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CHROMATOGRAPHIC AND BIOLOGICAL ASPECTS OF DDT AND ITS METABOLITES

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CONTENTS

1. INTRODUCTION

There is no question that DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (Dicophane) and its analogs have been the most important contemporary insecticides in scale of production and utility among pest control agents. Although first synthesized in 1874 by Zeidler[1], the insecticidal properties of DDT were first recognized by Muller at the Geigy Laboratories at Basel in 1939.

More than 4 billion pounds of DDT have been used since 1940, about 80% in agriculture and the remainder in the control of the insect vectors of primarily malaria, typhus, and the plague. The production of DDT in the U.S.A. reached a peak of about 160 million pounds in 1961. In this period DDT was registered for use in the U.S.A. on 334 agricultural commodities. Over the past fifteen years about 6×10^{10} g (140 million pounds) per year of DDT have been manufactured in the U.S.A., of which a considerable fraction is exported. The total global production per year was estimated to be in the order of 10^{11} g (ref. 2), of which all can be assumed to be released into the environment. Despite the fact the use of DDT has recently been severely curtailed in North America and Europe, it is evident that its utility in Asia, Africa, and South America is continuing. This combined with aspects of the ubiquity and persistence of DDT and its metabolites, plus the equivocal aspects of their toxicity, suggests continuing interests and evaluation in this important class of environmental agent.

DDT is most generally prepared via the condensation of 2 moles of chlorobenzene with 1 mole of chloral in the presence of condensing agents such as conc. sulfuric acid, oleum, hydrogen fluoride, anhydrous aluminum chloride, fluorosulfonic acid, etc. In industry, the most frequently employed method is condensation of chloral with chlorobenzene in the presence of conc. sulfuric acid or weak oleum at a temperature not higher than 20°. The technical grade product is a complex mixture of compounds in which the *p,p'*-isomer amounts to 75–76% (Table I shows the composition of 2 samples of technical grade DDT)[3]. Pure *p,p'*-DDT is a white crystalline substance melting at 108.50–109°; vapor pressure at 20° is 1.9×10^{-7} mm Hg and its solubility

TABLE I

COMPOSITION OF TECHNICAL DDT

Letters in parentheses refer to analytical methods as follows: (a) Isolation from technical DDT, (b) recrystallization from 75% aqueous ethanol previously saturated with *p,p'*-DDT, (c) fractional crystallization, (d) adsorption analysis and fractional crystallization, (e) isolation, supplemented by cryoscopic analysis on the residue.

Compound	*Sample 1: setting point, 91.2° (%)*	*Sample 2: setting point, 88.6 (%)*	*Sample 3: setting point, 91.4° (%)*	*Sample 4: by-product oil (%)*
1-Trichloro-2,2-bis-(*p*-chlorophenyl)-ethane (*p,p'*-DDT)	(a) 66.7 (b) 72.9	(b) 70.5 (c) 63.5 (d) 64.5 (e) 67.9	(a) 72.7 (b) 76.7	—
1-Trichloro-2-*o*-chlorophenyl-2-*p*-chlorophenyl-ethane (*o,p'*-DDT)	19.0	(c) 7.9 (d) 15.3 (e) 20.9	11.9[*]	74.8[**]
1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-DDD)	0.3	4.0	0.17[***]	—
1,1-Dichloro-2-*o*-chlorophenyl-2-*p*-chlorophenyl-ethane (*o,p'*-DDD)	—		0.044	—
2-Trichloro-1-*o*-chlorophenylethyl *p*-chlorobenzenesulfonate	0.4	1.85	0.57	0.11
2-Trichloro-1-*p*-chlorophenylethanol	0.2	—	—	—
Bis(*p*-chlorophenyl)sulfone	0.6	0.1	0.034	—
α-Chloro-α-*p*-chlorophenylacetamide	—	0.01	0.000	—
α-Chloro-α-*o*-chlorophenylacetamide	—	0.007	—	—
Chlorobenzene	—	—	—	2.44
p-Dichlorobenzene	—	—	—	0.73
1,1,1,2-Tetrachloro-2-*p*-chlorophenylethane	—	—	—	[§]
Sodium *p*-chlorobenzene-sulfonate	0.02	—	—	—
Ammonium *p*-chlorobenzene-sulfonate	—	—	0.005	—
Inorganic	0.1[§§]	0.04[§§§]	0.01[†]	—
Unidentified and losses	6.5	5.1	10.6	19.4

[*] This value does not represent all the *o,p'*-DDT present, as all oily fractions were not exhaustively studied.

[**] Miscellaneous fractions containing *p,p'*-DDT, *o,p'*-DDT, and *p,p'*-DDD.

[***] Includes 0.06% of *p,p'*-DDD isolated as such and 0.11% of the corresponding olefin.

[§] Isolated as nitro derivative from an oil mixture analyzing for a mixture of $C_8H_6Cl_4$ and $C_8H_5Cl_5$ and representing 2.54% of original material.

[§§] Qualitative tests for ferric, lead and magnesium carbonates were obtained.

[§§§] Insoluble in boiling 95% ethanol.

[†] Qualitative tests for ferric, ammonium, halide and sulfate ions were obtained.

in water is about 0.001 mg/l. The structures of DDT and related compounds are shown in Fig. 1.

Haller *et al.*[3] delineated the chemical composition of technical DDT in several commercial samples. Since technical DDT melts over a range, the setting point is used rather than the melting point to give an indication of its purity. Components isolated from technical grade DDT included: *p,p'*-DDT (66.7–76.7%); *o,p'*-DDT (7.9–20.9%), *p,p'*-DDD (0.17–4.0%); *o,p'*-DDD (0.044%); 2-trichloro-1-*o*-chlorophenylethyl-*p*-chlorobenzenesulfonate (0.11–1.85%); 2-trichloro-1-*p*-chlorophenylethanol (0.2%); bis-(*p*-chlorophenyl)sulfone (0.034–0.6%); (α-chloro-α-*p*-chlorophenylacetamide (0.006–0.01%); chlorobenzene (2.44%); *p*-dichlorobenzene (0.73%); sodium *p*-chlorobenzenesulfonate (0.02%); 1,1,1,2-tetrachloro-2-*p*-chlorophenylethane (trace) ammonium *p*-chlorobenzenesulfonate (0.005%); inorganic (0.01–0.1%); unidentified components (5.1–19.4%).

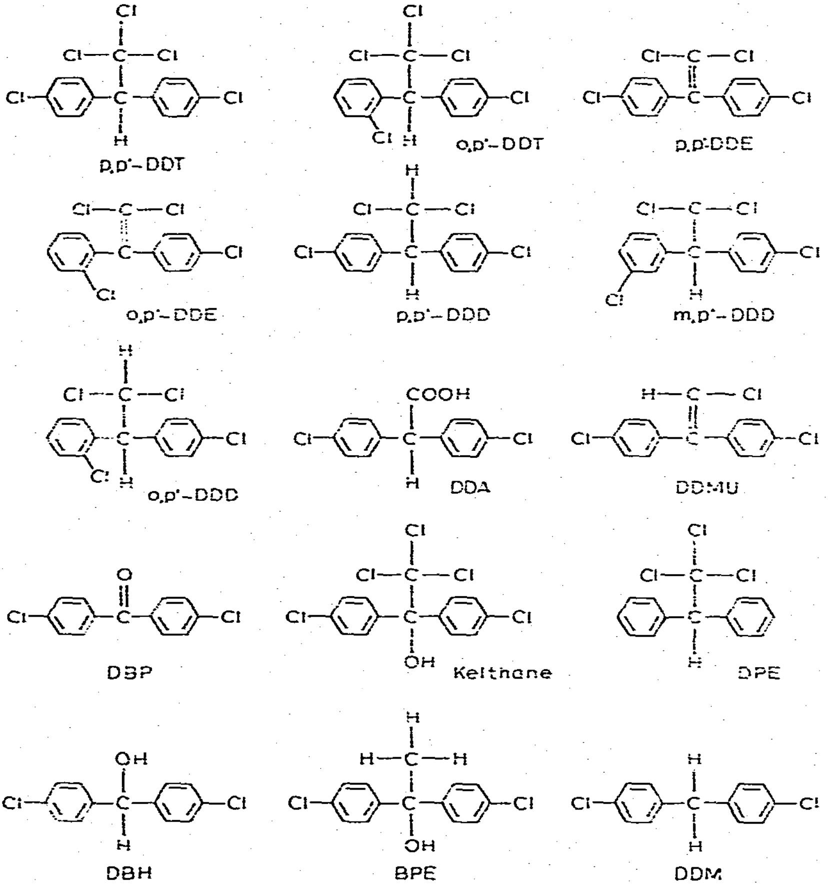
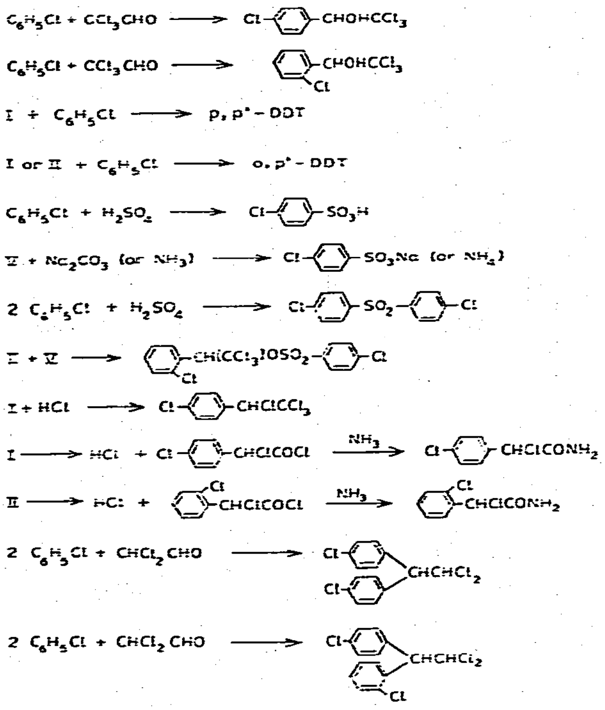


Fig. 1. Structures of DDT and related compounds.

The occurrence of the fourteen compounds which were isolated was explained from a consideration of the main and side reactions of chloral and chlorobenzene (and possible impurities in technical chloral and chlorobenzene) in the presence of sulfuric acid, followed by treatment of the product mixture with an alkaline wash material. These reactions can be summarized as follows:



I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII

Although some of the by products are active insecticides, none of them are as toxic as *p,p'*-DDT. The recovery of known compounds in the samples varied from 80.6 to 93.5%.

DDT has been used for the control of most phytophagous insects attacking fruits, vegetables, cotton, forest, livestock and home (including such household insects as houseflies, stable flies, mosquitoes, roaches, lice, bedbugs, ants, ticks and other insects). Agricultural products are available as wettable powders and emulsifiable concentrates. (The dusts range from 1–10% and sprays from 25–50%.) The principal formulations are 50% wettable powder, 25% emulsifiable concentrate, 5% dust and 10% aerosols. For use in the home, DDT is commonly available in aerosol form with the pesticidal synergist piperonyl butoxide and a pyrethrin for rapid knockdown.

DDT undergoes thermal decomposition above its melting point to produce DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene) and HCl. The reaction is catalyzed by ferric and aluminium chloride and by ultraviolet light. In solution DDT is readily dehydrochlorinated by alkalies or organic bases. Dehydrochlorination of DDT may proceed at temperatures as low as 50°. However, DDT is stable to aqueous alkalies, alcoholic permanganate and withstands acid oxidation[4]. The utility and chemistry[5], analysis in formulations and residues[6], and pharmacological[7] and epidemiological[8,9] aspects of DDT have been reviewed.

2. BIOLOGICAL AND TOXICOLOGICAL ASPECTS

The principal reasons for the concern regarding the potential hazard for human health represented by DDT and its metabolites are based on (a) their ubiquity and persistence in the environment, (b) their capacity to accumulate in living organisms, including man and their capacity to accumulate in the fetus and in mothers' milk, (c) the experimental evidence of DDT to increase tumor incidence in laboratory animals, (d) evidence of the mutagenic potential of DDT and its metabolites in mammalian and insect organisms, and (e) implication of DDT in the declining reproduction of several species of birds.

The acute oral median lethal dose (MLD) of DDT in various animal species is summarized in Table 2. Animals with large amounts of body fat appear less susceptible while those in a fasting state or receiving an inadequate diet and young animals are more susceptible to acute toxicity of DDT[7]. Acute toxicity in animals and man is evidenced by effects on the nervous system which produce signs and symptoms of increasing severity from apprehension, headache, anorexia, nausea, hyperexcitability to profound weakness, paresthesia, muscle fibrillation, and tremors, to myoclonsis, venticular fibrillation, respiratory arrest, convulsion, and coma to death[10]. The neurotoxic effects are generally agreed to be due to interference with nerve axons rather than synapses.

TABLE 2

ACUTE ORAL MEDIAN LETHAL DOSE (MLD) OF DDT IN VARIOUS ANIMAL SPECIES

Species	*Approximate MLD (mg per kg of body weight)*
Mice	150–250
Rats	150–250
Cats	150–300
Dogs	150–300
Guinea Pigs	300–500
Rabbits	300–500
Monkeys	>-200
Cows	>-300
Sheep	1000
Goats	1000
Man	About 500

DDT has been shown to cross the placenta in the mouse[11], dog[12], and rabbit[13], and since DDT residues have been detected in the aborted human fetus, placental transfer is likely in man[14]. DDT has been reported to induce hepatomas in mice of two strains following its continuous administration for 80 weeks (ref. 15). Kemeny and Tarjan[16,17] reported that the administration of crystalline *p,p'*-DDT at levels of 2.8 and 3.0 ppm in the diet to BALB/c mice for five consecutive generations resulted in a progressively increased incidence of tumors from the second generation onwards. The most frequent tumor types were leukemia, reticulum-cell sarcoma, carcinoma of the lungs and hemangio-endothelioma. Tomatis *et al.*[18] described the effect of long-term exposure to DDT of CF-1 mice. CF-1 minimal inbred mice were given technical DDT mixed into the diet at the dose levels of 2, 10, 50, and 250 ppm for the entire life span for two consecutive generations. Exposure of all 4 levels of DDT resulted in a significant increase in liver tumors in males (most evident at the highest levels used). In females, the incidence of liver tumors was slightly increased following the exposure to 10 and 50 ppm, while a marked increase was observed following exposure to 250 ppm. The age at death with liver tumors and the incidence of liver tumors appeared to be directly related to the dose of DDT to which the mice were exposed.

Fitzhugh and Nelson[18] reported the occurrence of hepatic cell tumors in four of 150 Osborne Mendel rats receiving DDT (81.8% *p,p'*-isomer and 18.2% *o,p'*-isomer) in the diet at levels of 200–800 ppm, while 11 other rats were described as showing adenomatoid hyperplasia of the liver. All 15 rats survived over 18 months and the age distribution for rats with liver tumors corresponded to that in untreated controls. Hepatomas occurred in about 1% of the untreated controls.

Shabad *et al.*[19] reported the early occurrence of hyperplastic foci in lung organ cultures of strain A fetal mice, the mothers of which were exposed to DDT.

The occurrence of hepatomas in rainbow trout fed a diet of 18 or 75 ppm of DDT has been reported by Halver[20]. However, in a few reports on rats and in one on Rhesus monkeys[21], as well as in one report on Syrian golden hamster[22], the long-term exposure to DDT was not shown to result in an increased incubence of tumors. The suggestion of the anti carcinogenic potential of DDT has also been advanced[23,24] and evidence for the antitumorigenic effects of DDT in mice (C57Bl) cited[25].

A number of reports have cited the genetic effects of DDT. The effects of environmental DDT and the genetics of natural populations in the fly *Drosophila pseudoobscura* were studied by Cory *et al.*[26], and it was suggested that DDT residues were indirectly responsible for alterations in the genetic content of populations. The conclusions were based on correlations between chromosomal changes in the fly described over the past 24 years in the Western U.S.A. by Dobzhansky and his colleagues[27–30] and the distribution patterns of DDT residues found by Cory *et al.*[26] throughout this area.

