 0.31–0.34; dimethyl, 0.47–0.49; diethyl, 0.56–0.60; diisobutyl, 0.72–0.76; dicyclohexyl, 0.71–0.76; diisooctyl, 0.78–0.90; and di(2-ethylhexyl) phthalate, respectively, 0.88–0.90. The R_F value of phthalic acid in this system was 0.00.

The TLC of plasticizers in PVC formulations was described by CAMPBELL *et al.*[68]. PVC was first dissolved in tetrahydrofuran, and methanol was added until the formation of a fine-granular precipitate was formed, which was then separated. The filtrate was evaporated, the residue chromatographed on Silica Gel G plates containing 5% eosin, and the plates developed with ethyl acetate–isooctane (15:85), methylene chloride, or diethyl ether–petroleum ether (1:4). Resorcinol–H_2SO_4, NaOH, or 2,6-dichloroquinone chloroimide–borax reagents were used for spot detection.

A TLC and GLC technique for the analysis of plasticizers in polyvinylidene chloride-mixed polymer films was described by GROEBEL[69]. TLC on silica gel using methylene chloride for development separated tributyl acetyl citrate, dibutyl sebacate, and di(2-ethylhexyl) phthalate (R_F values: 0.32, 0.38, and 0.58, respectively). Use of a column containing 20% polydiethylene glycol succinate, permitted the GLC separation of dibutyl sebacate, tributyl acetyl citrate and di(2-ethylhexyl) phthalate (Retention times: 3.80, 7.14, and 11.25 min, respectively).

The identification and separation[70] of a number of phthalates, sebacates,

azelates, adipates, phosphates, epoxides, epoxidized soybean oil, and chlorinated paraffin plasticizers of PVC was examined by TLC, using Silica Gel HF 245 + 366 with three mobile phases: (1) isooctane–ethyl acetate, (2) benzene–ethyl acetate, and (3) dibutyl ether–hexane. The plasticizers were identified, by determining their R_F values with reference to 4-dimethylaminoazobenzene, and by UV, IR, and color tests.

NELSON[71] studied the TLC behavior of eleven phthalate esters using five developing solvents. In addition to giving a positive Dragendorff test, the phthalates reacted with an anisaldehyde reagent. After spraying with anisaldehyde reagent, the plates were allowed to stand 1 h, and were then heated at 100° to give blue colors for alkyl and alicyclic phthalates, and red colors for diphenyl phthalates and di(*p-tert.*-butylphenyl) phthalate. The anisaldehyde had a limited utility value due to a variable sensitivity (10–25 mg).

7. GAS–LIQUID CHROMATOGRAPHY

DAL NOGARE AND SAFRANSKI[72] developed a high-temperature gas chromatograph for the qualitative and quantitative resolution and estimation of high-boiling organic mixtures. The partition columns were operated in the range of 150–350° for the resolution of hydrocarbon, ester, and glycol mixtures. The platinum-filament thermal conductivity detectors were operated at 10–100° higher than column temperature, to avoid condensation of high-boiling components. Mixtures of compounds boiling in the range of 150–450° were easily resolved on relatively short columns containing silicone grease or linear polyethylene as the partition medium, and thermal degradation was minimized by the all-glass construction and short residence time in the columns. The assembled apparatus consists of a carrier gas source and metering device, thermal-conductivity cells, column and heating jacket, and the necessary circuit for detecting and recording the detector cell signal (Figs. 8–10).

Fig. 11 illustrates the separation of polyethylene glycols and phthalates. Dimethyl, diethyl, diallyl, di-*n*-butyl, and diethoxyethyl phthalates were separated at a column temperature of 206° and detector temperature of 318°, using helium carrier

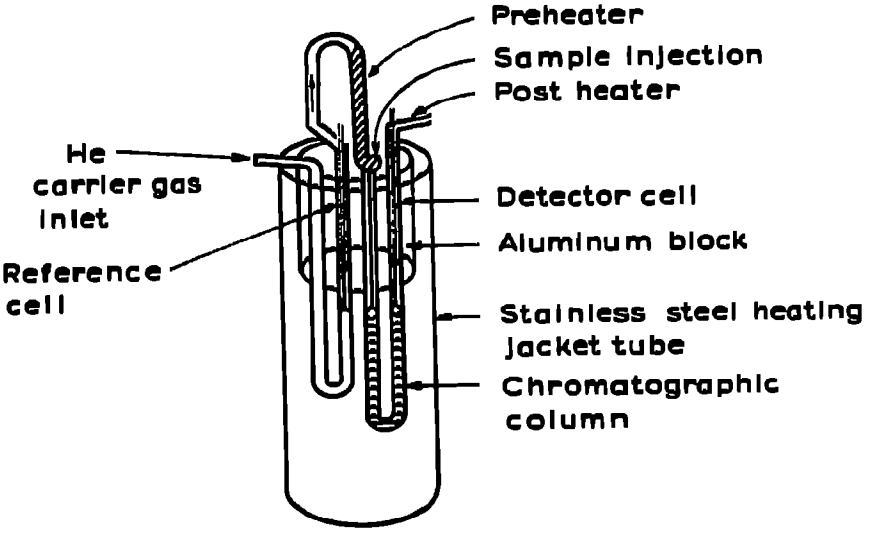


Fig. 8. Column and detectors.

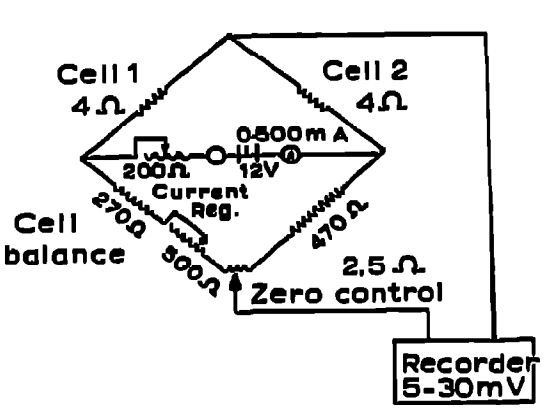


Fig. 9. Detector bridge circuit.

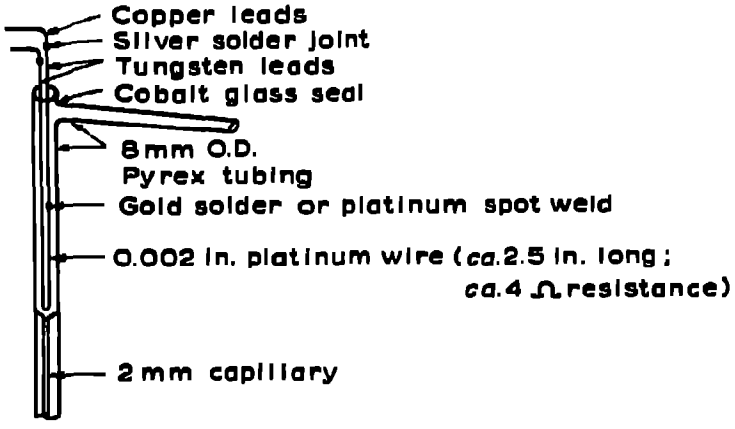


Fig. 10. Thermal conductivity cell.

gas at 124 ml/min. The separation was effected on a 23% silicone grease–Celite column (9 mm × 20 in.) in less than 10 min. The high sensitivity of the thermal conductivity cells is indicated by the fact that only 2 μl of the respective phthalate and glycol mixtures were used, with a 5-mV full-scale recorder for these chromatograms.

The identification and determination of seven plasticizers in nitrocellulose, vinylchloride and acrylic-type lacquers by temperature-programmed GC was reported by ESPOSITO[73]. The analysis was conducted on lacquer samples after treatment to remove the resin. The equipment used was a F&M Model 500 gas chromatograph equipped with a Brown Electronik recorder (Minneapolis-Honeywell) and a disc integrator (Disc Instruments). The column consisted of a 6 ft. × 1/4 in. copper tubing packed with 20% by weight of silicone grease on acid- and alkali-washed Chromosorb

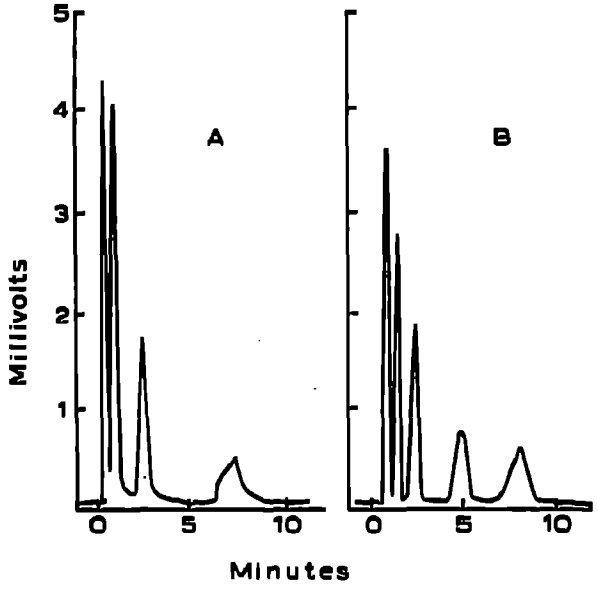


Fig. 11. GLC separation of polyethylene glycols and phthalates. A. Mono-, di-, tri-, and tetraethylene glycol (in order of appearance). Column temperature, 172°; detector temperature, 250°; helium carrier gas flow-rate, 20 ml/min. B. Dimethyl, diethyl, diallyl, di-*n*-butyl, and diethoxyethyl phthalates (in order of appearance). Column temperature, 206°; detector temperature, 318°; helium carrier gas flow-rate, 124 ml/min.