A study of a natural population of mice in the foothills of the Andes revealed an unprecedented number of genetic and chromosomal mutants with DDT suspected to be the causal agent[31]. Johnson and Jalal[32] recently reported DDT-inducted chromosomal damage in mice. DDT treatment ranging from 100 ppm (mg per kg of body weight), to 400 ppm in BALB/c mice was associated with significantly higher proportions of chromosomal abnormalities in the form of deletions, stickiness and rarely, ring and metacentric chromosomes. This investigation indicated that chromosomal damage to mice occurred frequently at dosages of 150 ppm or higher (LD_{50} is 550 ppm). Since the induction of chromosomal damage is closely associated with mutagenic events (point mutations) in mammals[33], DDT appears to be a potential mutagen. Markaryan[34] had previously reported significantly higher proportions of stickiness and chromosomal damage in mice on treatment with a single dose of DDT at 100 ppm.

Kelly-Garvert and Legator[35] described cytogenetic and mutagenic effects of DDT and DDE in a Chinese hamster cell line. In all experiments, DDE consistently produced a significant increase in the mutation frequency over the control level, while DDT proved inactive. The cytogenetic studies also indicated that DDE-treated cells had a significant increase in chromosome aberrations with exchange figures and chromatid breaks being evident while DDT produced no significant increase in chromosome abnormalities. The Chinese hamster cell populations exposed to DDE also manifested an increased number of polyploid cells over the control level.

Palmer *et al.*[36] reported that chromosome abnormalities were produced by DDT and its metabolites in a cultured mammalian cell line, however, Legator *et al.*[37] later reported no cytogenetic abnormalities were found in bone-marrow cells of rats treated with DDT.

Palmer *et al.*[38] recently reported that DDT was only marginally positive with respect to the dominant lethal test in rats. A statistically significant effect was found in the proportion of females having one or more dead implant after being mated during week 3 with males given DDT orally in a dose of 100 mg/kg; no significant effects were found in females mated with i.p.-treated males.

The long term effects of DDT on mammalian reproduction are unclear although DDT exerts a number of reproduction-related effects in mammalian species. These include an increased incidence of stillbirths in cows[39]; higher blood levels of DDT in female sea lions that abort compared to females with normal gestation[40]; and higher blood levels of *p,p'*-DDE, the principal stored metabolite of *p,p'*-DDT, in premature infants than in normal infants at term[41]. Treatment of pregnant rabbits at doses of 10 and 50 mg/kg of *p,p'*-DDT caused an increased incidence of prematurity, an increase in the number of fetal resorptions and a decrease in fetal weight[42]. It is interesting to note that these doses of DDT did not increase the incidence of chromosomal aberrations in the maternal or fetal tissue and exposure *in vitro* to levels as high as 100 μg/ml caused no abnormalities[42].

Residues of chlorinated hydrocarbon insecticides in our environment have been inplicated in the declining reproduction of several species of birds[43,44]. DDT in birds is believed to produce thinner eggshells with consequent egg breakage leading to decreased population[45]. Ottoboni[46] described the feeding of technical DDT (80% *p,p'*-DDT; 2% *p,p'*-DDE) at levels of 0, 20, and 200 ppm to Sprague–Dawley derived rats (begun with a weanling parent generation and terminated with weanling of third litters from the F_1 generation): DDT ingestion produced no apparent effects on fecundity of dams or viability of young. Comparison of total numbers of pregnancies in each group indicated there was no effect of DDT on fertility of 16- or 24-week-old rats, but data obtained for an individual family line in the experiment suggested the possibility of an adverse effect of DDT in matings of marginal fertility. The reproductive performance of 52-week-old rats indicated that DDT may also exert a protective effect against age decrement of the reproductive process. The 200 ppm DDT diet produced a significant increase in the occurrence of ringtail, a disease of obscure etiology that effects suckling rats.

Deichmann and Keplinger[47] and Ware and Good[48] reported studies relating to the effect of DDT, alone and in combination with other pesticides, on reproduction in mice. Neither decreased viability of mice[47] nor decreased fertility of the dams[48] was associated with DDT ingestion.

Chung *et al.*[49,50] reported on the ability of DDT or dieldrin to reduce or stimulate (depending on the concentration used) the synthesis of nucleic acid or protein in human strain HeLa cell cultures when used at concentrations of 0.5, 10, or 50 ppm and the change in synthesis of RNA and protein by subcellular fractions of HeLa cells exposed to these insecticides at the same concentrations. DDT and aldrin, when tested at 125 ppm with monolayer cultures of HeLa cells[51], produced a 42 and 48% decrease in the synthesis of DNA after 4.5 h of treatment while synthesis of RNA was significantly decreased by both aldrin and DDT at the same concentrations as above.

Robinson[52] reviewed the evidence regarding the residues or organochlorine insecticides in the general human population, the dynamics of the uptake, storage and elimination of these compounds in man and other vertebrates, their effects upon hepatic microsomal enzymes and the metabolites found in vertebrates.

The microsomal enzyme induction organochlorine insecticides have been described by Hart *et al.*[53,54], Conney[55], Street[56], Risebrough *et al.*[57] and Peakall[58].

The estimated average daily intakes of DDT-type compounds in the U.S.A. and the United Kingdom are about 0.001 mg per kg of body weight (ref. 59–61) and the corresponding intake of dieldrin (HEOD) in these two countries is in the range of 0.0001–0.0002 mg per kg of body weight (ref. 62–64). Robinson [52] stressed that hepatic microsomal induction effects that may arise from these exposures can only be assessed at present by extrapolation from animal experiments. However the ambiguity was cited in the extrapolation of animal trials to man since different exposures in terms of mg of insecticide per kg of body weight of rats and man on the one hand, and by comparing residues in adipose tissue on the other hand.

Induction of hepatic detoxifying enzymes by organochlorine insecticides has the potential of influencing several effects of these insecticides in the general environment, *e.g.* lowered chronic toxicity of certain insecticide combinations as the result of antagonism of insecticide residue storage in animal tissue due to accelerated metabolic degradation. Conversely, disturbed endocrine imbalance due to increased hormone turnover by induced enzymes can lead to physiological aberrations, *e.g.*, as that suggested to be the basis for egg fragility and poor reproduction in certain birds[58]. It has also been shown that DDT and dieldrin are additive at low doses in inducing testosterone metabolism in pigeon liver. Street *et al.*[64] recently reviewed the ecological significance of pesticide interactions. The authors assessed the significance of such effects depending on minimum effective levels, combined action of two or more pesticides and species sensitivities toward interaction effects. Table 3 shows the minimum oral levels for hepatic enzyme induction in the rat by a number of organochlorine insecticides.

3. ECOLOGICAL ASPECTS

The widespread distribution of chlorinated hydrocarbon residues as contaminants in the global ecosystem is an established fact[65–71]. Substances deposited in the biosphere and troposphere can and often are, as in the case of chlorinated hydrocarbon insecticides[71], transported thousands of kilometers from their original site of application. Their rates of utilization are such that they contaminate soil, water, and food in terms of parts per trillion to parts per million, the extent of pollution depending on the location and meterological conditions of a given community. Although pesticide contamination, as well as other forms of chemical contamination, can be generally reduced by photochemical oxidations, aqueous hydrolysis, and via biochemical degradations in micro-organisms, plants, and animals in the soil, detectable amounts of the more stable pesticides such as DDT and of their degradation products enter ground waters through leaching and erosion. Fig. 2 summarizes a general scheme for the cycling of pesticides in the environment and illustrates the movement of resi-

TABLE 3

MINIMUM ORAL LEVELS FOR HEPATIC ENZYME INDUCTION IN THE RAT BY ORGANOCHLORINE INSECTICIDES

Sex	*System*	*Test condition*	*Feeding time (days)*	*Min. dosage (ppm)*	*Compound*
M	aldrin epoxidase	microsomes	14	2–2.5	DDT
				~1	dieldrin
				~1	heptachlor epoxide
				>1–<5	aldrin
				>1–<5	heptachlor
M	Hexobarbital oxidase	homogenate	14	~5	DDT
	Pentobarbital oxidase	*in vivo*	14	~5–10	DDT
M	EPN* oxidn. O-demethylase N-demethylase	homogenate	7–21	~1 ~5	DDT toxaphene
F	Hexobarbital oxidase	*in vivo*	10	~1	DDT, dieldrin
	EPN* oxidn. O-demethylase	homogenate	15	>1–<5	DDT, dieldrin
F	dieldrin storage	*in vivo*	10	~1	DDT

* EPN = O-Ethyl-O-(4-nibrophenyl)-phenyl phosphorothionate.

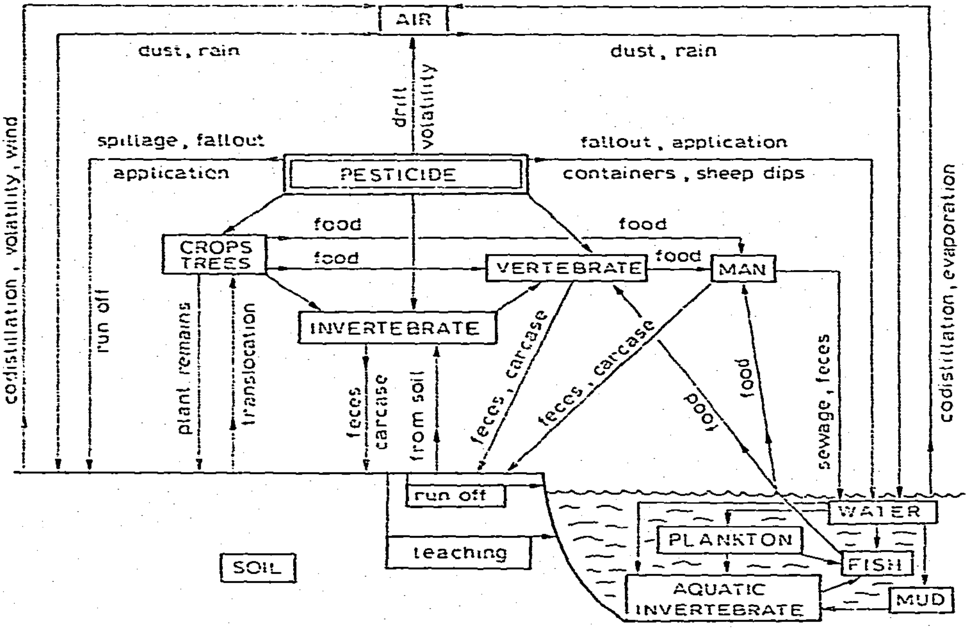


Fig. 2. Pesticide cycling in the environment.

dues through the various compartments of the environment while Figs. 3 and 4 illustrate some typical amounts of DDT found in the environment and degradative pathways of DDT in the environment, respectively. Tables 4 and 5 summarize the distribution of DDT, DDE, DDD residues collectively in the principal segments of the environment in terms of typical relative values, some maximum values and the bioconcentration of DDT residues in plants or animals from its environment, respectively[72].

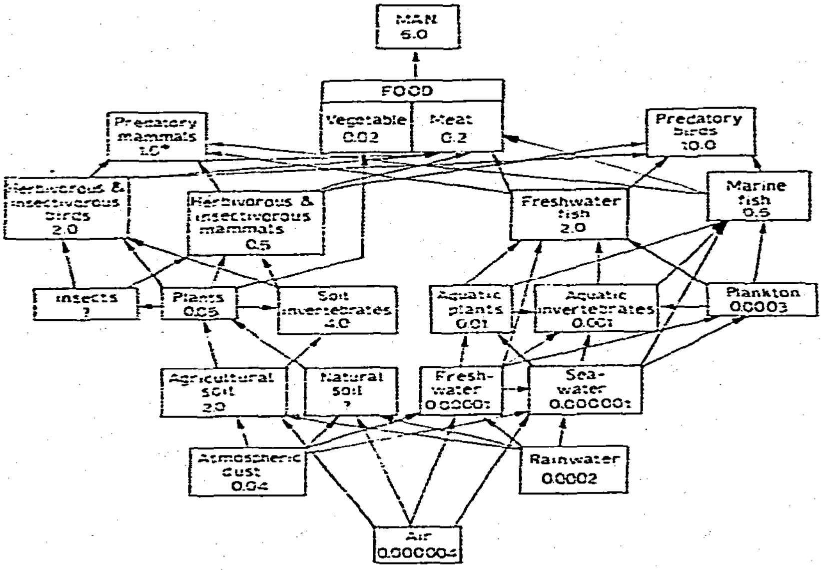


Fig. 3. Typical amounts of DDT (ppm) in the environment.

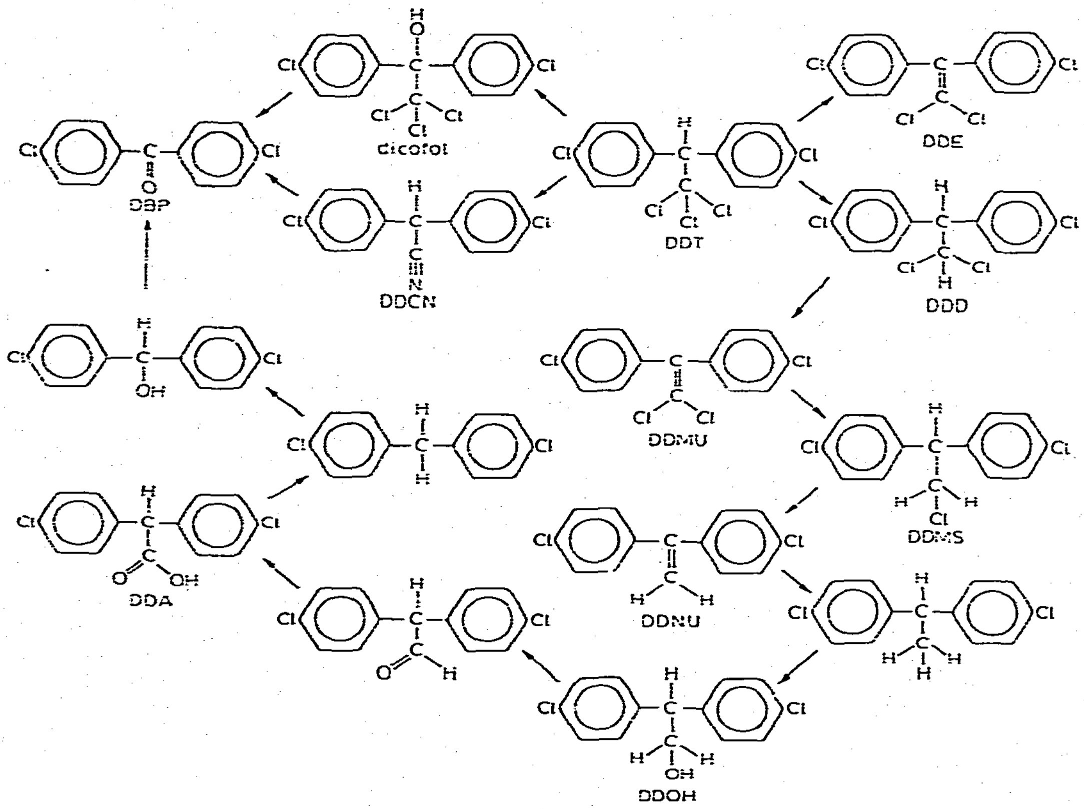


Fig. 4. Degradative pathways of DDT in the environment.

TABLE 4

DISTRIBUTION OF DDT-R* IN THE PRINCIPLE SEGMENTS OF THE EARTH'S ENVIRONMENT

	*Typical relative values*** *(ppm)*	*Some maximum values****
Air related		
Air	0.000004	$0.003–56.1 \times 10^{-12}$ (μg/m^3)
Atmospheric water (rain)	0.0002	$1–102 \times 10^{-12}$ (ng/l)
Atmospheric dust	0.04	45×10^{-9} (μg/l)
Water related		
Fresh water	0.00001	$< 1–109 \times 10^{-12}$ (ng/l)
Sea water	0.000001	$< 1 \times 10^{-12}$ (ng/l)
Plankton	0.0003	5 ppm
Aquatic vegetation (algae, vascular plants)	—	0.01–0.003 (75 by deposition) ppm
Aquatic invertebrates (shrimp, crayfish, mollusks, etc.)	0.001	0.14–151
Marine fish	0.5	
Freshwater fish	2.0	0.01–136 ppm
Aquatic mammals	—	8.3–23.3 ppm
Predatory birds (fish eaters) (herons, eagles, pelicans)	10.0	7.1–194 ppm
Aquatic birds	—	0.16–100 ppm
(herbivorous, insectivorous scavengers)		99.8 ppm
Herring gull eggs		227 ppm
Soil related		
Agricultural soils	2.0	0–131 ppm
Natural soils	?	0
Soil invertebrates (earthworms, slugs, beetles)	4.0	0–40 ppm
Plants	0.05	—
(soil treatment)	—	0.9–3.2 ppm
(foliage treatment)	—	179 (by deposition) ppm
Herbivorous and	0.5	
insectivorous mammals	0.05	0.01–126 ppm
Herbivorous and insectivorous birds	2.0	0.16–55.6 ppm
Predatory mammals	1.0	0.13–0.24 ppm
Predatory birds (owls, hawks, not including ospreys and eagles)	—	1.42–18.1 ppm
Man (adipose tissue)	6.0	$<$ 0.01–647 ppm
Man's vegetables	0.02	0.0002–0.05 ppm
Man's meat	0.2	0.05–0.28 ppm

* DDT-R is the composite of DDT, DDE and DDD residues.

** Assigned by Edwards[72].

*** Adapted from tables in Edwards[72].

The very factors that have made DDT such an effective insecticide (*e.g.*, its low vapor pressure, 1.5×10^{-7} mm at 20°; its low water solubility, 0.0012 ppm at 25°; its high fat solubility, *ca.* 100,000 ppm; and its general stability to photo-oxidation) all have ironically conspired to make DDT the prototype environmental pollutant. Both

TABLE 5

BIOCONCENTRATION OF DDT-R* RESIDUES IN PLANTS OR ANIMALS FROM ITS ENVIRONMENT**

Environment	*Plant and animal organism*	*Concentration factor (DDT-R residue organism divided by residue in environment)*	
		Maximum value observed	*Minimum value observed*
Soil	earthworm	73	0.67
	beetles	2.81	0.31
	slugs	3.70	2.33
	crop roots	0.13	0.04
	crop foliage	0.08	—
Water	sea squirt	1.000.000***	200***
	sea hare	178.000***	—
	eastern oyster, clam	70.000***	60
	shrimp	2.800***	280
	crabs	144	—
	crayfish	97	17
	snails	1.480***	—
	plankton	16.666***	250
	fish	829.300***	5-(1450)***
	fish (DDD)	9.214***	417
	algae	33	0.34
	aquatic plants	100.000***	0.45
Diet	pheasant	2.91	
	woodcock	4.5	2.6
	bald eagle		
	brain	0.1	
	liver	1.9	
	fat	35.7	

* DDT-R is the composite of DDT, DDE and DDD residues.
** Adapted from Edwards[72].
*** DDT may be present in excess of solubility in water.

DDT and its major metabolite, DDE, are concentrated in organisms in the trophic webs, increasing roughly tenfold in concentration stored in lipids and in percentage of DDE at each trophic level[69].

The remarkable extent to which relatively stable lipid-partitioning substances may concentrate through food chains is exemplified in the case of DDT in Lake Michigan. DDT is present in its waters at about 2 parts per trillion (0.000002 ppm), however, it has been shown that bottom muds contain an average of 0.014 ppm, amphipods 0.41 ppm, and fish such as coho and lake trout 3–6 ppm. Herring qulls, at the top of the food chain, contain as much as 99 ppm. The overall concentration from the water is approximately 5 million fold. The long-term effects of this process on the biota of the lake are admittedly difficult to estimate, but it has been suggested that they are likely to result in disturbing species composition[73].

The average rate of water retention in Lake Michigan is 30.8 years and the lake has a volume of 4,871 km^3 (ref. 74). It is suggested that even if no more DDT were to enter it from the contaminated water shed around it, the problem would not be corrected for several generations. The magnitude of this aspect of biosphere pollution by a very stable lipid-partitioning substance is illustrated by the calculation that only about 4.5 metric tons (5 tons) of DDT are required to produce the present concentration of DDT in solution in Lake Michigan[75].

The processes of accumulation are especially dramatic in fish and other aquatic poikilotherms where detoxification and elimination are very low. A highly significant correlation has been found between the age of lake trout, *Salvelinum hamaycush* and total body residues of DDT, DDE, and DDD which ranged from about 1 ppm at 1 year to as much as 28 ppm at 11 to 12 years of age[76]. It is important to note this long term accumulation in fish, together with the very long water retention times in water such as Lake Michigan (30.8 years) and Lake Superior (189 years) which re-emphasizes the dimensions of the contamination with DDT.

Over wide limits, the storage of DDT in fat of humans and in laboratory animals is directly proportional to intake[77], although it appears that a plateau is eventually attained. The average of U.S.A. inhabitants is 2.3–4.0 ppm of DDT and 4.3–8.0 ppm of DDE, respectively, in body fat. In India, where usage is higher, the respective figures are 16 ppm of DDT and 10 ppm of DDE. Storage of DDT and DDE in Eskimos (where there is no use of the chlorinated insecticide) is 0.8 ppm and 2.2 ppm, respectively. Warm-blooded animals slowly metabolize and excrete stored DDT, and its biological half-life in humans is about 0.5 year.

Residues of DDT have been shown to be persistent and pervasive, appearing in air[78–80] and in the lipids of most organisms[78,81–83]. Concentrations of DDT in certain of the earth's biota have reached toxic levels which have been attributed to the declines in populations of a number of carnivorous and scavenging birds and fish, and threatening significant contamination of human food chains[84–86]. DDT has a vapor pressure high enough to assure direct losses from plants and soil into the atmosphere which can result in ubiquitous residues; hence soils, air, and the waters of the oceans and the biota are all considered potential reservoirs for DDT residues. The residues of DDT have been shown to be extremely persistent in nature with estimates of their half-life ranging upward to 20 years and perhaps longer under certain conditions[87–90].

The vapor pressure of DDT at 20° is 1.5×10^{-7} mm Hg (ref. 91) producing an equilibrium concentration of DDT in the atmosphere of about 3×10^{-6} g/m^3 or about 2 ppb by weight. The dominant mechanism for the removal of DDT from the atmosphere is probably by rainfall. Concentrations in the U.S.A. and in England have been reported in the range of approximately 73–210 ppm (refs. 91 and 92).

It is estimated that agricultural soils in the U.S.A. contain an average concentration of DDT approaching 0.168 g/m^2 (1.50 lbs./acre; refs. 93–97). DDT is known to be quite stable in soil for long periods of time. Considerable amounts of DDT in soil have been detected in soil even 8–10 years after treatment. Non-agricultural soils were estimated to contain an average of 4.5×10^{-4} g/m^2 (refs. 93 and 94).

Four mechanisms have been suggested to account for most losses of DDT residues from soils: (1) volatilization, including losses by wind erosion of small particles from the soil surface; (2) removal by harvest of organic matter; (3) water run-off; and (4) chemical, including biotic degradation.

The total amount of DDT retained within the biota is considered small by comparison with the totals that can be terained in other pools within the biosphere and is also small by comparison with the annual amount of DDT produced. It is estimated that 10^9–10^{10} g of DDT are held within the biota worldwide (including residues in man)[98,99]. This is about $^1/_{30}$ of the amount produced in one year during the mid-1960's. The worldwide pattern of movement of DDT residues appears to be from the land through the atmosphere into the oceans and into the oceanic abyss.

Until recently, there had been no attempt to construct a model of the dynamics of DDT circulation throughout the ecosystem. Harrison *et al.*[73] applied a simplified model to an idealized ecosystem and concluded that the response time (98.2% of equilibrium attained) could be estimated as between four times the longest life-time and the sum of all the life-times of individual species. In their model, it was assumed that degradation and excretion were negligible. However, Bloom and Menzel[100] have shown that even very slow rates of degradation and excretion lead to shorter response times.

Cramer[101] recently developed a model to predict the global distribution and accumulation of DDT. The model considers the land, atmosphere, shallow sea, and aquatic plankton as reservoirs for DDT. Values for the rates of transfer between reservoirs and rates of chemical and biological degradation were estimated, and the parameters of the model were systematically varied in an attempt to determine the parameters of most critical importance to the predicted results. Two hypothetical alternative rates of future DDT usage were considered: (1) continued constant rate of DDT usage at the 1970 level and (2) zero rate of DDT usage after 1970. The time required to return to equilibrium after the termination of DDT usage could not be precisely determined but was estimated by Cramer[101] to be between 25 and 110 years with most of the decrease in the concentration of DDT, however, occurring in the early years. It was also suggested that the concentration of DDT in marine life is already near its equilibrium value and should not increase significantly in the future. The factors which were considered to be most important are the rates of biological and chemical degradation of DDT, both on land and in the sea, the amount of DDT which enters the atmosphere, the rate of transfer between the deep and shallow sea, and the rates of direct absorption of DDT by phytoplankton and zooplankton.

A model similar in many respects to that of Cramer[101] was recently described by Woodwell *et al.*[99]. The principal difference in the latter model is the emphasis placed on the role of the atmosphere as both a reservoir and means of transport of DDT. For example, Woodwell *et al.*[99] assumed very high values for the mean residence time of DDT in air and for the transfer rate of DDT from land to air which Cramer suggests are not substantiated by the generally low background levels of atmospheric DDT which have been reported[77].

A recent finding that may have significant environmental significance concerns the studies of Moilanen and Crosby[102] which suggest that DDT may be a source of polychlorinated biphenyls (PCB) by irradiation with ultraviolet light of the same wavelengths present in sunlight in the lower atmosphere (Fig. 5). Extrapolated to the atmosphere, the overall scheme of Moilanen and Crosby predicts that DDT vapor is converted to small amounts of DDD, which should accumulate in the biosphere, and to a great deal more DDE, which should degrade more slowly than DDT. DDE vapor in turn is converted at a moderate rate to dichlorobenzophenone (DBP) via 1-chloro-

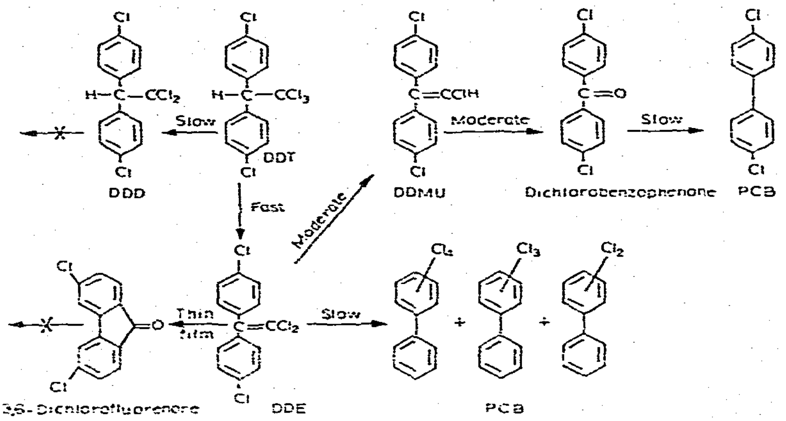


Fig. 5. Proposed scheme for the degradation of DDT vapor in sunlight[102].

2,2-bis(*p*-chlorophenyl)ethylene (DDMU), and then at a much slower rate to dichlorobiphenyl. Some of the DDE is also converted to 3,6-dichlorofluorenone (whose environmental fate is unknown), and to small amounts of di-, tri-, and tetrachlorobiphenyls. The degradation of DDT vapor as proposed by Moilanen and Crosby[102], if confirmed, could have profound effect in regard to the fate of pesticides in the environment. For example, atmospheric degradation of DDT by sunlight was considered to be unimportant in comparison to transport, although the residence time of DDT in the atmosphere is estimated to be approximately 4 years. It was suggested by Moilanen and Crosby[102] that much of the DDT and DDE may have been converted to other chemicals whose presence in the environment would not necessarily be suspicious because of the other sources for these products. It is of importance to note that although DDE and DDT should be accumulating in the biosphere since 2.8 × 10^9 kg of DDT have been dispersed since the early 1940's, global models of pesticide transport and monitoring of pesticide residues can account for only a small fraction of that amount.

4. ANALYSES OF ENVIRONMENTAL SAMPLES

The vapor pressure and relative volatility of some DDT isomers, impurities in the technical material and potential breakdown products have been recently described by Spencer and Cliath[103]. Vapor densities of DDT and related compounds with and without soils were determined by a gas saturation method in which nitrogen gas was passed through the insecticide-treated sand or soil at a sufficiently slow rate to insure equilibrium vapor saturation. The apparatus and the procedures have been previously described[104].

The *o,p'*- and *p,p'*-isomers of DDT, DDE and DDD in sand were analyzed by gas-liquid chromatography (GLC) following extraction from soil in benzene, evaporation of solvent, placing the sanding in 6 × 43 cm glass columns, followed by passage of dry nitrogen gas through the columns at 3.5 ml/min until sufficient materials had been trapped in hexane for analysis, thence concentrating in a Kuderna-Danish apparatus. A 1.83 m × 3 mm I.D. glass column was used, packed with 2% DC-200 (12,500 cst) on 80–90 mesh Anakrom ABS or 3% QF-1 on Anakrom ABS (80–90 mesh). Helium carrier gas flow-rate was 50 ml/min and the injection port, column and detector temperatures were 200°, 185° and 250°, respectively. The vapor pressure of *o,p'*-DDT was 7.5 times that of *p,p'*-DDT. At 30° the atmosphere above a surface deposit of technical grade DDT contained approx. 62% *o,p'*-DDT, 16% *o,p'*-DDE, 14% *p,p'*-DDE, and only 8% *p,p'*-DDT. When technical grade DDT was applied to moist soil at concentrations up to 20 µg/g the atmosphere in and above the soil contained approximately equal quantities of *o,p'*-DDT and *p,p-'*DDT, but at higher concentrations the ration of *o,p'*- to *p,p'*-DDT in the vapor phase increased. The primary breakdown product of DDT, *p,p'*-DDE, had a higher vapor pressure than the original compound *p,p'*-DDT, which suggests that much of the DDT now present in the soil may volatilize as DDE. Dieldrin applied with DDT did not affect DDT vapor density and the interaction between *o,p'*- and *p,p'*-DDT was very slight.

Because of its resistance to either microbial or chemical degradation, DDT tends to persist and accumulate in soils. It has been recently shown, however, that DDT degrades more rapidly in soils under biologically active, anaerobic conditions compared with well-aerated systems with DDD as the major product[105-107].

The chemical stability of DDT and related compounds in selected alkaline environments was studied by Smith and Parr[108]. DDT was stable in soil treated with anhydrous ammonia (pH 10.0) and in sterile, buffered, glass microbeads up to pH 12.0. The threshold pH for dehydrochlorination of DDT to DDE in microbeads was 12.5 with extensive conversion (70%) at pH 13.0 where the amount of applied DDT unaccounted for increased from 20% at 140 h to approx. 50% after 30 days, suggesting the formation of intermediates that were relatively stable in microbeads even at pH 13.0 where nearly complete recovery was obtained after 7 days. However, extended incubation to 28 days resulted in a gradual disappearance of DDE with only 88 and 74% accounted for at pH 10.0 and 13.0, respectively, suggesting active-dependent pH relationship for transformation under these conditions. Similar observations were observed in regard to the effect of pH on the dehydrochlorination of DDD to DDMU. While DDD was stable for extended periods at pH 10.0, it converted rapidly to DDMU at pH 13.0 and then tended to disappear with time.

DDT and other residues were assayed with a Micro-Tek Model GC-200R gas chromatograph equipped with a ^{63}Ni electron capture detector operated at 295° with the inlet at 215° and the oven at 195°. The column was a 180 cm × 6 mm coiled glass tube packed with 80–100 mesh Chromosorb W acid-washed DMCS coated with 3% OV-1; the carrier gas was nitrogen at a flow-rate of 120 cm^3/min.