W. The detector cell temperature was set at 300°, the detector cell current at 160 mA, the injection port temperature at 330°, and the helium flow rate at exit was 120 ml/min. The chromatographic column was heated to 210°, then programmed to a heating rate of 4°/min to 290°. The plasticizers were identified by calculating the retention times relative to dibutyl sebacate (Table 16).

TABLE 16

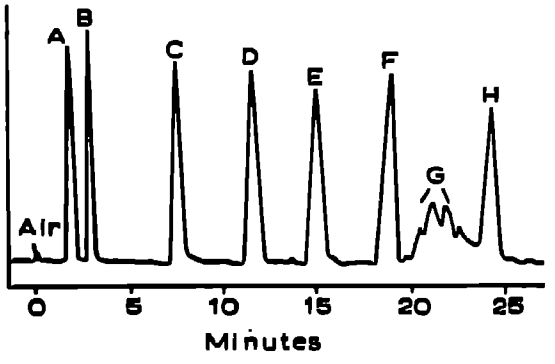
RELATIVE RETENTION TIME DATA FOR PLASTICIZERS OBTAINED BY GLC OF LACQUER SAMPLES

Plasticizer	*Rel. retention time*[a]
Dimethyl phthalate	0.16
Diethyl phthalate	0.26
Dibutyl phthalate	0.67
Butyl benzyl phthalate	1.32
Di(2-ethylhexyl) phthalate (DEHP)	1.65
Tricresyl phosphate	1.78–1.97[b]
Dioctyl sebacate	2.20

[a] Relative to dibutyl sebacate = 1.00.
[b] Several peaks are produced in this range.

The above separation of seven phthalate esters and tricresyl phosphate plasticizers and of dibutyl sebacate on silicone grease is illustrated in Fig. 12. All plasticizers produced one peak except tricresyl phosphate. Figs. 13 and 14 show the analysis of nitrocellulose lacquers containing phthalate plasticizers, *e.g.*, di(2-ethylhexyl) phthalate (DEHP), and diethyl and dibutyl phthalates. Fig. 15 shows the analysis of an acrylic-type lacquer plasticized with butyl benzyl phthalate. A vinyl chloride lacquer containing tricresyl phosphate is shown in Fig. 16. Both dibutyl sebacate and dibutyl adipate were used as internal standards with dibutyl sebacate being preferred for establishing relative retention times and for quantitative analysis.

GLC analysis of plasticizer esters has been accomplished using a Perkin-Elmer Model 116E chromatograph with a thermistor detector[74]. Two stationary phases were



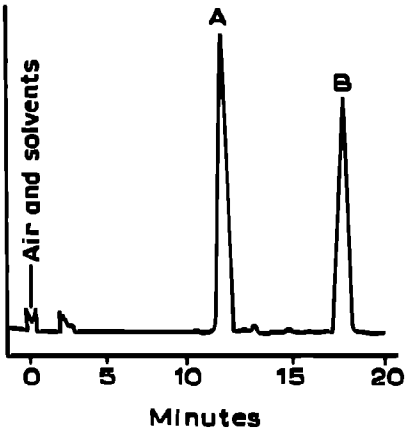
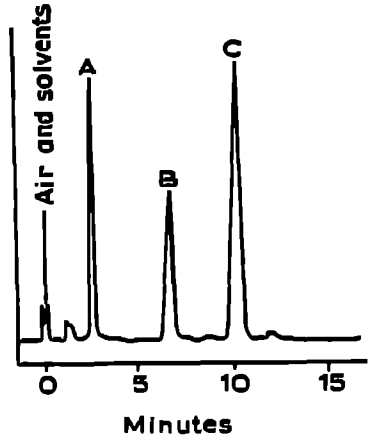


Fig. 12. GLC separation of plasticizers on a silicone grease column. (A) Dimethyl phthalate; (B) diethyl phthalate; (C) dibutyl phthalate; (D) dibutyl sebacate; (E) butyl benzyl phthalate; (F) di(2-ethylhexyl) phthalate (DEHP); (G) tricresyl phosphate; (H) di(2-ethylhexyl) sebacate. Fig. 13. GLC analysis of nitrocellulose lacquer. (A) Dibutyl sebacate (internal standard); (B) di(2-ethylhexyl) phthalate (DEHP).



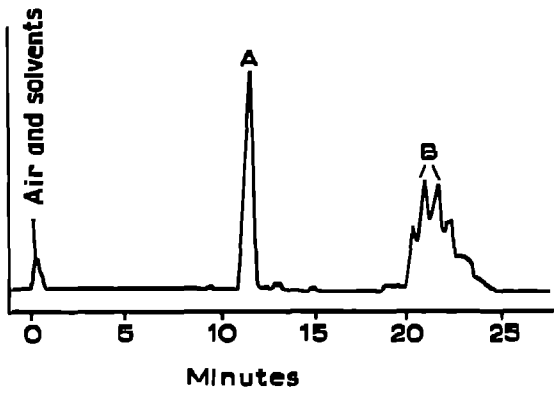


Fig. 14. GLC analysis of nitrocellulose lacquer containing two plasticizers. (A) Diethyl phthalate; (B) dibutyl phthalate; (C) dibutyl sebacate (internal standard).

Fig. 15. GLC analysis of acrylic-type lacquer. (A) Dibutyl adipate (internal standard); (B) dibutyl phthalate; (C) butyl benzyl phthalate.

used: 0.5% SE-30 and 0.5% neopentylglycol polysebacate. Analysis at 200° was possible for liquids with boiling points below 400°. Retention indices were reported for phthalic and adipic esters of normal- and branched-chain alcohols on two columns of different polarity.

The GLC determination of plasticizers, such as dioctyl phthalate or dioctyl adipate and related compounds, was analyzed on GC columns at 200–240° using SE-30 or polyneopentylglycol adipate on glass beads[75].

A special technique of mild pyrolysis of the sample in the injection port permitted analysis of the plasticizers without a preliminary extraction step. The chromatograph used was a Perkin-Elmer Model 116E with a thermistor katharometer detector. Two columns were used, both copper tubing, 4 mm I.D., 6 mm O.D. One 2-m column was packed with 0.5% SE-30 on glass beads, previously etched for a few minutes by concentrated HF and washed. The sieved beads were 125–160 μ. The other column (3-m long) was packed with 0.5% polyneopentylglycol adipate on the etched glass beads. Hydrogen was used as carrier gas with a flow rate of about 60 ml/min.

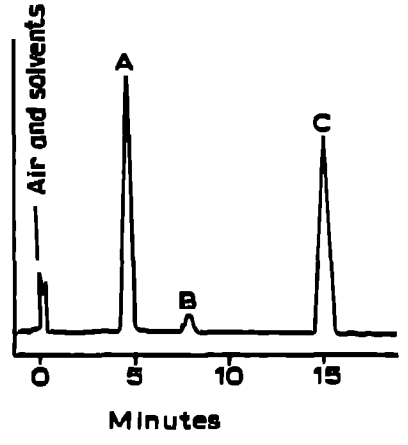


Fig. 16. GLC analysis of vinyl chloride lacquer. (A) Dibutyl sebacate (internal standard); (B) tricresyl phosphate.

The main part of the electrical circuit for the pyrolysis unit, is a platinum coil welded to two copper wires very similar to those used in the pyrolysis of polymers[76]. The device is fitted to the chromatograph in place of the conventional silicone rubber cap. The platinum wire is 35 mm × 0.4 mm in diameter and the coil is 2 mm O.D. (resistance, 0.02 Ω).

Fig. 17 shows the chromatogram obtained with a sample of a PVC, containing tri-*n*-butyl phosphate, di-*n*-butyl phthalate, and di-*n*-butyl sebacate, via initial pyrolysis, and the corresponding chromatogram obtained by injections of a liquid mixture of the three esters. For approximately the same quality of solute, peaks are narrower and higher when the liquid was injected than when the plasticizer was obtained on pyrolyzing the polymer. The pyrolysis peaks tail somewhat, while with liquid injection they were Gaussian. Fig. 18 shows the chromatograms obtained on an apolar and a polar column for samples of the same material. PVC plasticized with diesters may be identified only by the comparison of retention data on the two columns.

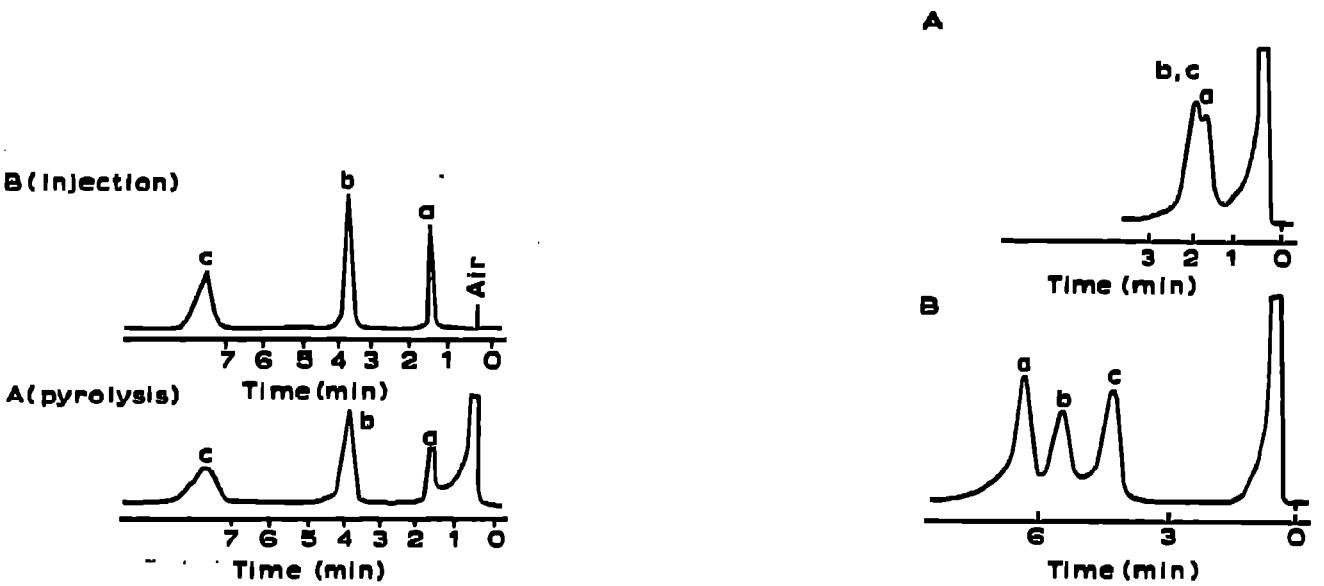


Fig. 17. GLC on an apolar column.
A. Analysis by pyrolysis of a sample of PVC plasticized with: (a) tri-*n*-butyl phosphate; (b) di-*n*-butyl phthalate; and (c) di-*n*-butyl sebacate.
B. Analysis of a mixture of the same plasticizers, using conventional injection with a syringe.

Fig. 18. GLC analysis of a sample of plasticized PVC on A = apolar column and B = polar column. Peaks: (a) diethyl phthalate; (b) dimethyl sebacate; (c) tri-*n*-butyl phosphate.

Table 17 shows the retention indices of some plasticizers. In the first row the values were computed according to the earlier described technique of ZULAICA AND GUIOCHON[77]. In the second row they were measured from pure compounds injected as liquids, and in the third row they were measured from the pyrolyses of plastic products containing known plasticizers at different concentrations. The difference between the last two values are less than ±1% of the indices.

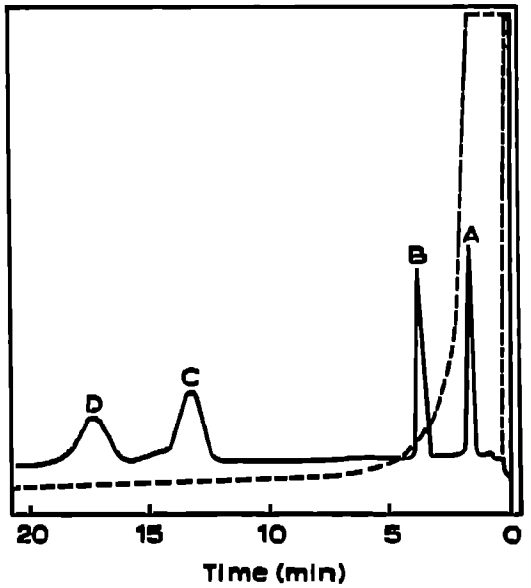
Dialkyl phthalates, at concentrations of 1–10 p.p.m. in aqueous solutions, have been separated and determined by GLC after extraction into hexane[78]. The instrument used was an Aerograph 1520 equipped with a 1:1 stream splitter. The two equal

TABLE 17

COMPUTED AND MEASURED GLC RETENTION INDICES (*RI*) OF PLASTICIZERS

Compound	*RI value*			
	Computed	*Measured*		
		Injection	*Pyrolysis*	*Pyrolysis–injection*
Dimethyl phthalate	1457	1450	1469	19
Diethyl phthalate	1587	1583	1594	11
Di-*n*-butyl phthalate	1926	1932	1920	−12
Di(2-ethylhexyl) phthalate (DEHP)	2482	2470	2496	26
Tri-*n*-butyl phosphate	1663	1648	1633	−15
Di-*n*-butyl sebacate	2169	2150	2156	6
Di(2-ethylhexyl) adipate	2412	2391	2400	9

effluent gas streams were monitored by flame ionization (FID) and electron capture (ECD) detectors, to demonstrate the difference in response to both sample and solvent. The column (5 ft. × 1/8 in. O.D.) comprised 1% Carbowax 20M on DMCS Chromosorb G, 80–100 mesh, coated with 1% polyvinyl pyrrolidone. The column was conditioned overnight at 230° in a stream of nitrogen, followed by 10 days at 190°. Nitrogen used as carrier gas at a flow rate of 20 ml/min, was dried by passing through molecular sieve 5A. Fig. 19 illustrates a chromatogram (at a column temperature of 190°) of a 4-μl mixture of diethyl, dibutyl, di(2-ethylhexyl) (DEHP), and di(3,5,5'-trimethylhexyl) phthalates, each at a concentration of 1 p.p.m. Fig. 20 illustrates an example of the



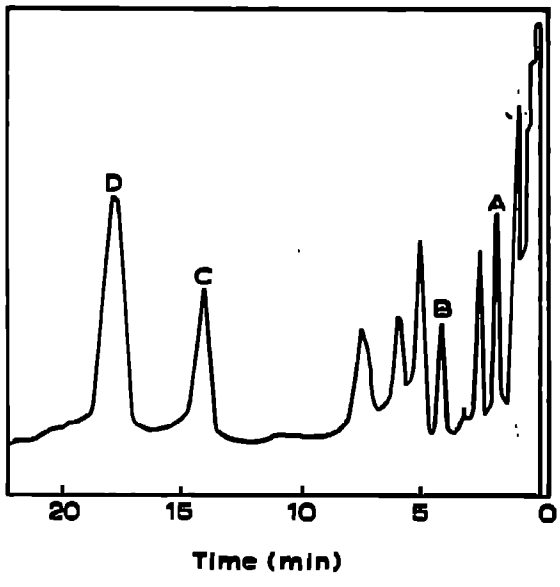


Fig. 19. Relative GLC response to ECD (———) and FID (— — —) of a 4-μl mixture of (A) diethyl phthalate; (B) dibutyl phthalate; (C) di(2-ethylhexyl) phthalate (DEHP); and (D) di(3,5,5'-trimethylhexyl) phthalate (each at a concentration of 1 p.p.m.).
GLC conditions: column (5 ft. × 1/8 in. O.D.), 1% Carbowax 20 M on DMCS Chromosorb G, 80–100 mesh, coated with 1% polyvinyl pyrrolidone; temperature, 190°; nitrogen flow-rate, 20 ml/min; instrument, Aerograph 1520; sensitivity of ECD and FID, 1 × 8 and 0.1 × 16, respectively.

Fig. 20. Typical example of the type of chromatogram, obtained by GLC under same conditions as in Fig. 19, after extraction of a polyvinyl chloride-type plastic. (A) Diethyl phthalate; (B) dibutyl phthalate; (C) di(2-ethylhexyl) phthalate (DEHP); and (D) di(3,5,5'-trimethylhexyl) phthalate.

type of chromatogram, obtained from an actual sample by using only the ECD, and it shows that di(3,5,5'-trimethylhexyl) phthalate and DEHP are easily separated. A better separation of the lower-boiling dialkyl phthalates could be affected by GLC at a lower column temperature than 190°.

The GLC of a number of commercial plasticizers was elaborated by COURTIER[70]. Commercial samples of di-*n*-octyl, di(2-ethylhexanyl) and diisooctyl phthalates showed the presence of several C_8-alcohols, produced in the oxo process. The chromatogram of the phthalate made from C_7-, C_8-, and C_9-alcohols from the oxo process, showed twenty peaks. Chromatograms of ethyl hexyl and dioctyl azelates showed the presence of acids from adipic to undecylenic acid.

A study was described by COURTIER[80] of the purity control of DEHP: the separation and identification of light impurities as well as the analysis of plasticizers of higher molecular weight, *viz.* trimellitic esters. A high-temperature apparatus was used, and a CuO furnace operated at 700°, was included, which converted the eluted compounds to CO_2 and H_2O. CO_2 was detected by a thermistor catharometer, and the water was absorbed in $CaCl_2$. The columns used were 2 m × 4 mm I.D. copper tubing, containing silicone grease or SE-30 on Chromosorb W (60–80 mesh) or Chromosorb G, respectively. Diisooctyl phthalates from three different sources were resolved and the impurities detected. Impurities found in DEHP were: air, ethylhexene, ethylhexanol, phthalic anhydride, ethyl hexyl ether, and ethyl hexyl benzoate. Analogous results were obtained for trimellitic esters.