Tessari and Spencer[109] described air sampling and GLC analysis of pesticides in the environment. The pesticides were adsorbed onto nylon-chiffon cloth (0.25 or 0.5 m^2) impregnated with ethanediol and held vertically in a wooden frame and exposed to the atmosphere for 5 days. The pesticides were extracted with acetone–hexane (1:1), cleaned up on alumina and Florisil columns and analyzed by GLC with microcoulometric and flame photometric detection for organophosphorus compounds. Levels found were 0.5–50 µg of *p,p'*-DDT per sq. meter of cloth. The technique was used for monitoring air inside and outside the homes of people handling pesticides.

Södergren[110] described the determination of chlorinated hydrocarbon residues in airborne fallout with application to the analysis of the input and output of chlorinated residues in a fresh water ecosystem in south Sweden. Aliquots of fallout extract were determined by electron capture gas chromatography. The samples were run on three columns with 4% SF-96, 8% QF-1 and SF-96–QF-1 (3:1) as the stationary phases on 100–120 mesh Gas Chrom P. The temperature of the injection block, column and detector were 225°, 175° and 190°, respectively. The identity of the more important peaks in the chromatograms was confirmed by chemical methods. *p,p'*-DDT was hydrolyzed to *p,p'*-DDE by boiling with alkali solutions; *p,p'*-DDE which interfered with some of the PCB peaks was decomposed by oxidation with chromic acid.

The functions of estuarine sediments as a pollutant sink and as a pollutant bank were assessed by Albone *et al.*[111] in relation to the fate of DDT in the environment. *p,p'*-DDT was degraded more slowly *in situ* in Severn estuary sediments than when incubated in sediment samples maintained under hydrogen in the laboratory. These transformations were compared with the more extensive degradation of DDT on incubation in anaerobic sewage sludge. In all incubations, metabolites included *p,p'*-DDD. Extracts were measured initially by GLC using a Perkin-Elmer F-11 chromatograph with a ^{63}Ni electron capture detector and a 6 ft. × 1/4 in. silanized glass column with a mixed phase of 1.5% OV-17 and 1.95% QF-1 on 80–100 mesh Diatoport S; column temperature, 200° isothermal; nitrogen carrier gas at 75 ml/min; and subsequently by thin-layer chromatography (TLC) using Kieselgel G or GF$_{254}$ with *n*-hexane–ethyl acetate–acetic acid (15:1:0.01) or *n*-hexane–diethyl ether–acetic acid (1:4:0.1).

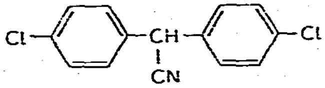
Although the use of organochlorine pesticides in Britain is 55 × 10^4 kg/year, it has been estimated[112] that Britain's rivers annually transport some 1 × 10^4 kg of these compounds to the sea at levels averaging 180 ppb.

Residue levels of persistent chlorinated hydrocarbons (mainly DDT and its metabolites as well as the polychlorinated biphenyls) in open ocean biota, especially those occupying lower positions in the food chain, are generally expected to be low, *e.g.*, in the ppb range. Because of limitations in sample availability and handling problems, composite samples for analysis are usually in the range of 25–100 g. Hence, sample extracts require concentration to a small volume, often from about 500 ml to less than 5 ml. Gas chromatographic analysis also has to be operated at very high sensitivity in order to detect such low levels of chlorinated hydrocarbons. Giam and Wong[113] recently reported on the problems of background contamination from chemicals, materials, and equipment used in the analysis of open ocean biota for chlorinated hydrocarbons.

A Tracer Model MT 220 gas chromatograph equipped with a ^{63}Ni electron capture detector was used with a 6 ft. × 1/4 in. O.D. U-shaped glass column packed with 5% DC-200 on HP Chromosorb W (80–100 mesh). Nitrogen was used as the carrier gas at a flow-rate of 60 ml/min and the injector, oven and detector temperature were 225°, 200°, and 275°, respectively. The operating sensitivity was about 0.05 ng heptachlor epoxide giving 50% full-scale deflection (f.s.d.). Contaminants which were found to interfere with the GLC analysis of chlorinated hydrocarbons included: Florisil, sharkskin filter paper, glasswool, aluminum foil, reagent grade Na_2SO_4 and NaCl, nanograde petroleum ether, distilled water, PTFE equipment and PTFE-rubber laminated discs. It was recommended that where applicable, heating the materials, equipment and chemicals at 300–350° overnight was effective in removing the contaminants responding to the electron capture detector.

Low concentrations of organochlorine pesticides have been found in waters from most areas of the world, higher levels being found in streams draining watersheds in which pesticides have been applied[114,115]. Although these small quantities of pesticides are often unnoticed in terms of water quality, they can lead to detrimental effects in aquatic organisms exposed to these waters[116], leading to concentrative movement up the food chain. For example, levels as high as 90 ppm of DDT have been reported in Coho salmon (*Oncorhynchus kisutch*)[117].

The formation of bis(*p*-chlorophenyl)acetonitrile (*p,p'*-DDNC) (I) in anaerobic sewage sludge has recently been reported by Jensen *et al.*[118] and Albone *et al.*[119], with separation and confirmation of product being accomplished in both studies by gas chromatography–mass spectrophotometric procedures. In the studies of Jensen *et al.*[118] one liter of activated sludge was fed with 100 mg of *p,p'*-DDT fortified with [^{14}C]DDT (5 μCi) and containing 4.0% DDD and 3.1% DDE and incubated with stirring for 8 days at 20° in an atmosphere of nitrogen. One-milliliter samples were mixed with 1 ml of methanol and shaken with 15 ml of hexane for 1 h, then analyzed on a Varian 1400 gas chromatograph fitted with an electron capture detector and a 160 cm × 0.18 cm I.D. all glass column filled with 4% SF-96 methyl silicone oil on 100–120 mesh silanized Chromosorb W. The oven temperature (*ca.* 180°) gave a retention time of 20 min for DDT with a nitrogen flow-rate of 20 ml/min. The DDT was found to be rapidly consumed with a half-life of only 7 h partly under transformation to DDD, DBP, DDMU and DDCN. The original low content of DDE disappeared within 48 h. DDCN was isolated from the sludge by first centrifugation at 25,000 rpm yielding complete sedimentation and no ^{14}C-activity in the water phase. The centrifugate was air dried (22.5 g) on filter paper ground before Soxhlet extraction with acetone–hexane (1:1). The ^{14}C activity recovered was 2.5 μCi (13%). The concentrated extract was separated on a partly deactivated aluminum oxide column (7 oz., 5% water). Elution with 160 ml of hexane gave almost pure DDD, while elution with diethyl ether in hexane (10%, 250 ml) gave a fraction continuing the new DDT transformation product (9% of initial ^{14}C activity) contaminated with traces of DDT, DDD, DDMU and DBP. A final elution with 400 ml of ethanol gave a fraction of unidentified products adding up the recovery of ^{14}C activity to 40%. The DDCN was further purified by preparative TLC using 1 mm silica gel HF$_{254}$ and diethyl ether in hexane (20%) as eluant. The chromatogram was covered with a Kodak X-ray film for 3 days and after development the R_F value of DDCN was determined by extension of spots corresponding to human DDT metabolites such as DDA (R_F 0.0), DBP (R_F 0.39), DDD (R_F 0.48), DDMU (R_F 0.64) and DDT (R_F 0.84) and verified by GLC. An extract of the zone containing DDCN (R_F 0.18) was analyzed on an LCB 9000 combined gas chromatograph–mass spectrometer using the column system described above. The mass spectrum with molecular ion at *m/e* 261 (isotopic pattern *m/e* 261:263:265 with relative intensities 1.0:0.62:0.10) abundant ion at *m/e* 226 (*m/e* 226:228 with relative intensities 1.0:0.33), *m/e* 191 and *m/e* 190 as the abundant ions were essentially identical with a mass spectrum from an authentic sample of bis-(*p*-chlorophenyl)-acetonitrile (m.p. 90–91°, synthesized according to Grummit and Marsh[120]). The tendency of DDCN to undergo antioxidation forming DBP (*p,p'*-dichloro-diphenyl-benzophenone) in ethanolic potassium hydroxide, and its conversion to bisphenyl acetonitrile was found to be suitable for confirmation of DDCN at low concentrations. In the studies of Jensen *et al.*[118] it could not be determined whether DDCN was formed via a metabolic pathway or originated from a direct reaction with substances present in the sludge. DDCN was also found (0.6 ppm by dry weight) by the authors in a sediment layer of the Lake Malaren in Sweden. DDCN has not previously been found in nature, probably due to its polar properties which complicate separation from common substances, its low chemical stability and relatively low response to electron capture detection (about one-fourth of that of DDD) as well as its tendency to form DBP on treatment with bases. This latter tendency is a reaction typical of the dicofol-like substances also formed from DDT in different biological systems.



DDCN (I)

In the studies of Albone *et al.*[119] anaerobic sewage sludge was incubated for 88 days at 37° with a total of 7.45 mg [^{14}C]*p,p'*-DDT (4.7 μCi) and 20 g minced beef added (to enhance microbiological activity). The solid residue was extracted rapidly three times in the cold with isopropanol–heptane (4:1) using a modified procedure of Dole and Meinertz[121], and a total of 1.5 μCi (32% of total label) was recovered. The Dole–Meinertz extract was first examined by GLC using a Perkin-Elmer Model F-11 instrument fitted with a ^{63}Ni electron capture detector and a 6 ft. × $^1/_4$ in. silanized glass column with a mixed phase of 1.5% OV-17 and 1.95% QF-1 on 80–100 mesh Diatoport S (column temperature 200°, isothermal). TLC using Kieselgel F with *n*-hexane–ethyl acetate–acetic acid (15:1:0.01) revealed 3 zones of activity: Zone A (R_F 0.3–0.6) including *p,p'*-DDT and *p,p'*-TDE; zone B (R_F 0.1–0.3) and zone C (R_F 0.0–0.1) in the ratio 62:29:9. TLC zone B was found to give only one major peak on further electron capture–GLC analysis (retention time relative to *p,p'*-DDT, 0.62). High resolution probe mass spectrometry using an AEI-MS902 instrument indicated the compound TLC zone B to be DDCN, confirmed by comparison of *p,p'*-DDCN synthesized by the method of Skerrett and Woodcock[122], m.p. 90–95°. As in the previously cited studies of Jensen *et al.*[118], it was not known whether DDCN was produced microbiologically or chemically in the nitrogen-rich alkaline environment or anaerobic sewage sludge. Possible routes suggested include: via *p,p'*-DDA → amide → nitrile or more directly from *p,p'*-DDT.

The analysis of pesticides in water has been reviewed by Hindin *et al.*[123]. Chlorinated insecticides are of low solubility in water and hence they may be extracted with water-immiscible solvents such as hexane or benzene. However, the extraction of large volumes of water to achieve the necessary sensitivity requires time-consuming batchwise procedures.

Rosen and Middleton[124] removed the pesticides from 2000 l of water with a carbon filter and desorbed them from carbon by Soxhlet extraction with chloroform. Recoveries of BHC, chlordane, DDT, aldrin, TDE, and lindane were in the range of 75–86% at the 2.5 ppm level, but some were lower below the 1 ppm level. Kahn and Wayman[125] described a simple apparatus that could be used for the continuous extraction of several hundred liters of water with petroleum spirit and obtained a 83–100% recovery at the 0.2–340 ppb level with a range of compounds. Organochlorine pesticide residues in water sediment, algae, and fish in Hawaii during the period 1970–1971 were investigated by Bevenue *et al.*[126], rainwater, drinking water and non-potable waters in Hawaii were found to contain chlorinated insecticide residues in the low parts-per-trillon range. Dieldrin, *p,p'*-DDT and lindane were the pesticides most prevalent. Pentachlorophenol was present in samples from sewage fallout. The ratio of chlorinated pesticide residues in algae, sediment, and fish from the same canals was 1:4.000:9.000:32,000 respectively. The following two gas chromatographs were used for analyzing the water and sediment samples. The first was a Varian Aerograph Model 204 with a concentric tritium electron capture detector and a 6 ft. × $^1/_8$ in. borosilicate glass column: column temperature, 190°: injector and detector temperature, 200°: and carrier gas argon–methane (90:10) at a flow-rate of 75 ml/min. Four types of column packing were used, *viz.*, (1) 3% SE-30 on 80–100 mesh Chromosorb W, AW, DMCS; (2) 3% QF-1 and 2% DC-200 on 80–100 mesh Chromosorb W, AW, DMCS: (3) 4% SE-30 and 6% QF-1 on 80–100 mesh Chromosorb W, HP: and (4) 1.5% OV-17 and 1.95% QF-1 on 100–200 mesh Supelcoport. The retention times of the pesticides on these columns relative to aldrin are given in Table 6. The limits of detectability of the pesticides ranged from about 0.05 ppt (lindane the most sensitive) to 0.5 ppt (DDT the least sensitive). Dieldrin was detected at about 0.2 ppt. This was an approximate range only because detection limits varied with the degree of purity of the water sample on the degree of success in cleaning up non-potable water, sediment, and biota samples.

TABLE 6

RETENTION TIMES OF PESTICIDES IN GAS CHROMATOGRAPHIC COLUMNS RELATIVE TO RETENTION TIME OF ALDRIN

Compound	*3% SE-30*	*3% QF-1 + 2% DC-200*	*4% SE-30 + 6% QF-1*	*1.50% OV-17 + 1.95% QF-1*
	Chromosorb W, AW, DMCS, 80–100 mesh	*Chromosorb W, AW, DMCS, 80–100 mesh*	*Chromosorb W, HP, 80–100 mesh*	*Supelcoport, 100–120 mesh*
Lindane	0.43	0.57	0.54	0.64
Heptachlor	0.77	0.81	0.81	0.81
Aldrin	1.00	1.00	1.00	1.00
Heptachlor epoxide	1.26	1.47	1.43	1.62
p,p'-DDE	1.97	1.92	1.95	2.43
Dieldrin	2.26	2.19	2.19	2.60
p,p'-DDD	2.60	2.79	2.76	3.89
p,p'-DDT	3.46	3.36	3.44	4.69
Pentachlorophenol ethyl ether			0.57	0.55
Pentachlorophenol methyl ether			0.46	0.46

The mechanism of conversion of DDT to DDD by *Aerobacter aerogenes* was described by Plimmer *et al.*[127]. The conversion proceeded by direct reductive dechlorination without the intermediate formation of DDE in still cultures of *Aerobacter aerogenes*. Deuterated DDT was used to determine whether an unsaturated intermediate was formed during the reaction. Retention of the deuterium atom in the product would exclude the possibility of a two-step reaction involving DDE. Under anaerobic conditions, 92% conversion of DDT to DDD was measured in the ether extract. The product was identified by mass spectroscopy, vapor-phase chromatography and TLC on silica gel. Mass spectra were obtained by volatilizing the DDD directly from silica gel in the probe of the mass spectrometer. The parent peak occurred at *m/e* 319 due to the contribution of the species $C_{14}H_9D^{35}Cl_4$, indicating that the deuterium atom originally present at the 2 position in DDT was retained throughout the reaction.

Mass spectral studies were conducted on a Bendix Model No. 12 mass spectrometer using a direct inlet probe. GLC was performed on a Wilkens Aerograph Model 204B gas chromatograph equipped with a flame ionization detector and a 5 ft. × $^1/_8$ in I.D. stainless-steel column containing 5% SE-30 on Chromosorb W, AW, DCMS, at a column temperature of 212°. The carrier gas (nitrogen) flow-rate was 30 ml/min. The identity of the products was established by co-chromatography with authentic samples on silica gel G (Merck) plates developed with carbon tetrachloride–cyclohexane (1:1). Compounds were visualized by ultraviolet irradiation. The R_F values for DDT and DDD were 0.87 and 0.68, respectively.