Mixtures of phthalates; dioctyl succinate, adipate, azelate, and sebacate; ethyl linoleate, oleate, and stearate; and several maleates were analyzed by HOSHI *et al.*[81]. Two columns of lower-content liquid phase, such as 1.4% neopentyl glycol sebacate or 1.2% SE-30 on Diasolid-M were used to separate these esters. The vapor pressures of these esters were calculated from the relative retention time (t_R) *vs.* diethyl phthalate or dioctyl adipate used as the standard substance, and the latent heat values (ΔH) were calculated by using the Clausius–Clapeyron equation. The corrected t_R values were not affected by the kind of columns, but depended on the difference of ΔH between the samples and the standard substance.

The determination of dimethyl, diethyl, and dibutyl phthalates in small arms double-base propellants has been reported by NORWITZ AND ABATOFF[82]. The phthalates were extracted from the propellant with methylene chloride, and an aliquot passed through a SE-30 column at 200° for dimethyl and diethyl phthalates, and at 230° for dibutyl phthalate. Triacetin was used as the internal standard in the determination of dimethyl and diethyl phthalates, while dimethyl sebacate was used as the internal standard for the determination of dibutyl phthalate. A single-column gas chromatograph was used equipped with a thermal conductivity cell (Aerograph Model A90) and a 6 ft. × 1/4 in. O.D. column containing 20% SE-30 on a.w. Chromosorb W (60–80 mesh). The injection port and detector temperatures were both 275°, and the carrier gas was helium at 35 ml/min.

The peaks obtained at a column temperature of 210° for a mixture of dimethyl, diethyl, and dibutyl phthalates (and the internal standard triacetin) in methylene chloride are shown in Fig. 21. The actual retention times for the above compounds were: 2.75, 4.0, 12.0 and 1.75 min, respectively. The relative retention times of dimethyl, diethyl, and dibutyl phthalate were: 1.57, 2.29, and 6.86 min, respectively.

TUNSTALL[80] described the GLC of plasticizers in propellant compositions. A

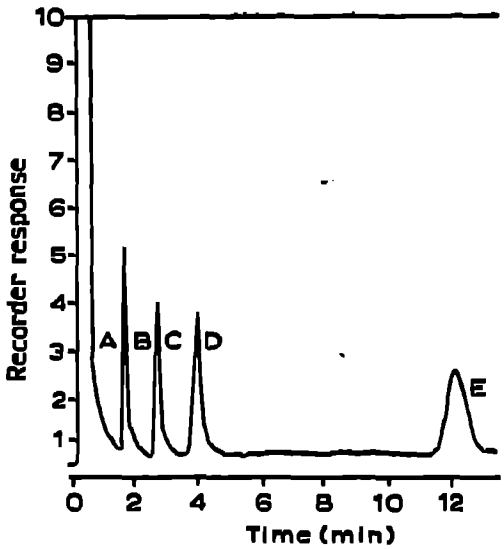


Fig. 21. GLC chromatogram of a methylene chloride solution containing: (A) methylene chloride; (B) triacetin (internal standard); (C) dimethyl phthalate; (D) diethyl phthalate; and (E) dibutyl phthalate.

Varian Aerograph 1522/1B dual-flame-detector gas chromatograph and a 1-mV Speedomax W recorder fitted with a Model 224 Disc Instruments Integrator, were used. The operating conditions and relative retention data are summarized in Tables 18 and 19. Relative response factors were obtained for each of the plasticizers, using dimethyl phthalate as internal standard, after it had been established that there was no interference from stabilizers, their degradation products, or from nitroglycerine.

TABLE 18

GLC CONDITIONS FOR PROPELLANT SAMPLE PLASTICIZER ANALYSIS

Column	2 m × 1/8 in s.s. tubing
Column packing	5% Antarox CO-990[a] on 80–100 mesh AW DMCS Chromosorb G
Column temperature	185°
Injector temperature	275°
Detector temperature	275°
Carrier gas	nitrogen at 25 ml/min
Sample size	0.5 μl

[a] Nonyl phenoxy polyethyleneoxy ethanol.

TABLE 19

RELATIVE RETENTION DATA OBTAINED BY GLC OF PROPELLANT SAMPLES

Compound	*Rel. retention time*[a]
Triacetin (TA)	0.46
Diacetin (DA)	0.74
Dimethyl sebacate (DMS)	0.89
Dimethyl phthalate (DMP)	1.00
Diethyl phthalate (DEP)	1.28

[a] Relative to dimethyl phthalate = 1.00.

The small proportions of diacetin present in stock triacetin used for manufacture, did not change under the chromatographic conditions given in Table 18. Propellant samples were extracted with methylene chloride, extracts concentrated almost to dryness, internal standard added, contents made up to 25 ml with methyl ethyl ketone, a suitable aliquot chromatographed, and the plasticizer content determined by peak area measurement. A typical chromatogram of an aged propellant extract is shown in Fig. 22.

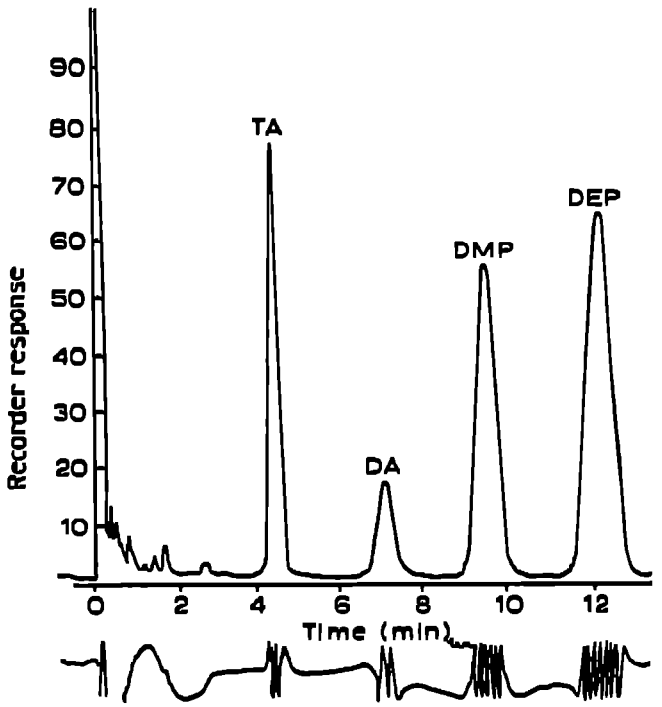


Fig. 22. GLC chromatogram of an aged propellant extract. GLC conditions and plasticizer abbreviations listed in Table 18 and 19, respectively.

Adequate separation has been achieved in the past with a polypropylene sebacate column. The disadvantage of this liquid phase, however, was a high bleed rate at the temperature, required to give reasonable retention times for analysis, which was only 10° below its maximum operating temperature of 200°. However, polypropylene sebacate did completely resolve dimethyl phthalate and dimethyl sebacate, eluted in that order, and could be used for the analysis of compositions containing both of these esters. Antarox CO-990 has now generally replaced polypropylene sebacate because of its lower bleed rate at an operating temperature, 40° below its maximum of 225°, and because of improved separation of triacetin and diacetin.

Diethyl phthalate is a commonly employed denaturant. It is widely used since it seldom interferes with the intended use of the preparation, and may be a preferred component because of its utility as a perfume fixative, a plasticizer or an insect repellant. In Great Britain the inclusion of diethyl phthalate is mandatory for perfumes made with industrial-methylated spirits (α-grade)[84], and the minimum proportion of diethyl phthalate accepted is 1% by volume of the preparation.

The determination of diethyl phthalate in ethanolic preparations such as per-

fumes, lacquers, deodorants, varnishes and paints was described by HANCOCK *et al.*[85]. Three methods were evaluated: A method suitable as a screening test for large numbers of samples involved the use of direct GC. Another method involved a simple clean-up of the sample followed by column chromatography and determination of the ester by its absorption in the UV. An additional method was based on hydrolysis of the ester, and then gravimetric determination of the phthalic acid after conversion to phthalanil.

A Perkin-Elmer Model 800 gas chromatograph equipped with an FID, was used with stainless-steel columns (9 ft. × 1/8 in. O.D. and approx. 0.1 in. I.D.) containing fluorosilicone oil, FS1265–HMDS Chromosorb W, 80–100 mesh, (1.5:98.5), or alternatively, SE-30–HMDS Chromosorb W, 80–100 mesh, (1.5:98.5). The carrier gas was nitrogen at a flow rate of 30 ml/min. The temperature of the column was 165° for fluorosilicone oil and 150° for SE-30. The inlet block temperature was 250°.

Fig. 23 illustrates successive chromatograms of a variety of ethanol preparations including perfumes, lacquers, deodorants, and varnishes. Relative retention times of dimethyl, diethyl, dibutyl, and di(2-ethylhexyl) phthalates, isopropyl myristate, and methyl salicylate on fluorosilicone oil and SE-30 are listed in Table 20.

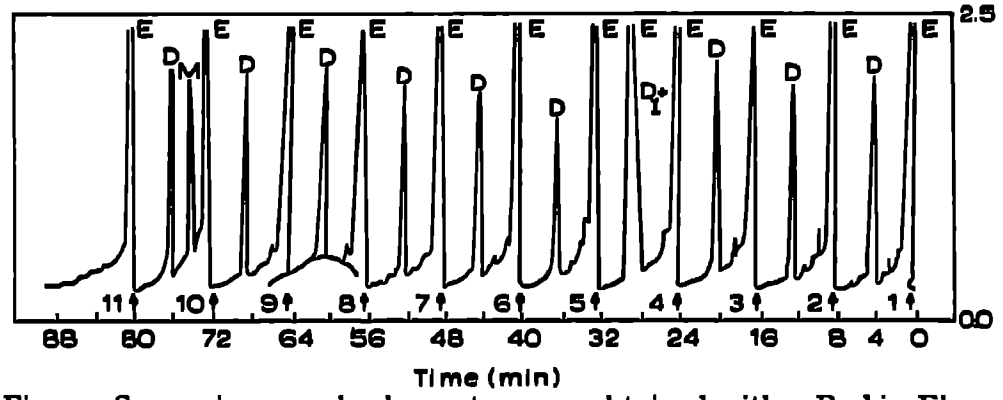


Fig. 23. Successive sample chromatograms obtained with a Perkin-Elmer Model 800 gas chromatograph FS1265–HMDS Chromosorb W (1.5:98.5); nitrogen flow, 30 ml/min; temperature, 165°. 1, 2, 3 = Perfumes; 4 = after-shave lotion containing isopropyl myristate; 5, 6 = hair lacquer; 7 = deodorant; 8 = nail varnish remover; 9 = eau de cologne; 10 = surgical spirit containing methyl salicylate; 11 = eau de cologne with diethyl phthalate absent. E = Ethanol; D = diethyl phthalate; I = isopropyl myristate; M = methyl salicylate;

TABLE 20

RELATIVE RETENTION TIMES[a] FOR SUBSTANCES FROM FIG. 23 ON TWO COLUMNS

Substance	*FS1265*	*SE-30*
Dimethyl phthalate	0.65	0.59
Diethyl phthalate	1.00	1.00
Dibutyl phthalate	3.20	5.70
Di(2-ethylhexyl) phthalate (DEHP)	38.2	—
Isopropyl myristate	0.98	3.0[b]
Methyl salicylate	0.02	—

[a] Relative to diethyl phthalate = 1.00.

[b] Commercial samples of isopropyl myristate often contain an impurity with a relative retention time on SE-30 of 1.15.

Column chromatography utilized alumina, 100–200 mesh (Brockman 1–2), with a mixture of light petroleum–diethyl ether (60:40) used for elution. The eluate fraction containing the diethyl phthalate was evaporated to dryness, taken up in ethanol and measured at 227 nm.

Table 21 compares the results from GC, gravimetric and titration methods for the determination of diethyl phthalate in a variety of ethanolic preparations.

GC analyses of the phthalate esters have generally employed isothermal conditions[83,86,87], with only limited use of temperature programming for a few of the plasticizers. One of the major handicaps of isothermal column operation has been the necessity of employing either two different columns or two different temperatures, for the identification of both the original plasticizer and the products obtained from it by hydrolysis and esterification.

TABLE 21

COMPARISON OF RESULTS FROM GC, GRAVIMETRIC AND TITRATION METHODS

Type of ethanolic preparation	*Diethyl phthalate (%)*			*Remarks*
	GC method	*Gravimetric method*	*Titration method*	
Perfume spray	0.7	0.71	0.9	—
Perfume spray	1.0	1.10	1.0	—
Perfume	1.0	1.00	2.4	
Hair oil ("Honey and Flowers")	nihil	nihil	—	Top layer.
	0.8	1.00	—	Lower layer.
Pre-electric shave lotion	1.3	1.39	1.4	Contains isopropyl myristate.
Eau de cologne	0.9	0.80	1.2	—
Hair dressing	0.95	0.90	1.2	—
Aerosol perfume	1.2	1.2	1.9	—
Perfume	0.6	0.74	1.1	—
Hair lacquer	0.7	—	2.0	Contains diethyl and dibutyl phthalates.
Hair tonic	nihil	nihil	nihil	Contains castor oil.
After-shave lotion	0.8	0.84	—	Contains isopropyl myristate.
Hair tonic	0.85	0.80	—	Contains glycerin.
Hair lacquer	1.7	1.9	—	Contains polyvinyl pyrrolidone.
Eau de cologne	0.90	0.86	—	Contains benzyl acetate, 2-phenylethanol and methyl naphthyl ketone.
Bay rum	0.75	0.86	—	—
Eau de cologne	0.25	0.38	—	—
Hair dressing	0.70	0.66	—	Contains xylol, salicylic acid, resorcinol and hexachlorophene.
Lavender water	0.80	0.78	—	—

KRISHEN[88] recently described the programmed GC for identification of a variety of ester plasticizers used in PVC and other polymers. The GC unit was a Hewlett-Packard 5750B dual-flame-ionization chromatograph equipped with a Moseley-Hewlett-Packard 7127A 1-mV recorder. Two stainless-steel columns, each 6 ft. × 1/8 in. O.D. packed with 10% UCW-98 on 60–80 mesh Diatoport S, were employed in the

dual operation mode. The initial column oven temperature was 100° and after 4 min of isothermal operation, the temperature was programmed at a rate of 8°/min to a max. of 330°, and the final temperature held constant for 8 min. The injection block and detector temperatures were maintained at 270°. The helium, hydrogen, and air pressures were 60 (Flowrator 0.8), 14, and 30 p.s.i.g., respectively. Samples of the plasticizers were dissolved in tetrahydrofuran and then injected into the gas chromatograph. When the plasticizers were present in PVC, the polymer sample was first dissolved in tetrahydrofuran, insoluble components allowed to settle out, and a sample of the clear solution chromatographed. A 1% solution of the polymer was suitable for this purpose, when the plasticizer content of the polymer was between 10 and 40%.

Fig. 24 shows a gas chromatogram of a mixture of ester plasticizers. Most of the

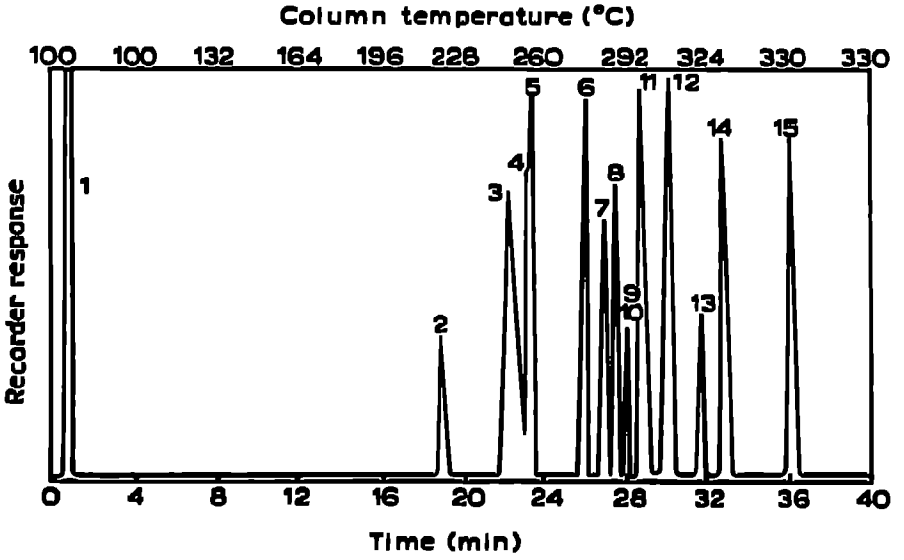


Fig. 24. Gas chromatogram of ester plasticizer mixture. 1 = Tetrahydrofuran; 2 = trithyl citrate; 3 = methylphthalyl ethyl glycolate; 4 = ethylphthalyl ethyl glycolate; 5 = dibutyl phthalate; 6 = dibutyl sebacate; 7 = acetyl tributyl citrate; 8 = butylphthalyl butyl glycolate; 9 = butyl benzyl phthalate; 10 = trioctyl phosphate; 11 = di(2-ethylhexyl) adipate; 12 = di(2-ethylhexyl) phthalate (DEHP); 13 = di(2-ethylhexyl) azelate; 14 = di(2-ethylhexyl) sebacate; 15 = di-*n*-decyl phthalate.

commonly-used materials easily separated by temperature-programmed GC and could be identified by their retention times. The retention times calculated relative to di(2-ethylhexyl) phthalate (DEHP) are shown in Table 22.

Although the relative retention times of the plasticizers are helpful for the identification of plasticizers, the complexity of mixtures normally encountered, generally necessitates hydrolysis and esterification to obtain information about the components of plasticizers. Simple hydrolysis (using lithium metal in methanol, and refluxing the mixture for 2 h) followed by esterification to the respective methyl esters, thence GC permitted the facile identification of these products.

Fig. 25 depicts a gas chromatogram of alcohols and of methyl esters of acids of the commonly-used plasticizers. The identification of unknown alcohols and methyl esters of acids, facilitated by their relative retention times are given in Table 23.