Pfaender and Alexander[128] attempted to establish how $[^{14}C]$DDT would be transformed in samples from natural ecosystems containing highly heterogeneous microbial communities. Extracts of Hydrogenomonas sp. cells converted DDT to DDD, DDMS and DBP and several other products under anaerobic conditions. *p*-Chlorophenyl acetic acid was formed when whole cells and oxygen were subsequently added, showing that enzymes of a single organism could convert DDT to ring-cleavage products. A strain of Arthrobacter also grew on *p*-chlorophenyl acetic acid, indicating that the action of two bacteria led to extensive biodegradation of DDT. The Arthrobacter formed *p*-chlorophenyl glycol aldehyde and other products from *p*-chlorophenyl acetic acid. The major metabolites formed by microbial communities of sewage and fresh water containing sediment was DDD and DBP and small amounts of DDMS and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene. No *p*-chlorophenyl acetic acid was accumulated: added *p*-chlorophenyl acetic acid was rapidly decomposed by the sewage microflora.

TLC and GLC were used for isolation and identification of ether extracts of DDT and its degradation products. Silica gel S sheets containing a fluorescent indicator (Brinkmann) were used with 2% acetone–hexane and 10% chloroform–methanol as developing solvents. Spots were visualized by viewing the sheets under UV light at 270 nm. GLC was performed with an Aerograph Model 1740-20 instrument equipped with electron capture and hydrogen flame ionization detectors. The separation of DDT and the products formed from it anaerobically was achieved using a 183 × 0.3 cm Pyrex glass column containing 10% DC-200 on acid-washed Chromosorb W at 180° with electron capture detection. *p*-Chlorophenyl acetic acid and its metabolites were separated with the same packing material in a 183 × 0.3 cm stainless-steel column using hydrogen flame ionization detection. The operating temperatures for the detector and injector were 195° and 205°, respectively. The flow-rates were 110 and 30 ml/min for the glass and stainless-steel columns, respectively.

The utility of DDT dehydrochlorinase for the identification of DDT in soil was outlined by Gooding *et al.*[129]. Soil extracts were treated with DDT dehydrochlorinase (extracted from DDT-resistant houseflies) in order to convert DDT to DDE under relatively mild conditions so that non-DDT moieties would unlikely be destroyed. The DDE thus obtained was analyzed by GLC using a Varian Aerograph 1200 chromatograph with a 250 mCi ^3H foil detector and a 90 cm × 3 mm O.D.

Pyrex glass column packed with 5% Dow-11 on 60–80 mesh Chromosorb W, HMDS. The temperatures of the column, injector and detector were 190°, 185° and 200°, respectively, and nitrogen carrier gas was used at a flow-rate of 15 ml/sec. A comparison of the retention times of the peaks in the material extracted from soil with the retention times of PCB standards suggested that most of the contaminants were PCBs. It was found, however, that although PCBs interfered with the routine quantitation of DDT, they did not interfere with the enzymatic confirmation of DDT.

Shin *et al.*[130] described an empirical attempt to identify actively adsorbing fractions such as soils, soil fractions and biological materials which may have significant ecological and structural relationships in the soil with regard to the absorption of *p,p'*-DDT. DDT in the hexane extracts of the absorbing fractions was identified by elution time and estimated by peak area using a Beckman GC-5 gas chromatograph with an electron capture detector and a 3 mm × 1.83 m borosilicate glass column packed with 2% DC-11 on Gas Chrom Q: helium was the carrier gas.

In 1966, Terriere *et al.*[131] reported that about 88% of the DDT remaining in the soil of two orchards (Hood River and Medford, Oregon), which had been treated since 1946, was in the upper six inches and it was concluded that contamination of the land adjacent to the orchards was slight and that losses in underground water were negligible. The further fate of these residues was recently summarized by Kiigemagi and Terriere[132] in 1972. Between 1946 and 1964 the Hood River orchard was treated with 388 pounds of DDT and 14 pounds of dicofol (kelthane: 4,4'-dichloro-α-(trichloromethyl)benzhydrol) per acre and with additional treatments through 1967 totalled 414 pounds of DDT and 22.1 pounds of dicofol. The Medford orchard received a total of 169 pounds of DDT and 14.32 pounds of dicofol from 1947–1966. The soil samples tested were composites made of subsamples from the drip zone of 5 trees, *e.g.*, 5 cores per sample and the samples were taken with a $^3/_4$ inch soil auger at 0–6, 7–12, 13–24, and 25–36 inch levels. The samples were extracted by shaking for 1 h with a 1:1 mixture of acetone–hexane, the extracts were washed free of acetone and dried with anhydrous sodium sulfate. They were analyzed without cleanup by electron capture gas chromatography using a column of QF-1–Dow-11 (2:1) on 100–200 mesh HP Chromosorb W at 180° (other details of the analytical method were described earlier[131]). Resolution of DDT analogs and metabolites was good except that when residue levels were high TDE appeared as a shoulder on the larger *p,p'*-DDT peak. Dicofol was converted to its thermal decomposition product DBP. To measure the soil levels of dicofol and DBP, aliquots of the extracts were given a preliminary cleanup by passage through an activated Florisil column and eluted with 300 ml of hexane–diethyl ether (3:1). The eluates were concentrated and chromatographed on alumina-coated plates which were developed with carbon tetrachloride–hexane (2:1). The R_F values in this system were 0.11 for dicofol and 0.50 for DBP. The absorbent was scraped from the plates in the appropriate areas, extracted with acetone and quantified by electron capture GLC. The amount determined by this TLC procedure agreed with those indicated by the DBP peaks in the original GLC analysis of the soil extracts. The sensitivity of the analytical method was 0.01 ppm for all compounds and recovery averaged 89%. An examination of the levels of DDT analogs and metabolites showed the largest losses occurred in *p,p'*-DDT residues and the largest gains in dicofol–DBP residues. This latter finding suggests two possibilities, *e.g.*, (a) In soil dicofol–DBP residues are more stable than DDT, or (b) they are metabolites or degradation products of DDT. The latter possibility is supported by Lichtenstein *et al.*[133] who reported traces of dicofol in soil treated earlier with DDT. One of the more interesting aspects of the study of Kiigemagi and Terriere[132] was the similarity in the residue trends seen in the two locations. The net loss in the 5 year period in the Hood River soils amounted to 18% and in the Medford soils to 17%. This was in spite of differences in climate, soil type and cultural practices. It was suggested that the DDT residues in these soils attained a threshold level within a few years after its regular use began. The decline in total residues is proceeding at a rate of about 3.5% per year, based on those present in 1965, with an additional loss of the amounts added since 1965. The decline of DDT residues in soils is confirmed by a Mississippi study reported by Smith[134] which showed that an initial residue of 2300 ppm declined by 30–50% in a 20 year period.

Photo-oxidation of DDT and DDE in methanol has been reported by Plimmer *et al.*[135], who suggested that photolytic generation of free radicals may abstract hydrogen from solvent, react with oxygen, or abstract hydrogen from unreacted substrate. Further decomposition of short-lived intermediates yields many compounds. Oxidation products included benzoic acids, aromatic ketones and chlorinated phenols. DDE was also found to undergo photocyclization to yield dichlorofluorene derivatives. Methanolic solutions of DDE or DDT (*ca.* 1.0 g/l) were irradiated with a 450-Watt mercury lamp in a water-cooled quartz housing and a suitable filter eliminated light of short wavelength (Corex $>$ 2600 Å or Pyrex $>$ 2800 Å) and oxygen or nitrogen was bubbled through the solution in separate experiments. Products were obtained by chromatography of the methanolic solution after it was concentrated in a rotary evaporator. Acids and phenols were removed by extraction with NaOH, the NaOH extract acidified, and the free acids and phenols extracted into diethyl ether. Acids were then extracted from the ether with $NaHCO_3$. Portions of the free acids and phenols thus obtained were methylated with diazomethane before GLC analysis. The neutral extract was fractionated by chromatography on a silica gel column and each fraction was examined by TLC and mass spectrometry. A Perkin-Elmer Model GS270 instrument was equipped with a 30 m open tubular capillary column (0.051 cm I.D.) coated with SE-30 on Chromosorb W (scot). Table 7 lists the photolysis products of DDT and DDE in methanol. A 10% yield of 3,6-dichlorofluorenone was obtained by irradiation of a methanolic solution of DDE (wavelength $>$ 2600 Å).

The irradiation of DDT with ultraviolet and visible light under a variety of conditions has been reported[135–137]. The chain dechlorination of DDT in alcohol solution induced by ionizing radiation has been reported by Evans *et al.*[138].

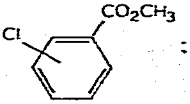
Kimbrough and Gaines[139] studied the fate of DDT residues stored in the lipids of foods processed by γ irradiation. The radiation products of a 1 : 2 mixture of technical DDT and tristearin in water is shown in Table 8. Technical DDT is composed of 85–90% *p,p'*-DDT, 10–15% *o,p'*-DDT and traces of several other compounds including: *p,p'*-DDE and *o,p'*-DDE[3]. DDT was found to be fairly resistant to ionizing radiation. The analysis of gaseous radiation products was carried out by gas chromatography. Hydrogen was determined on alumina with argon as carrier gas, and nitrogen was determined on a molecular sieve with helium as carrier gas. Analysis of solid reaction products was carried out by GLC on SE-30 at 250° with nitrogen as carrier gas and by TLC on silica gel with hexane as solvent. The strips were detected by spraying with 0.5% silver nitrate in ethanol, then dried 5 min at 100°, resprayed

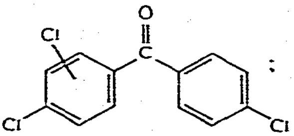
TABLE 7

PHOTOLYSIS PRODUCTS OF DDT AND DDE IN METHANOL

R is ClC_6H_4. The compounds listed represent the volatile components of the photolyzate separated by gas chromatography; m = present in moderate amounts; + = detected; — = not detectable; Ph = phenyl.

m/e	*Structure*	*DDT*		*DDE*	
		O_2	*N_2*	*O_2*	*N_2*
Neutral fraction					
136	$PhCO_2CH_3$	+	—	—	—
140	RCHO	+	—	+	—
170	RCO_2CH_3	+	—	m	m
180	$PhC{=}CH_2$	—	—	—	—
184	$RCH_2CO_2CH_3$	—	—	+	+
204	(a)*	+	—	+	—
214	$PhRC{=}CH_2$	—	+	+	+
222	R_2	—	m	+	+
226	$Ph_2CHCO_2CH_3$	—	±	—	—
232	$PhRCHOCH_3$	—	—	—	—
236	R_2CH_2	—	—	—	+
248	$R_2C{=}CH_2$	—	—	—	+
248	(g)*	—	—	m	
250	R_2CO	—	m	+	m
260	$PhRCHCO_2CH_3$	m	+		
266	R_2CHOCH_3	—	+	—	+
280	(c)*	—	—	+	
282	$R_2C{=}CHCl$	—	—	+	m
284	$PhRCHCHCl_2$	m	—	—	—
284	(b)*		—	—	
294	$R_2CHCO_2CH_3$	m	—	—	+
314	(d)*	—	—	—	
316	$R_2C{=}CCL_2$ (DDE)	—	m	+	+
318	$R_2CHCHCl_2$ (DDD)	m	—	—	—
324	$R_2C(OCH_3)CO_2CH_3$	—	—	m	
338	Unknown	—	—	m	
352	R_2CHCCl_2 (DDT)	+	+		
Acidic and phenolic compounds (methylated)					
142	$ROCH_3$	—		—	
170	RCO_2CH_3	—		+	—
176	$Cl_2C_6H_3OCH_3$	—		+	+
204	$Cl_2C_6H_3CO_2CH_3$	—		+	
210	$Cl_3C_6H_2OCH_3$	+		—	—
228	(e)*	—		—	
280	(f)*	—		—	
294	$R_2CHCO_2CH_3$	+		+	

* Structures: (a) = 

; (b) = 

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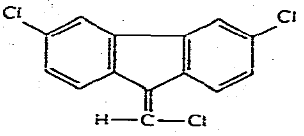
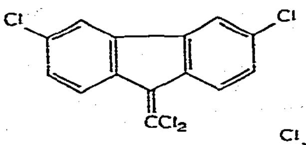
(c) = 

; (d) = 

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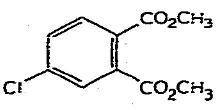
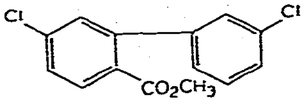
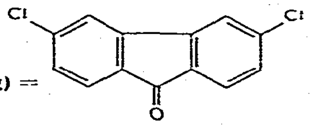
(e) = 

; (f) = 

; (g) = 

with a mixture of 0.2% bromophenol blue and 0.15% silver nitrate in ethyl acetate–ethanol (1:1) and dried 10 min at 100°.

The degradation of [^{14}C]DDT on silica gel G chromatograms has been recently reported by Ernst[140]. TLC of [^{14}C]DDT on silica gel G and silica gel GF$_{254}$ using two solvent systems, (A) *n*-heptane with 1% acetone[141] and (B) benzene–methanol–acetic acid (80:14:7), followed by examination and irradiation with UV light at 254 and 366 nm gave rise to the formation of polar substances. The most effective source was shortwave UV light, when even after 15-min periods (as used in the detection of separated compounds), gave rise to 4 components. Table 9 lists the R_F values of [^{14}C]-DDT and unlabeled DDA, DDD, DDT, and DDE as well as components I–IV in determining solvent systems A and B. Studies by a more sensitive method such as radioscanning led to the conclusion that in the investigation of metabolic or other degradation pathways with [^{14}C]DDT, the evaluation of silica gel chromatograms will not be conclusive if appropriate precautions are not followed, *e.g.*, working in the dark and repeated purification of the [^{14}C]DDT batches to remove interfering material.

TABLE 8

RADIATION PRODUCTS OF 1:2 MIXTURE OF TECHNICAL DDT AND TRISTEARIN IN WATER

The *G* value is the radiation chemical yield expressed as the number of molecules or ions of product liberated per 100 eV of absorbed energy.

Product	*G value*	*Method of analysis*
Hydrogen	5.7 including that which reacted with the O$_2$ present	GC on alumina with argon carrier gas
p,p'-DDE*	0.04*	GC on SE-30, nitrogen carrier gas
o,p'-DDE**	0.004**	GC on SE-30, nitrogen carrier gas
DDT-Dimer***	0.001***	TLC
DDT-Tristearin*** addition product, hexane soluble	0.001***	TLC
DDT-Tristearin hexane insoluble	0.2	

* The *G* value for the formation of this material was obtained from irradiation of pure *p,p'*-DDT-tristearin which contained no *p,p'*-DDE before irradiation.

** The *G* value for the formation of this material was obtained from irradiation of pure *o,p'*-DDT-tristearin which contaiend no *o,p'*-DDE before irradiation.

*** The *G* value was estimated from the size and intensity of the silver nitrate–bromophenol blue spot compared to spots of known concentration of DDT.

McKinney *et al.*[142] described the synthesis and reactions of a proposed DDT metabolite, 2,2-bis(*p*-chlorophenyl)acetaldehyde (DDAA). GLC, NMR and mass spectrometry were utilized for the identification of DDAA. A Varian Aerograph 1525B chromatograph was equipped with a tritium detector and a glass column of 1% orthophosphoric acid and 3% DEGS on 30–60 mesh Chromosorb W. Low-resolution mass spectra were obtained with an LKB 9000 gas chromatograph–mass spectrom-

TABLE 9

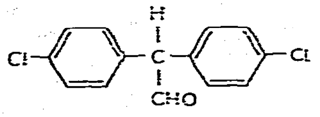
TLC OF DDT AND ITS DEGRADATION PRODUCTS ON SILICA GEL G

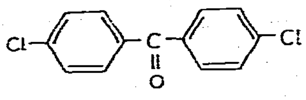
Solvent A: *n*-heptane with 1% acetone; solvent B: benzene-methanol-acetic acid (80:14:7).

Compound	R_F	
	Solvent A	*Solvent B*
DDA	0.0	0.7
DDD	0.25	0.9
DDT	0.36	0.9
DDE	0.55	0.9
Component I	0.0	0.0
Component II	0.0	0.7
Component III	0.0	0.8
Component IV	0.0	0.9

eter. TLC was used to separate DDAA from the DBP reaction byproduct. Eastman Chromagram Type 6060 sheets with fluorescent indicator were developed in hexane–chloroform–methanol (3:2:1) and the spots were detected under UV light (R_F values of 0.59 and 0.71 for DDAA and DBP, respectively).

2,2-Bis(*p*-chlorophenyl)acetaldehyde was suggested to be a logical precursor of 2,2-bis(*p*-chlorophenyl)acetic acid (DDA). It had been earlier shown by Peterson and Robison[143] that 2,2-bis(*p*-chlorophenyl)ethanol (DDOH) could be obtained from *in vivo* transformations of other known DDT metabolites and it was suggested by McKinney *et al.* that the occurrence of DBP, DDA and DDOH in DDT metabolism is strong supporting evidence for the intermediacy of 2,2-bis(*p*-chlorophenyl)acetaldehyde. However, it was noted that the detection of DBP *per se in vivo* would be extremely difficult because of its instability and reactivity, and it was believed that DDAA would more likely be stabilized by interaction with functional groups, *e.g.*, interaction with amino acids to form Schiff bases which might be incorporated into proteins, than chemically binding DDT residues and simulating storage.


(DDAA)


(DBP)

The chromatographic behavior of DDT and fourteen related compounds, metabolites, analogs and degradation products on commercially available precoated aluminum oxide TLC chromatoplates was investigated by Bishara *et al.*[144,145] using 33 solvent systems. The color and/or fluorescence response of the DDT-type compounds and their sensitivity to the chromogenic reagent ammoniacal silver nitrate and 2-phenoxy ethanol in acetone was also described. Table 10 lists the solvent systems and their development times on aluminum oxide (Type E) F_{254} plates. Table 11 lists the R_F values × 100 of DDT and some related compounds developed by ascending one-dimensional TLC on aluminum oxide (Type E) F_{254} plates. Table 12 records the color and/or fluorescence response of DDT and some related compounds on alu-

TABLE 10

SOLVENT SYSTEMS AND THEIR DEVELOPMENT TIMES ON ALUMINIUM OXIDE (TYPE E) F_{254} PLATES

Number	*Composition*	*Time (min)*
I	*n*-hexane	31
II	*n*-hexane–benzene (90:10)	31
III	*n*-hexane–chloroform (90:10)	36
IV	*n*-hexane–chloroform (80:20)	36
V	*n*-hexane–chloroform–acetic acid (90:10:1)	36
VI	*n*-hexane–diethyl ether–acetic acid (90:10:1)	34
VII	*n*-hexane–ethyl acetate (90:10)	26
VIII	*n*-hexane–ethyl acetate–acetic acid (80:20:2)	36
IX	*n*-hexane–ethyl propionate (90:10)	31
X	*n*-hexane–carbon disulfide (90:10)	29
XI	*n*-hexane–ethylene dichloride (90:10)	32
XII	*n*-hexane–acetone (95:5)	34
XIII	*n*-hexane–acetone–acetic acid (95:5:1)	32
XIV	*n*-hexane–acetone–diethyl ether (80:10:10)	27
XV	*n*-hexane–ethanol–acetic acid (90:10:1)	35
XVI	*n*-hexane–ethanol (80:20)	40
XVII	*n*-hexane–ethanol–acetic acid (80:20:2)	50
XVIII	*n*-hexane–propionic acid (95:5)	46
XIX	*n*-hexane–propionic acid (90:10)	60
XX	*n*-hexane–acetic acid (90:10)	34
XXI	ethanol (absolute)	106
XXII	methanol (absolute)	55
XXIII	cyclohexane	72
XXIV	cyclohexane–carbon tetrachloride (90:10)	65
XXV	cyclohexane–acetone (95:5)	70
XXVI	cyclohexane–acetic acid (90:10)	70
XXVII	*n*-pentane	34
XXVIII	*n*-pentane–carbon disulfide–acetic acid (80:20:2)	35
XXIX	*n*-pentane–acetone (95:5)	29
XXX	*n*-pentane–methanol (50:50)	60
XXXI	*n*-pentane–propionic acid (99:1)	45
XXXII	*n*-pentane–propionic acid (90:10)	64
XXXIII	*n*-pentane–ethanol–acetic acid (90:10:1)	42

minum oxide (Type E) F_{254} TLC plates and Table 13 lists the limit of detectability of DDT and its fourteen related compounds on aluminum oxide (Type E) F_{254} TLC plates developed with *n*-pentane–methanol (50:50). Fig. 6 illustrates two-dimensional TLC chromatograms of DDT and eleven related compounds.

Depending on the general chromatographic mobilities and separation of the fifteen DDT-type compounds on aluminum oxide plates, they could be classified into three groups, *viz.*, the non-polar compounds exhibiting higher R_F values, *e.g.*, *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, DDMU, DPE, and DDM; the polar compounds having lower mobilities, *e.g.*, DDA, kelthane, DBH and BPE; and an intermediate group consisting of one polar, DBP, and three non-polar, *p,p'*-DDD, *m,p'*-DDD, *o,p'*-DDD, compounds with mobilities in between the preceding two groups. Substitution of the less polar chlorine atom with a more polar hydrogen atom on the dichloromethyl

Solvent systems*	*p,p'*-DDT	*o,p'*-DDT	*p,p'*-DDE	*o,p'*-DDE	*p,p'*-DDD	*m,p'*-DDD	*o,p'*-DDD	DDA	DDMU	DBP	Kelthane	DPE	DBH	BPE	DDM
I	25	35	41	36	10	11	12	0**	32	2	0	27	0	0	35
II	48	55	61	56	28	29	30	0	55	8	2	44	0	0	54
III	69	72	75	72	52	52	52	0	67	24	3	63	0	0	67
IV	76	76	77	75	64	64	64	0	74	45	8	72	2	4	75
V	67	69	72	69	52	53	54	0	70	69	7	66	1	2	71
VI	67	70	75	68	51	51	50	0	72	45	10	66	2	4	70
VII	70	70	75	70	63	61	58	0	74	61	23	66	6	13	69
VIII	78	79	83	79	77	76	74	6	83	75	53	77	27	39	80
IX	69	71	76	69	60	60	57	0	73	56	19	67	4	9	71
X	35	44	49	45	16	18	19	0	48	4	0	35	0	0	42
XI	58	63	65	62	42	43	46	0	62	18	3	55	0	0	61
XII	60	64	71	64	43	41	39	0	69	50	8	60	2	5	67
XIII	63	66	71	64	48	47	46	0	68	55	16	62	4	9	67
XIV	73	74	77	72	65	64	61	0	75	68	48	77	24	36	76
XV	83	84	85	83	78	78	79	5	83	81	58	82	46	55	83
XVI	84	84	84	83	80	79	80	0	84	82	75	84	71	74	83
XVII	87	88	89	87	81	81	81	20	87	83	64	86	59	63	86
XVIII	59	68	73	69	39	39	40	8	67	34	34	60	33	34	67
XIX	92	94	96	93	83	83	84	32	92	82	72	91	61	70	93
XX	78	80	80	77	69	70	71	17	78	67	43	74	24	35	78
XXI	82	82	82	80	81	81	81	0	81	81	81	81	80	81	82
XXII	80	80	80	77	79	79	79	5	78	78	79	79	79	79	80
XXIII	35	46	50	45	13	15	16	0	40	2	0	36	0	0	39
XXIV	40	51	52	48	16	18	20	0	44	3	0	42	0	0	42
XXV	71	74	77	72	59	57	56	0	74	64	21	70	5	13	72
XXVI	77	77	78	77	72	72	74	30	78	72	56	78	34	47	78
XXVII	27	40	43	42	10	10	11	0	35	2	0	32	0	0	35
XXVIII	70	75	77	75	51	52	53	2	74	20	4	69	4	4	75
XXIX	65	69	76	68	48	46	44	0	72	57	7	64	2	5	70
XXX	85	85	85	84	84	84	84	4	80	83	84	84	83	84	84
XXXI	54	67	74	69	22	24	26	0	61	6	6	54	6	6	62
XXXII	sf***	sf	sf	sf	94	94	94	35	sf	94	92	sf	83	92	sf
XXXIII	93	94	96	94	90	90	90	7	96	93	75	94	67	70	92

* The numbering of solvent systems corresponds to Table 10.

** Migration from point of application could not be detected.

*** Compound migrated solvent front.

TABLE 12

COLOR AND/OR FLUORESCENCE RESPONSE OF DDT AND SOME RELATED COMPOUNDS ON ALUMINUM OXIDE (TYPE E) F_{254} TLC PLATES

(*A*) Viewing in short-wavelength UV light; (*B*) viewing in long-wavelength UV light; (*C*) spraying with chromogenic reagent and viewing like *A*; (*D*) spraying with chromogenic reagent and viewing like *B*; (*E*) spraying with chromogenic reagent followed by exposing to short-wavelength UV light for 5 min and viewing like *A*; (*F*) like *E*, except viewing as *B*; (*G*) like *E*, except viewing in visible light; (*H*) spraying with chromogenic reagent followed by exposing to short-wavelength UV light for 15 min and viewing like *A*; (*I*) like *H*, except viewing as *B*; (*J*) like *H*, except viewing in visible light; (*K*) spraying with chromogenic reagent followed by exposing to short-wavelength UV light for 30 min and viewing like *A*; (*L*) like *K*, except viewing as *B*; (*M*) like *K*, except viewing in visible light; (*N*) exposing to short-wavelength UV light for 30 min, then like *K*; (*O*) exposing to short-wavelength UV light for 30 min, then like *L*; (*P*) exposing to short wavelength for 30 min, then like *M*. Abbreviations: B = blue; F = fluorescence; G = gray; N = negative; P = purple; Y = yellow; dk = dark; ft = faint; lt = light.

No.	*Compound*	*Method of detection*															
		A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*	*M*	*N*	*O*	*P*
1	*p,p'*-DDT	lt P	N	N	ft Y	P	ft YBF	dk G	dk P	ft BF	dk G	dk P	P	dk G	dk P	dk P	dk G
2	*o,p'*-DDT	lt P	N	N	ft Y	P	ft YBF	dk G	dk P	ft BF	dk G	dk P	P	dk G	dk P	dk P	dk G
3	*p,p'*-DDE	dk P	N	ft P	ft Y	P	ft YBF	dk G	P	ft YBF	dk G	dk P	YBF	dk G	dk P	P	dk G
4	*o,p'*-DDE	dk P	N	ft P	ft Y	P	ft YBF	dk G	P	ft YBF	dk G	dk P	YBF	dk G	dk P	P	dk G
5	*p,p'*-DDD	lt P	N	N	ft Y	BF	dk BF	G	BF	dk BF	G	BF	dk BF	G	dk PF	dk BF	dk G
6	*m,p'*-DDD	lt P	N	N	ft Y	BF	dk BF	G	BF	dk BF	G	BF	dk BF	G	dk PF	dk BF	dk G
7	*o,p'*-DDD	lt P	N	N	ft Y	BF	dk BF	G	BF	dk BF	G	BF	dk BF	G	dk PF	dk BF	dk G
8	DDA	lt P	N	N	ft Y	ft B	ft BF	ft G	ft P	ft YBF	lt G	lt P	YBF	lt G	P	YBF	G
9	DDMU	dk P	ft Y	ft P	YF	BF	BF	lt G	BF	BF	lt G	BF	BF	lt G	BP	BF	dk G
10	DBP	dk P	ft Y	ft P	YF	lt P	YF	N	lt P	YF	N*	P	YF	N	P	YF	G
11	Kelthane	lt P	N	N	ft Y	P	P	dk G	dk P	P	dk G	dk P	P	dk G	dk P	dk P	dk G
12	DPE	lt P	N	N	ft Y	P	ft YBF	dk G	P	P	dk G	dk P	P	dk G	dk P	dk P	dk G
13	DBH	lt P	ft Y	N	ft Y	ft B	ft YBF	ft G	lt P	ft YBF	lt G	P	lt YBF	lt G	P	YBF	lt G
14	BPE	lt P	ft Y	N	ft Y	ft B	ft YBF	ft G	lt P	ft YBF	lt G	lt P	lt YBF	lt G	lt P	lt YBF	lt G
15	DDM	lt P	N	N	ft Y	P	ft YBF	G	P	ft YBF	G	P	lt YBF	G	dk P	lt YBF	dk G

* Spot starts to appear as light gray within 1–2 h.

group of the three DDD isomers increased their polarity compared with the rest of the non-polar compounds resulting in lower mobilities.

In an earlier study reported by Bishara *et al.*[145] it was shown that when DDT, DDE, DDD, DDA and DDMU were chromatographed on aluminum oxide (Type E) F_{254} (Brinkman) plates and multiply developed with 6 solvent systems and detected with ammoniacal silver nitrate[146], multiple spots were observed for each compound. It was suggested that they were due to degradation caused by exposure to ultraviolet *per se* as well as to the possible effect of alumina which can catalyze reactions such as isomerization of double bonds and ester hydrolysis[147].

Abou-Donia and Menzel[148] described the separation and identification of DDT-type compounds by a system combining TLC, GLC, and infrared (IR) spectroscopy. R_F values for DDT and 13 other compounds, most of which have been reported as DDT-breakdown products, were determined in 27 solvent systems. Three columns in combination with electron capture detection were elaborated for the analysis of the DDT-type compounds. Micro amounts of these compounds were then identified by IR, using KBr. TLC was performed on silica gel G with detection achieved using 2% *o*-toluidine in acetone followed by UV irradiation until spots appeared. Table 14 lists the R_F values of 14 DDT-type compounds obtained with 27 solvent systems. These compounds could be classified into two groups according to their separation by TLC, *viz.*, non-polar compounds: *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDD, *o,p'*-DDD, DDE, DDMU, DDMS, DDNU, and DDM; and polar compounds: DDA, DDOH, DBH, kelthane and DBP. The non-polar group can be further subdivided into three subgroups: (1) the unsaturated compounds: DDE, DDMU, and DDNU; (2) *p,p'*-DDT, *o,p'*-DDT and DDM; and (3) *p,p'*-DDD, *o,p'*-DDD and DDMS. Polar compounds also could be separated into three groups: (1) DDA; (2) DDOH and DBH; and (3)

TABLE 13

LIMIT OF DETECTABILITY (μg) OF DDT AND ITS FOURTEEN RELATED COMPOUNDS ON ALUMINIUM OXIDE (TYPE E) F_{254} TLC PLATES DEVELOPED WITH *n*-PENTANE-METHANOL (50:50)

No.	*Compound*	*Concentration (μg)*
1	*p,p'*-DDT	0.05
2	*o,p'*-DDT	0.05
3	*p,p'*-DDE	0.05
4	*o,p'*-DDE	0.05
5	*p,p'*-DDD	0.05
6	*m,p'*-DDD	0.05
7	*o,p'*-DDD	0.05
8	DDA	0.20
9	DDMU	0.50
10	DBP	0.50
11	Kelthane	0.05
12	DPE	0.05
13	DBH	0.10
14	BPE	0.25
15	DDM	0.25

kelthane and DBP. The best system for the separation of polar compounds from each other as well as from non-polar compounds was 50% chloroform–methanol (2:1) in *n*-hexane (final ratio of *n*-hexane–chloroform–methanol (3:2:1)). Good resolution of *p,p'*-DDT, *p,p'*-DDD, DDE, DBP, kelthane, DDOH, and DDA was obtained using a two-dimensional system consisting of 10% diethyl ether in *n*-hexane and *n*-hexane–chloroform–methanol (3:2:1) (Fig. 7). For GLC, a Micro-Tek MT instrument equipped with a ^{3}H electron capture source was used and three U-shaped 6 ft. × $^{1}/_{4}$ in. O.D. glass columns were employed: 5% QF-1, 5% SE-30 + 5% QF-1, and 10% DC-200, all absorbed on 80–100 mesh Chromosorb W. All columns were conditioned at 250° with purified nitrogen at 70 ml/min for 24 h. The operating temperatures were: column, 195°; detector, 200°; and injector block, 230°. Optimal nitrogen flow-rates varied with the column: 70 ml/min for 5% QF-1, 100 ml/min for 5% SE-30 + 5% QF-1 and 120 ml/min for 10% DC-200. The retention times of the DDT-type compounds relative to aldrin are listed in Table 15. No single column separated all 14 compounds. The QF-1 column did not resolve DDM and DDNU and *o,p'*-DDT and DDMS. DDE and DDP had very close retention times (Fig. 8). When the 10% DC-200 column was used, all the pairs of compounds with overlapping peaks on the polar column QF-1 were resolved, *e.g.*, DDM–DDNU, DBP–DDE and *o,p'*-DDT–DDMS. However, DDE and *o,p'*-DDD were not resolved (Fig. 9). All the compounds except DDE and DDMS were resolved using a 5% SE-30 + 5% QF-1 column (Fig. 10). Tables 16–18 list the characteristic IR absorption frequencies (cm^{-1}) of saturated DDT-, unsaturated DDT-, and polar DDT-type compounds, respectively.