Phthalic anhydride (PAA) is manufactured by catalytic oxidation of naphthalene and *o*-xylene, which process allows the possible formation of impurities such as

TABLE 22

RELATIVE RETENTION TIMES FOR ESTER PLASTICIZERS[a]

Plasticizer	*Rel. retention time*
Phthalates	
Dibutyl	76.9
Butyl benzyl	92.8
Di(2-ethylhexyl) (DEHP)	100.0
Di-*n*-decyl	120.0
Adipates	
Di(2-ethylhexyl)	95.0
Azelates	
Di(2-ethylhexyl)	105.4
Citrates	
Triethyl	62.5
Acetyl tributyl	89.1
Sebacates	
Dibutyl	85.9
Di(2-ethylhexyl)	108.7
Glycolates	
Methylphthalyl ethyl	73.6
Ethylphthalyl ethyl	76.4
Butylphthalyl butyl	91.3
Phosphates	
Trioctyl	92.8

[a] For gas chromatogram, see Fig. 24.
[b] Relative to DEHP = 100,00.

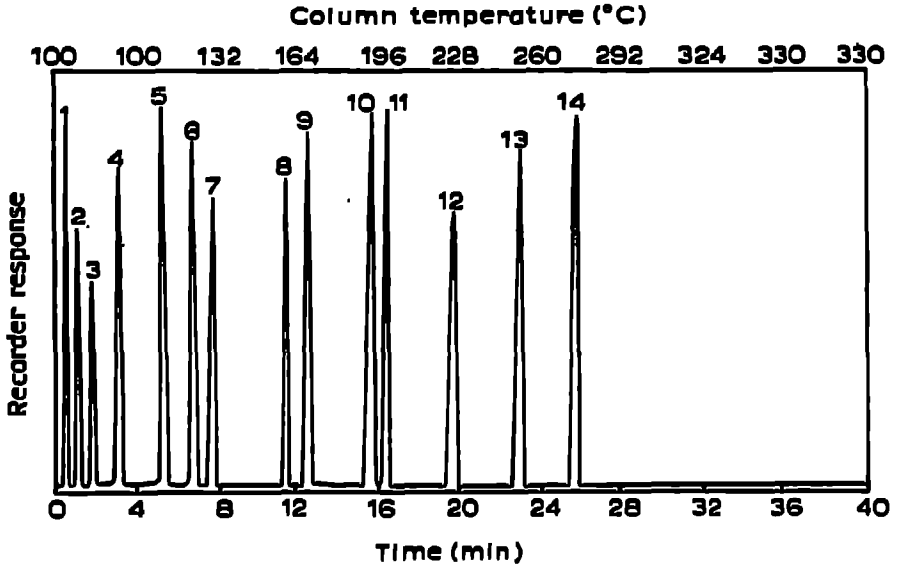


Fig. 25. Gas chromatogram of alcohols and of methyl esters of acids of ester phasticizers. 1 = Methanol; 2 = butanol; 3 = pentanol; 4 = hexanol; 5 = heptanol; 6 = 2-ethylhexanol; 7 = octanol; 8 = dimethyl adipate; 9 = decanol; 10 = dimethyl-*o*-phthalate; 11 = dodecanol; 12 = tetradecanol; 13 = methyl palmitate; 14 = methyl stearate.

TABLE 23

RELATIVE RETENTION TIMES FOR HYDROLYSIS PRODUCTS[a] OF ESTER PLASTICIZERS

Component	*Rel. retention time*[b]
Methanol	0.034
Butanol	1.82
Pentanol	3.98
Hexanol	8.04
Heptanol	15.5
2-Ethylhexanol	21.0
Octanol	24.5
Dimethyl adipate	37.0
Decanol	40.0
Dimethyl-*o*-phthalate	51.1
Dodecanol	53.0
Tetradecanol	64.3
Methyl palmitate	76.1
Methyl stearate	84.8

[a] For gas chromatogram, see Fig. 25.
[b] Relative to DEHP = 100.00.

napthhalene, maleic and citraconic anhydrides, benzoic and *o*-toluic acids, phthalide as well as unreacted *o*-xylene. The identification and quantitation of impurities in PAA, is of importance for quality control because of stringent specifications for PAA plasticizer and resin use.

The quantitative analysis of the impurities usually found in PAA was achieved by CUCARELLA AND CRESPO[80], using a SE-30–Carbowax 20M column and a hydrogen flame detector. The PAA was esterified with methanol, and chromatograms were obtained using phenoxy acetic acid as an internal standard. An F&M Model 700 gas chromatograph was used equipped with an F&M Model 240, temperature programmed, and a Minneapolis-Honeywell 1-mV recorder. The column employed was a stainless-steel tubing of 240 cm × 6.3 mm, packed with 28% SE-30 and 2% Carbowax 20M on 60–80 mesh a.w. Chromosorb W. The column was conditioned at 220° with nitrogen flowing, until a stable baseline was obtained. The operating conditions were: detector and injection port temperature, 250°; column temperature, 175° isothermal or programmed as indicated; flow-rates: nitrogen, 25 ml/min, hydrogen, 30 ml/min, and air, 300 ml/min.

Fig. 26 illustrates the temperature-programmed separation of *o*-xylene, maleic acid dimethyl ester, citraconic acid dimethyl ester, benzoic acid methyl ester, *o*-toluic acid methyl ester, naphthalene, phenoxyacetic acid methyl ester, phthalide, and phthalic acid methyl ester. The temperature was programmed as follows: 13 min isothermal at 175°, then increasing 2°/min until 200° and later on isothermally. Attenuation was necessary, usually × 100 or × 200. Table 24 lists the retention times, temperatures, and the area correction factors for each of the above substances.

The qualitative and quantitative determination of the various related substances commonly found in PAA (obtained from the naphthalene conversion process) is of importance because of the many use applications of the phthalate esters. For example, plasticizers for PVC medical application (*e.g.*, blood bags, heart and kidney machine tubing, etc.) as well as for food wraps must contain a minimum of impurities.

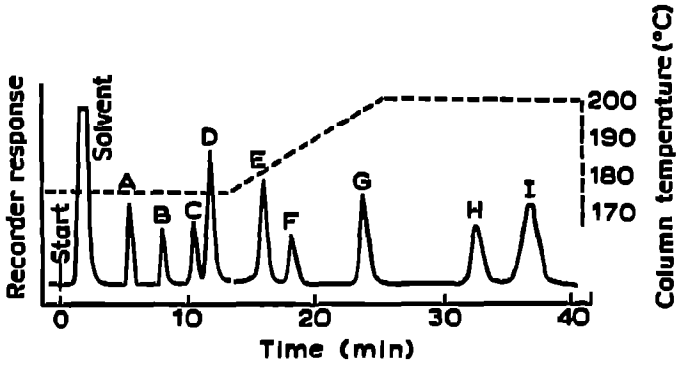


Fig. 26. (———) Temperature-programmed GLC separation of (A) *o*-xylene; (B) maleic acid dimethyl ester; (C) citraconic acid dimethyl ester; (D) benzoic acid methyl ester; (E) *o*-toluic acid methyl ester; (F) naphthalene; (G) phenoxyacetic acid methyl ester; (H) phthalide; and (I) phthalic acid methyl ester. (— — —), the temperature program.

TABLE 24

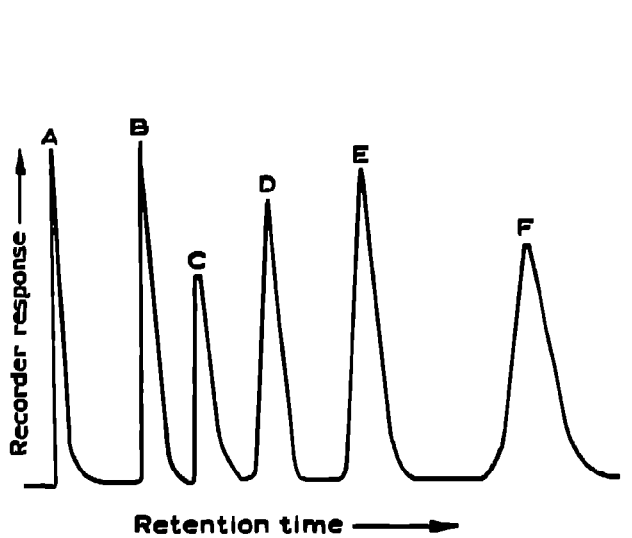
RETENTION DATA FROM TEMPERATURE-PROGRAMMED GLC SEPARATION[a]

Substance	*Retention temp. (°C)*	*Retention time (min)*	*Rel. retention time*[b]	*Area correction factor*
o-Xylene	175	5.5	0.23	0.48
Maleic acid dimethyl ester	175	8.5	0.35	1.65
Citraconic acid dimethyl ester	175	10.5	0.44	0.95
Benzoic acid methyl ester	175	12	0.50	0.60
o-Toluic acid methyl ester	181	16	0.67	0.54
Naphthalene	186	18.5	0.77	0.42
Phenoxyacetic acid methyl ester	197	24	1.00	1.00
Phthalide	200	33	1.38	0.88
Phthalic acid dimethyl ester	200	38	1.58	—

[a] GLC chromatogram and temperature program illustrated in Fig. 26.
[b] Relative to phenoxyacetic acid methyl ester = 1.00.

TRACHMAN AND ZUCKER[90] described the quantitative determination of maleic anhydride, benzoic acid, naphthalene, and 1,4-naphthoquinone in PAA by GLC. Separation of all the related compounds was achieved by isothermal analysis using a silicone SF-96 column and a hydrogen flame detector. An F&M Model 1609 gas chromatograph was employed with a Brown Electronik recorder (Minneapolis-Honeywell) and a Disc chart integrator (Disc Instruments). The operating conditions were: injection port temperature, 250°; column temperature, 220°; and detector cell temperature, 200°; helium flow rate, 60 ml/min (30 p.s.i. at inlet). The column (6 ft. × 1/4 in. s.s.) was packed with 30% silicone SF-96 on 60–80 mesh a.w. Chromosorb W. Fig. 27 shows a chromatogram of a synthetic mixture of PAA and related components that could be present from the naphthalene process of PAA synthesis (*viz.* maleic anhydride, *o*-dichlorobenzene, benzoic acid, naphthalene, PAA and 1,4-naphthoquinone). Retention data as well as relative retention data (relative to solvent peak *o*-dichlorobenzene) are presented in Table 25.

A considerable amount of PAA is also prepared via an *o*-xylene conversion process. The column and operating parameters used above were also applicable for the



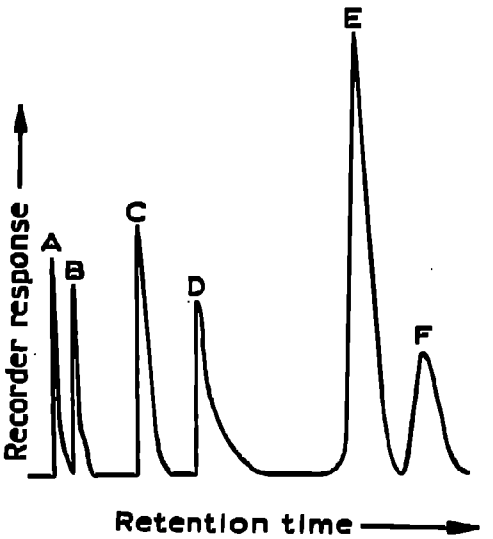


Fig. 27. GLC chromatogram of phthalic anhydride (PAA) and related components (synthetic mixture) that could be present from the naphthalene process. A = maleic anhydride; B = *o*-dichlorobenzene; C = benzoic acid; D = naphthalene; E = phthalic anhydride (PAA); F = 1,4-naphthoquinone.

Fig. 28. GLC chromatogram of phthalic anhydride (PAA) and related impurities that could be present from the *o*-xylene process. A = maleic anhydride; B = *o*-xylene; C = *o*-dichlorobenzene; D = benzoic acid; E = phthalic anhydride (PAA); F = phthalide.

TABLE 25

GLC RETENTION DATA FOR PAA AND POSSIBLE IMPURITIES

Component	*Retention time (min)*[a]	*Rel. retention time*[b]
Acetone (if used)	1.1	0.24
Maleic anhydride	2.4	0.52
o-Dichlorobenzene	4.6	1.00
Benzoic acid	6.2	1.35
Naphthalene	8.4	1.83
Phthalic anhydride (PAA)	11.4	2.48
1,4-Naphthoquinone	16.3	3.54

[a] Measured from inject.
[b] Relative to solvent peak *o*-dichlorobenzene = 1.00.

analysis of PAA and expected impurities from the *o*-xylene process. Fig. 28 illustrates a chromatogram of PAA and the expected impurities from this process, and shows that a good separation can be obtained for phthalide, *o*-xylene, PAA, maleic anhydride, and benzoic acid.

The use of vapor-phase chromatography in the production analysis of PAA was described by MORENO AND MENDOZA[91]. Acidic impurities were esterified with diazomethane, and analyzed on a column of 30% SE-30 on Chromosorb W (60–80 mesh) at a programmed temperature from 80–200° at 12°/min. Adequate separations and quantitative estimation of impurities, such as naphthalene, benzoic acid, maleic and phthalic acids, and 1,4-naphthoquinone were obtained in 8 min.

8. LIQUID–LIQUID CHROMATOGRAPHY

Recent advances have suggested, that the speed and efficiency of liquid chromatography (LC) are rapidly approaching that of GC. A number of high-efficiency LC supports have recently been introduced, including Zipax (DuPont's CSP support)[92,93], Corasil I and II, and Durapak (Waters Associates)[94]. With the exception of Durapak, these materials, in the micron particle range, consist of particles with a solid core and thin porous coating. This unique combination permits very high coefficients of mass transfer. Durapak consists of conventional liquid phases such as β,β'-oxydipropionitrile, chemically bonded to a rigid porous bead. The Zipax material has been reported to have a relatively inert surface[92].

MAJORS[95] has recently described the high-speed LC of a number of antioxidants and plasticizers, using solid core supports. Fig. 29 shows a schematic of the high-pressure LC system employed. The unit employed a Whitey micro-regulating high-pressure feed pump (Whitey Research Tool Co., Oakland, Calif.) equipped with an 11-mm plunger, capable of a max. flow rate of 7.3 l/h and output pressure of 5,000 p.s.i. For most experiments up to 2,500 p.s.i. output pressure, an ALC-100 LC pulse damper (Waters Associates) was used. To insure saturation of the carrier liquid when doing liquid–liquid chromatography, a presaturator column was placed before the chromatographic column. It consisted of an 8 in. × 3/8 in. O.D. stainless steel tube, packed with 62–100 μ Porasil A (Waters Associates) and loaded with 5% by weight of the same liquid phase as used in the chromatographic column. The detectors employed in the system were: (1) a Refracto-Monitor Model 1103 (Laboratory Data Control (LDC), Danbury, Conn.), with a cell volume of 3 μl and interchangeable prisms to cover solvent refractive indices from 1.31 to 1.55; and (2) UV Monitor Ultraviolet Absorbance (LDC) with an 8-μl cell volume and min. absorbance range of 0.02 O.D. units. The detector output was monitored by a 10-mV recorder. The columns employed were 2.1 m (I.D.) × 0.125 in. (O.D.), precision-bore stainless steel.

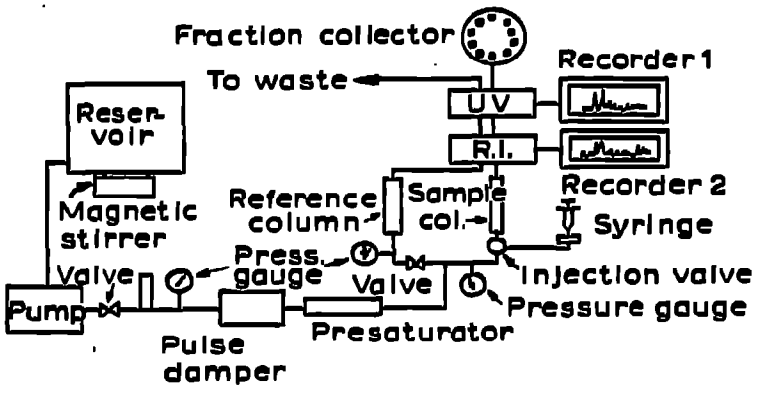


Fig. 29. Schematic of high-pressure liquid chromatographic (LC) system.

Figs. 30 and 31 show the separation of didecyl, dibenzyl and decyl benzyl phthalates using Zipaz and Corasil I supports, respectively. These plasticizers cannot be directly determined in less than 8 min on Zipax with HETP values less than 1 mm as summarized in Table 26. At the same flow rate, the separation times on Corasil were considerably longer and the plate heights greater although not excessive.

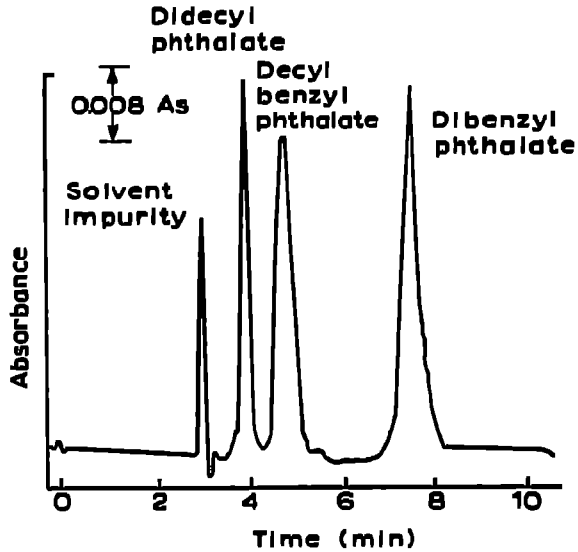


Fig. 30. LC separation of phthalate plasticizers using Zipax support column, 1 m × 2.1 mm I.D.; packing, 0.5% β,β'-oxydipropionitrile on 20–37 μ Zipax support; carrier, isooctane; flow-rate, 0.50 ml/min.; sample, 10.6 μl of a mixture of 0.40 μl/ml each of didecyl phthalate and decyl benzyl phthalate, and 0.35 mg/ml of dibenzyl phthalate in heptane. As = absorbance unit.