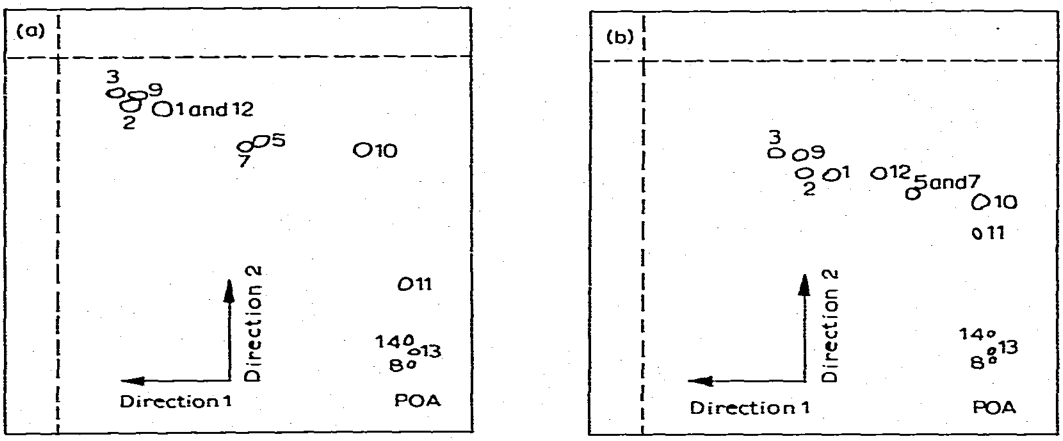


Fig. 6. Two-dimensional thin-layer chromatograms of DDT and eleven related compounds. The plates were developed three times with *n*-hexane in direction 1 and twice with *n*-hexane–diethyl ether–acetic acid (90:10:1) in direction 2 (130 ml per solvent system). The numbers of the spots correspond to Table 13. (a) Aluminum oxide (type E) F_{254} thin-layer plate, the mixture spotted at the point of application (POA) contained 1 μg of each compound; (b) Silca gel F_{254} thin-layer plate, 4 μg of each compound were spotted.

The column partition chromatography of DDT and a number of its homologs

TABLE 14

R_F VALUES OF FOURTEEN DDT-TYPE COMPOUNDS ON SILICA GEL TLC PLATES, USING VARIOUS SOLVENT SYSTEMS

The "eluting solvents" were combined with the specified "background" solvent; 200 ml solvent system was used for development.

"Eluting" solvents	*p,p'-DDT*	*o,p'-DDT*	*DDE*	*p,p'-DDD*	*o,p'-DDD*	*DDMU*	*DDMS*	*DDNU*	*DDM*	*Kel-thane*	*DBP*	*DDA*	*DDOH*	*DBH*
n-Hexane "background" solvent														
Chloroform, 25%	0,40	0,45	0,51	0,31	0,32	0,48	0,31	0,53	0,46	0,16	0,16	—	—	—
Chloroform, 10%	0,30	0,34	0,61	0,17	0,17	0,36	0,17	0,40	0,30	0,03	—	—	—	—
Chloroform–methanol (2:1), 10%	0,75	0,69	0,78	0,66	0,64	0,75	0,78	0,76	0,68	0,34	0,64	0,04	0,13	0,15
Chloroform–methanol (2:1), 50%	0,86	0,86	0,87	0,84	0,84	0,86	0,84	0,86	0,86	0,68	0,80	0,34	0,53	0,55
Carbon disulfide, 10%	0,33	0,36	0,43	0,17	0,17	0,37	0,19	0,43	0,37	—	—	—	—	—
Diethyl ether, 10%	0,41	0,55	0,66	0,36	0,31	0,59	0,39	0,61	0,51	0,16	0,23	—	—	—
Petroleum ether, 10%	0,30	0,36	0,45	0,17	0,15	0,36	0,15	0,42	0,31	—	—	—	—	—
Ethyl acetate, 10%	0,75	0,81	0,88	0,68	0,69	0,86	0,68	0,86	0,82	?	0,68	—	—	0,17
Acetonitrile (std)	0,37	0,43	0,53	0,25	0,23	0,49	0,29	0,55	0,43	0,23	—	—	—	—
Acetone, 5%	0,60	0,59	0,63	0,47	0,45	0,68	0,53	0,69	0,63	0,36	0,48	0,03	0,03	0,15
Acetone, 10%	0,43	0,46	0,59	0,30	0,28	0,52	0,36	0,55	0,47	0,16	0,32	—	—	0,04
Dimethyl sulfoxide (std)	0,34	0,30	0,51	0,16	0,16	0,33	0,15	0,39	0,33	—	—	—	—	—
n-Butanol, 10%	0,63	0,67	0,73	0,61	0,59	0,69	0,59	0,69	0,67	0,55	0,34–0,03 0,53–0,30	—	0,36	0,40
Isopropanol, 10%	0,67	0,65	0,72	0,65	0,61	0,67	0,71	0,71	0,72	0,54	0,32–0,01 0,80–0,30	—	0,34	0,45
Propylene glycol (std)	0,21	0,30	0,36	0,12	0,14	0,32	0,14	0,36	0,30	—	—	—	—	—
2-Methoxyethanol (std)	0,33	0,39	0,45	0,22	0,22	0,44	0,25	0,47	0,41	0,18	—	—	—	—
Ethylene glycol (std)	0,21	0,28	0,35	0,12	0,12	0,29	0,16	0,41	0,32	—	—	—	—	—
Ethanol, 10%	0,73	0,70	0,79	0,66	0,62	0,73	0,69	0,75	0,73	0,44	0,26	0,1–0,26	0,42–0,61	0,32
Methanol, 5%	0,82	0,84	0,84	0,75	0,71	0,84	0,77	0,84	0,80	0,42	0,66	—	—	0,14
Methanol, 10%	0,71	0,71	0,78	0,56	0,55	0,73	0,62	0,78	0,71	0,19	0,21–0,62	—	—	—
Acetic acid, 5%	0,54	0,56	0,65	0,38	0,37	0,58	0,56	0,63	0,55	0,14	0,19	—	—	0,50
Acetic acid, 10%	0,34	0,38	0,46	0,20	0,20	0,42	0,24	0,51	0,59	0,10	0,26	—	—	—
Iso-octane "background" solvent														
Chloroform–methanol (2:1), 50%	0,64	0,64	0,66	0,59	0,57	0,63	0,57	0,66	0,64	0,33	0,19	—	0,17	—
Diethyl ether, 10%	0,38	0,38	0,41	0,31	0,27	0,45	0,34	0,47	0,43	0,20	0,03	—	—	—
Chloroform "background" solvent														
Methanol, 33%	0,86	0,80	0,59	0,86	0,86	0,86	0,86	0,84	0,88	0,84	0,86	0,62	0,79	0,84
Chloroform	0,89	0,90	0,90	0,88	0,91	0,88	0,91	0,88	0,88	0,81	0,74	0,41	0,41	0,50

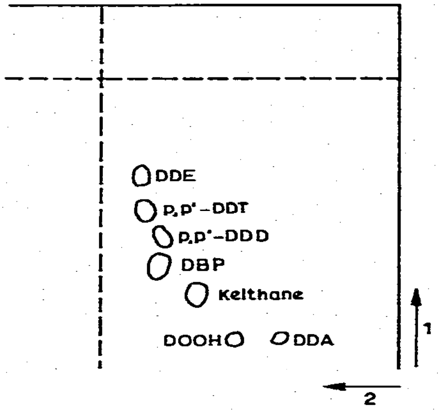


Fig. 7. Two-dimensional systems for separation of seven DDT-type compounds by TLC: Solvent system for direction 1, 10% diethyl ether in *n*-hexane: solvent system for direction 2, 50% chloroform-methanol (2:1) in *n*-hexane.

and analogs, DDE, DDD, DBP, kelthane and FW-152 (4,4'-dichloro-α-dichloromethylbenzhydrol) was achieved by Gillett *et al.*[149] using 2,3,4-trimethyl pentane (mobile phase) and 3-methoxy propionitrile (stationary phase) on cellulose powder. Measurement of the absorbancies of the effluent fractions at 235 and 268 nm served as the primary quantitative and qualitative assay.

Separation of DDT and related compounds has been achieved on a silicic acid

TABLE 15

RELATIVE RETENTION TIMES OF DDT-TYPE COMPOUNDS BY ELECTRON CAPTURE GAS CHROMATOGRAPHY

Compounds	*Relative retention time*		
	*5% QF-1, 70 ml/min**	*5% SE-30 + 5% QF-1, 100 ml/min***	*10% DC-200, 120 ml/min****
p,p'-DDT	4.48	3.65	3.24
o,p'-DDT	2.96	2.92	2.57
DDE	2.30	2.00	1.89
p,p'-DDD	4.09	2.70	2.43
o,p'-DDD	2.78	2.18	1.89
DDMU	1.83	1.58	1.45
DDMS	2.96	2.00	1.66
DDNU	0.91	0.81	0.78
DDM	0.91	0.70	0.62
DBP	2.26	1.28	0.99
Kelthane	2.26	1.28	0.99
DDOH	3.43	2.44	1.25
DBH	2.26	1.28	0.99

* Aldrin reference retention time, 3.7 min.
** Aldrin reference retention time, 12.8 min.
*** Aldrin reference retention time, 12.2 min.

TABLE 16

THE CHARACTERISTIC INFRARED ABSORPTION FREQUENCIES (cm^{-1}) OF SATURATED DDT-TYPE COMPOUNDS

Abbreviations: st. = stretching; sk. = skeletal; i.p.d. = in-phase deformation; o.p.d. = out-of-phase deformation; def. = deformation; vib. = vibration. Absorbance designations: w = weak; m = medium; s = strong; vs = very strong.

Compound	*Aromatic*		*C_6H_5–H* *–CH o.p.d.*	*CH_2*			*CH def.*	*(Aryl)$_2$–CR_1R_2 sk. st.*	*C–Cl st. vib.*
	–CH st.	*C–C sk. st.*		*i.p.d.*	*o.p.d.*	*def.*			
p,p'-DDT	3030 (w) 1500 (vs)	1600 (s)	810 (m)				1370 (w)	1188 (m) 1250 (m)	740 (vs) 755 (vs) 785 (vs)
o,p'-DDT	3030 (w)	1600 (w) 1500 (m)	755 (s) 825 (w)				1370 (vs)	1255 (vs)	835 (m)
p,p'-DDD	3030 (m)	1600 (s) 1500 (vs)	810 (vs)				1323 (w)	1188 (m) 1222 (m) 1250 (m)	780 (vs) 715 (vs)
o,p'-DDD	3030 (m)	1600 (m) 1500 (vs)	750 (s) 810 (s)				1323 (m)	1190 (w) 1212 (w) 1222 (m) 1248 (w)	785 (vs)
DDMS	3030 (w)	1600 (m) 1500 (vs)	815 (vs)	2970 (w)	3060 (w)	1465 (vs)		1185 (m) 1230 (m)	750 (vs) 785 (s)
DDM	3030 (w)	1600 (m) 1500 (vs)	815 (vs)	2910 (w)	2960 (w)	1458 (m)		1185 (w) 1210 (w) 1240 (w)	785 (vs)

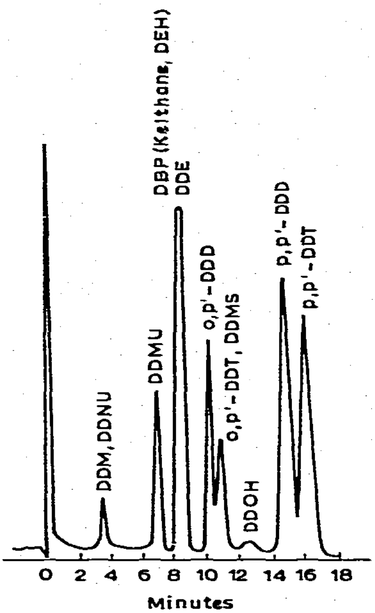
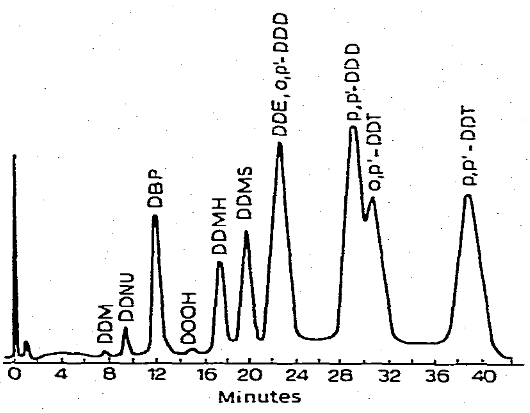


Fig. 8. Electron capture GLC on DDT-type compounds, using a 5% QF-1 on Chromosorb W and nitrogen flow-rate of 70 ml/min.



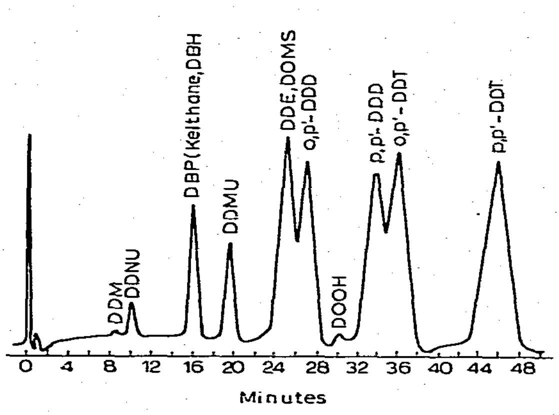


Fig. 9. Electron capture GLC of DDT-type compounds, using 10% DC-200 on Chromosorb W and nitrogen flow-rate of 120 ml/min.

Fig. 10. Electron capture GLC of DDT-type compounds, using 5% SE-30 + 5% QF-1 on Chromosorb W and nitrogen flow-rate of 100 ml/min.

column using gradient elution[150]. Table 19 lists the column conditions for the separation and purification of some DDT-type compounds and metabolites. Fig. 11 illustrates a chromatogram and conditions for the elution of a mixture of DDE, DDT, DDD, kelthane, DBP, and DDA. Although kelthane and DBP were not completely separated, they could be resolved by continuing the benzene gradient instead of using pure benzene elution. The above separations were achieved using a water jacketed

TABLE 17

THE CHARACTERISTIC INFRARED ABSORPTION FREQUENCIES (cm^{-1}) OF UNSATURATED DDT-TYPE COMPOUNDS

For abbreviations see Table 16.

Compound	*Aromatic*		*C_6H_5–H* *o.p.d.*	*C=C* *st.*	*=CH*		*=CH_2*	*C–Cl* *st. vib.*
	C=C sk. st.				*o.p.d.*	*i.p.d.*		
DDE	1585 (vs)	1500 (vs)	828 (vs)	1650 (w)				795 (vs) 855 (s)
DDMU	1600 (m)	1500 (vs)	810 (vs)	1615 (m)	812 (vs)	1375 (m)		755 (vs) 835 (s)
DDNU	1600 (m)	1500 (vs)	845 (vs)	1625 (w)			905 (vs) 1015 (vs)	760 (m) 840 (vs)

column 22 cm × 1.85 cm I.D. packed with silicic acid (Mallinckrodt, 100 mesh A.R.).