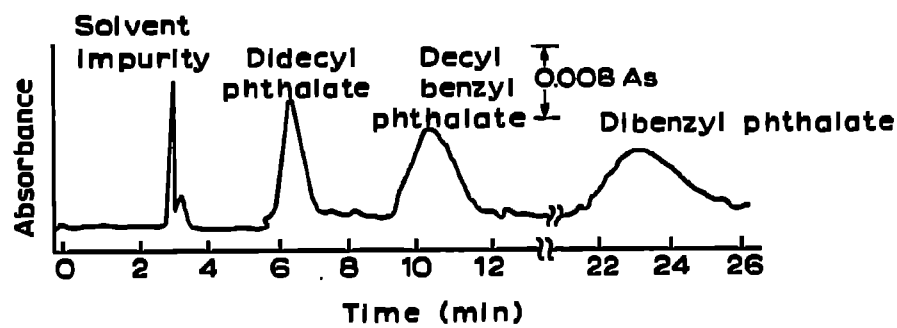


Fig. 31. LC separation of phthalate plasticizers using Corasil I support. For column, packing, carrier, flow-rate, and sample concn., see Fig. 30. As = absorbance unit.

TABLE 26

EVALUATION OF SOLID CORE SUPPORTS FOR LC OF PHTHALATE PLASTICIZERS

Plasticizer	*Zipax support*		*Corasil I support*		*Deactivated Corasil II support*	
	V_R *(ml)*	*HETP (mm)*	V_R *(ml)*	*HETP (mm)*	V_R *(ml)*	*HETP (mm)*
Didecyl phthalate	2.0	0.38	3.1	1.9	3.1	1.8
Decyl benzyl phthalate	2.4	0.93	5.1	3.3	4.7	2.7
Dibenzyl phthalate	3.8	0.51	11.6	1.9	8.4	2.5

The separation of a mixture of phthalate plasticizers from the bulk polymer has been accomplished using LC[96]. An ALC/GPC 301 (Waters Associates) apparatus was used with both differential refractometer and UV photometer detectors. Fig. 32 shows a chromatogram of a mixture of diisodecyl, diisooctyl, and dibutyl phthalates from a bulk polymer. Figs. 33 and 34 show the peak height response curves for the three

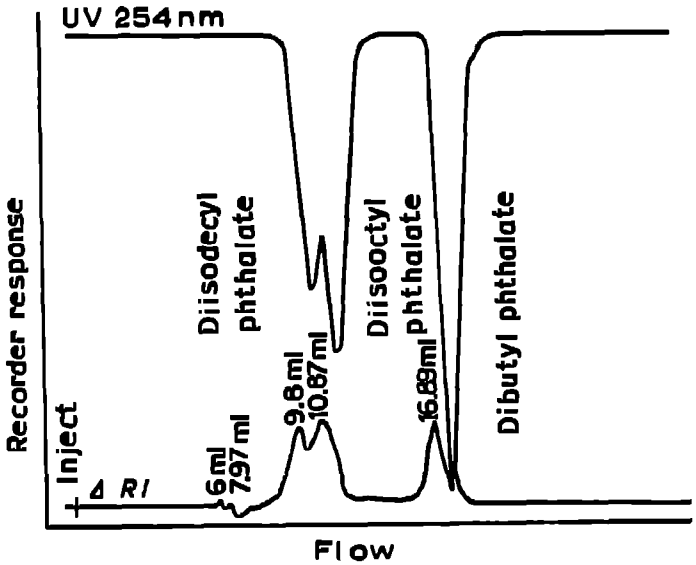


Fig. 32. Chromatogram of a mixture of phthalate plasticizers separated from bulk polymer.

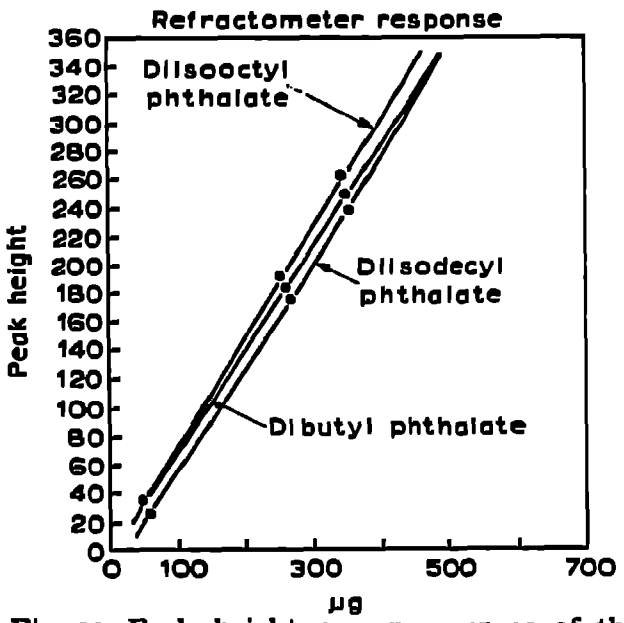


Fig. 33. Peak height response curves of three phthalate esters obtained by differential refractometry.

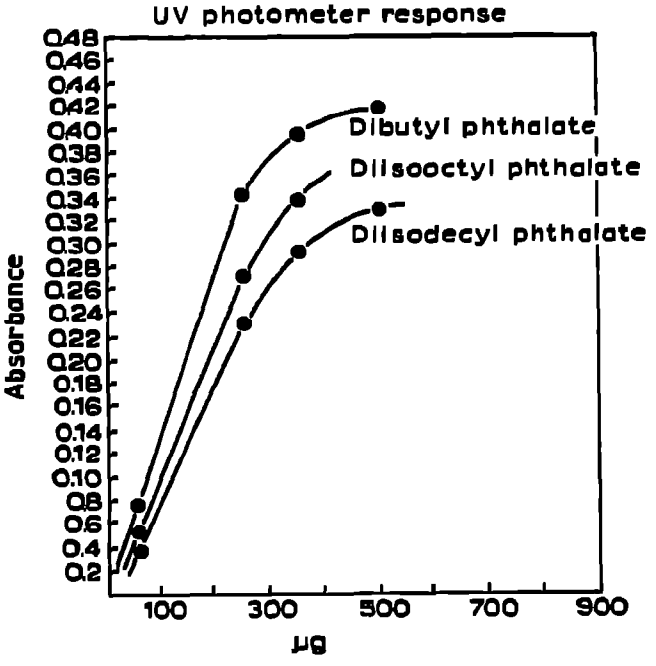


Fig. 34. Peak height response curves of three phthalate esters obtained by UV photometry.

phthalate plasticizers, obtained with the differential refractometer and UV photometer, respectively. Minimum detectability at twice the noise level was about 10 μg for either method of detection. The high selectivity afforded by the column packing Corasil II is demonstrated by the separation of the isomers di(2-ethylhexyl) phthalate (DEHP) and diisooctyl phthalate (Fig. 35).

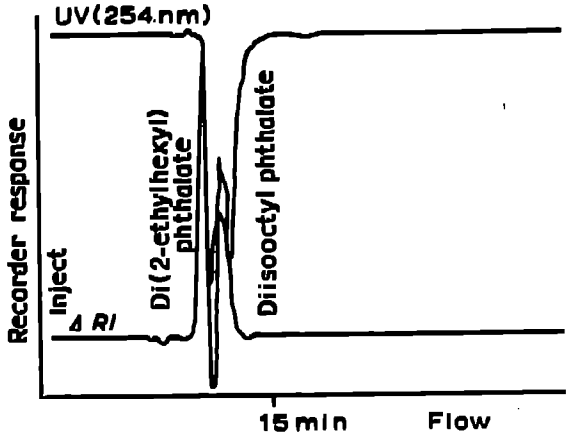


Fig. 35. LC separation of isomeric phthalate esters by Corasil II.

9. MISCELLANEOUS CHROMATOGRAPHIC AND ANALYTICAL METHODS

BURNS[97] described the use of paper impregnated with a polyvinyl chloride-water dispersion, for the separation of an homologous series of phthalates and phosphates, using potassium permanganate or a universal indicator as coloring reagents.

The identification of the components of plasticizers, by combining a column chromatographic separation with a subsequent IR analysis of the various fractions has been reported by CACHIA[98].

Retention data for a number of phosphates, phthalates and related plasticizers on Apiezon K at 283° using short columns with high load (30% on Celite 545), has been reported by LEWIS AND PATTON[99]. COOK *et al.*[100] separated dibutyl, butyl benzyl, and dibenzyl phthalates on a very short column (9 in.) of 25% silicone grease.

The analysis of phthalate esters has also been carried out by polarographic[101], fluorescence[102], gravimetric[85], spectrophotometric (of phthalic acid at 284 nm)[103], and mass spectroscopic techniques[104].

Because of the apparent facility of the phthalate esters to be leached from a variety of plastic devices (*e.g.*, tubing, gloves, vials, caps, bottles, etc.) it is well to stress the stringent precautions required both for its analyses as well as its biological elaboration. A further precaution is raised in regard to the purity of reagent solvents. For example, ASAKAWA AND GENJIJA[105] isolated and identified dibutyl and di(2-ethylhexyl) (DEHP) phthalates from benzene and chloroform, and diisobutyl and isobutyl phthalates from hexane and petroleum ether. In addition, they extracted dimethyl, diethyl, and dibutyl phthalates from polyethylene buckets, and dibutyl phthalate from plastic tape at the neck of chloroform bottles.

10. SUMMARY

The chromatographic and biochemical aspects of the phthalate esters have been reviewed with focus on their increasing occurrence in the environment, their physical and biological properties as well as the salient chromatographic (gas–liquid, thin-

layer, liquid–liquid) procedures that have been employed for their separation and identification.

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