Armour and Burke[151] described the separation of PCB5 from DDT and its analogs and other chlorinated pesticides by column chromatography on silicic acid–Celite. PCBs were eluted from the column with petroleum ether prior to elution of pesticides with a mixture of acetonitrile, hexane and methylene chloride. Determinations of PCB and pesticides could be made on the separate column eluates without cross-interference. Recoveries of Aroclors 1254 and 1260 and of several chlorinated pesticides through the separation method ranged from 76–100% and 80–107%, respectively. The method was suggested to be applicable to samples prepared by multipesticide residue methodology and should be applicable to sample extracts prepared for GLC by usual procedures.

GLC utilized an electron capture detector and 6 ft. × 4 mm I.D. glass columns of either (1) 10% DC-200 on 80–100 mesh Chromosorb W, HP, or (2) 1:1 mixture of 15% QF-1 plus 10% DC-200 on 80–100 mesh Chromosorb W, HP. The operating conditions were: nitrogen flow-rate, 120 ml/min; column and detector temperature, 200°; injection temperature, 225°. The concentric design electron capture detector was operated at dc voltage to produce $^1/_2$ full-scale recorder deflection for 1 ng heptachlor epoxide when full-scale deflection was 1×10^{-9} amp.

The effect of water content of silicic acid on elution and separation of Aroclor 1260 and DDT analogs is shown in Fig. 12. Silicic acid was heated at 130° for several days before equilibrating with 0.10% (w/w) water. A mixture of Aroclor 1260, *o,p'*- and *p,p'*-DDT, *p,p'*-DDE, and *p,p'*-TDE was eluted from columns of silicic acid of various moisture contents using petroleum ether as the eluent. The maximum margin of separation of PCB from pesticides with good reproducibility of elution pattern was obtained with silicic acid containing 3% water. A more polar eluant was required for complete elution of *p,p'*-DDT and *p,p'*-TDE when the water content was 5% or less. Aroclor 1260 was completely eluted with 200-ml of petroleum ether; 250 ml of eluant was required for complete recovery of Aroclor 1254. When more than 5% water was added, activity was decreased to the extent that retentiveness was lost. An adsorbent column of 20 g of silicic acid (containing 3% added water) plus 5 g of Celite was determined to be the best for separating PCB from DDT and analogs. The separation was accomplished by eluting PCB with 250 ml of petroleum ether followed by elution of DDT and analogs with 200 ml of acetonitrile–hexane–methylene chloride

TABLE 18

THE CHARACTERISTIC INFRARED ABSORPTION FREQUENCIES (cm^{-1}) OF POLAR DDT-TYPE COMPOUNDS

For abbreviations see Table 16.

Compound	*Aromatic*		*–H*	CH_2	C_6H_5–*OH*	*O–H*	*C–O*	*CH*	*(Aryl)*$_2$–	*C=O*	*C–Cl*
	C=C sk.	*st.*	*o.p.d.*	*st. def.*	*st.*	*def.*	*st.*	*def.*	CR_1R_2		*st. vib.*
DDOH	1650 (w)	1500 (m)	820 (s)	3010 (w) 1410 (m)	3470 (s)	1015 (s)	1222 (s)	1370 (s)	1230 (s)		780 (m)
DBH	1600 (vs)	1500 (vs)	810 (vs)		3400 (s)	1095 (s)	1200 (s)	1328 (m)	1248 (m)		765 (vs)
Kelthane	1600 (s)	1500 (vs)	835 (vs)		3600 (s)	1160 (s)	1405 (s)		1170 (s)		775 (vs)
DBP	1600 (vs)	1495 (s)	833 (vs)							1670 (s)	758 (vs)
DDA	1600 (m)	1500 (vs)	810 (vs)		3000 (s) 2600 (s)	947	1415	1328 (s)	1170 (s) 1175 (s) 1228 (vs) 1252 (w)	1735 (vs)	755 (vs)

TABLE 19

COLUMN CONDITIONS FOR THE SEPARATION AND PURIFICATION OF SOME DDT-TYPE COMPOUNDS AND METABOLITES

Water added to silicic acid (%)	*Solvent system*	*Compound or metabolite chromatographed*
Adsorption		
3, 5, 10	eluted *n*-hexane	DDE, *o,p'*-DDT, *p,p'*-DDT
0, 2, 3, 5, 10	benzene gradient on *n*-hexane	DBP, phenol*, ketone*
3, 10	carbon tetrachloride gradient on *n*-hexane	DDE, *o,p'*-DDT, *p,p'*-DDT
3	benzene gradient on carbon tetrachloride–*n*-hexane mixture	kelthane, ketone*, DBP, DDD
5, 10	diethyl ether gradient on *n*-hexane	DBP, DDA, phenol*, carboxylic acid*
Partition and mixed		
5	acetonitrile stationary phase 50% v/w of silicic acid, eluted *n*-hexane	kelthane, DBP
10	acetonitrile stationary phase 65% v/w of silicic acid, eluted *n*-hexane	phenol*
5	acetonitrile stationary phase 50% v/w of silicic acid, eluted benzene gradient on *n*-hexane	kelthane, phenol*
3, 5	acetonitrile stationary phase 50% v/w of silicic acid, eluted acetonitrile gradient on benzene, starting with 2% acetonitrile in benzene	kelthane, ketone*
0	dioxane stationary phase 50% v/w of silicic acid, eluted benzene gradient on *n*-hexane	DBP, phenol*

* Unidentified metabolite of DDT with this group indicated.

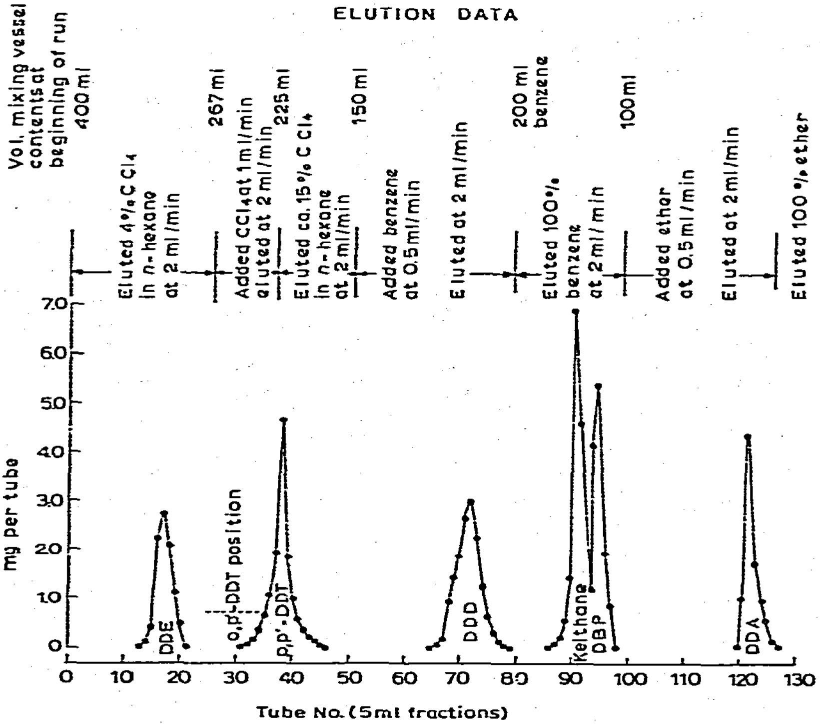


Fig. 11. Chromatogram and conditions for the elution of a mixture of DDE, DDT, DDD, kelthane, DBP and DDA.

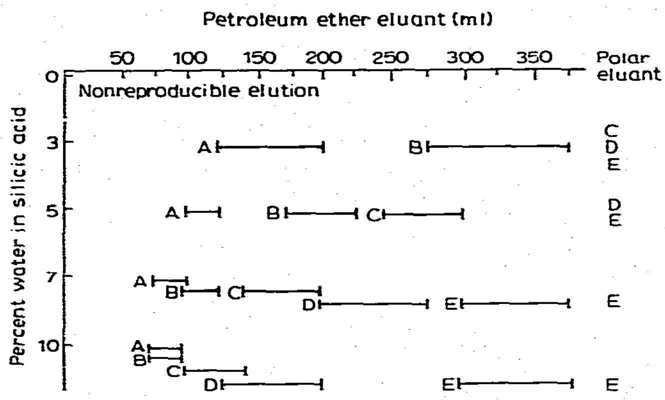


Fig. 12. Effect of silicic acid water content on elution and separation of Aroclor 1260 and DDT and analogs. 25-ml fractions of petroleum ether eluate analyzed by GLC; respective compound found present in volumes crossed by horizontal line. Various polar eluants used to elute pesticides C, D, and E. A = Aroclor 1260; B = *p,p'*-DDE; C = *o,p'*-DDT; D = *p,p'*-DDT; E = *p,p'*-TDE.

(1:19:80). DDT and analogs were eluted by the first 100–125 ml of the second eluant. In addition to *o,p'*- and *p,p'*-DDT, *p,p'*-DDE and *p,p'*-TDE, complete recovery of lindane, heptachlor, heptachlor epoxide, dieldrin and endrin was obtained by the second elution. Aldrin was eluted with PCB, and with the exception of dieldrin and endrin, all these pesticides and PCB were found in the 6% diethyl ether–petroleum ether–Florisil eluate. Recoveries of Aroclors 1254 and 1260 and pesticides are shown in Table 20. Aroclors 1221, 1232, 1262, 5442, and 5460 were also completely separated

TABLE 20

RECOVERIES AND SEPARATION OF AROCLORS AND CHLORINATED PESTICIDES ADDED TO A SILICIC ACID COLUMN

Chemical	*Amount added (μg)*	*Average found (%)*	*Number of trials*	*Range (%)*
*Eluate 1**				
Aroclor 1254	40	95–100**	10	95–100**
Aroclor 1260	20–40	95–100**	18	95–100**
Aldrin	1	101	4	100–103
*Eluate 2****				
Lindane	0.5	96	4	94–100
Heptachlor	1	93	4	88–98
Heptachlor epoxide	1	100	4	96–104
Dieldrin	1	104	2	102–106
Endrin	1	94	4	93–96
p,p'-DDE	5	99	18	80–105
o,p'-DDT	10	98	14	91–103
p,p'-DDT	10	98	18	90–106
p,p'-TDE	5	97	18	87–107

* Eluant: 250 ml petroleum ether.

** Recoveries estimated; no Aroclor present in second eluate.

*** Eluant: 200 ml acetonitrile–hexane–methylene chloride (1:19:80).

from pesticides by the petroleum ether elution. The multicomponent pesticides, chlordane and toxaphene were eluted by the polar eluant and did not interfere with PCB.

The analysis of residues and metabolites of DDT in the presence of PCBs is complicated by the difficulty of separating the two groups of compounds. Reynolds[152] and Armour and Burke[151] utilized liquid–solid fractionation for the separation of the DDT compounds prior to analysis by GLC.

The oxidation of DDE and DDMU to dichlorobenzophenone has also been utilized to effect a better separation of the DDT compounds from the PCB components by liquid–solid fractionation.

The oxidation of DDE to dichlorobenzophenone has been used by Mulhern *et al.*[153] to eliminate the interference of DDE in the TLC estimation of PCB. The oxidation of DDE to dichlorobenzophenone was first described by Haller *et al.*[3] and this reaction was subsequently used by Wichmann *et al.*[154] for the colorimetric analysis of DDT and DDE. (The parent DDT was determined by dechlorination to DDE followed by oxidation.)

Miles[155] effected the separation of DDT compounds from interfering PCBs by oxidation of DDE and DDMU to the more polar dichlorobenzophenone. DDT and *o,p'*- and *p,p'*-DDD were dehydrochlorinated utilizing 1,5-diazobicyclo[5.4.0]undec-5-ene (Aldrich Chem.) (5% in benzene) and the resulting olefins were oxidized by chromic acid in acetic acid. In subsequent Florisil fractionation, the interfering PCB compounds, which were relatively unchanged by the dehydrochlorination and oxidation, were eluted in the hexane fraction, while the dichlorobenzophenones were eluted by benzene. GLC determined recoveries of *p,p'*-DDE and *p,p'*-DDT added to fish extracts at 5 ppm each were 82.5 ± 2.1% and 86.0 ± 2.2%, respectively. The procedure has been successfully used to determine DDE, DDD and *o,p'*- and *p,p'*-DDT in the presence of large amounts of PCB in samples of bottom mud and fish in streams receiving effluent from urban areas. The procedure has also been used to measure DDE and DDT as low as 0.01 ppm each in dams. Aroclors 1254 and 1260 were examined by GLC to determine if they produced peaks which might interfere with the analysis of *o,p'*- and *p,p'*-dichlorobenzophenones by the above procedure. No shift in peak retention times was noted when these two Aroclors were analyzed by GLC before and after dehydrochlorination and oxidation.

The facile conversion of *p,p'*-DDT and *p,p'*-DDD to predominantly DCS (*trans-p,p'*-dichlorostilbene) with DDNU (1,1-bis(*p*-chlorophenyl)ethylene), DDMU (1,1-bis(*p*-chlorophenyl)-2-chloroethylene) and DBP (*p,p'*-dichlorobenzophenone) as secondary products under acidic conditions (pH 3.7) using chromous chloride as the reducing agent has been described[156].

The above products were analyzed by GLC utilizing an Aerograph Hy-Fi Model 600D with a ^3H electron capture detector with operating parameters as those previously reported by Chau and Cochrane[157].

Fig. 13 illustrates a chromatogram of *p,p'*-DDT after $CrCl_2$ reaction and Table 21 gives the relative retention times of *p,p'*-DDT and its reaction products by electron capture GLC. It is of interest to note that DCS has been isolated and identified as a constituent of mainstream cigarette smoke which was obtained from tobacco products containing *p,p'*-DDT or *p,p'*-DDD[158].

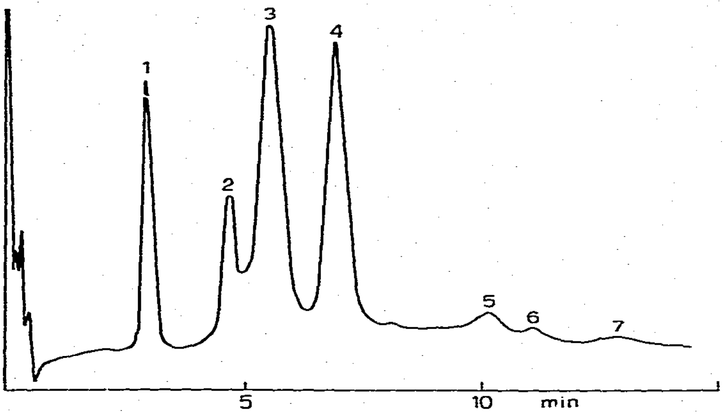


Fig. 13. Chromatogram of *p,p'*-DDT after $CrCl_2$ reaction. For explanation of peak numbers see Table 21.

TABLE 21

RELATIVE RETENTION TIMES OF *p,p'*-DDT AND ITS REACTION PRODUCTS BY ELECTRON CAPTURE GAS CHROMATOGRAPHY

The peak numbers of the reaction products refer to Fig. 13.

Reaction product	*Retention time (Aldrin = 1)*	*m.p. of isolated products (°C)*	*Assignments*
Peak 1	0.82	88–89	DDNU
Peak 2	1.29	149–150	DBP
Peak 3	1.51	67–68	DDMU
Peak 4	1.92	177–178	DDC
Peak 5	2.80	—	*p,p'*-DDD*
Peak 6	3.02	—	*o,p'*-DDT*
Peak 7	3.50	—	*p,p'*-DDT*

* Characterized by comparative TLC and GLC retention times with authentic samples.

5. ANALYSES OF METABOLIC, FOOD, AND TOBACCO SAMPLES

The chemical modification and excretion of DDT in animals[159] and man[160] is well established. Initial degradation of DDT may proceed either by dehydrochlorination, yielding DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene or by substitution of hydrogen for one chlorine atom, yielding DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane[142,161,162]. DDD readily degrades further through a series of intermediates to the excretable DDA (bis(*p*-chlorophenyl acetic) acid and is rarely found as a stored metabolite in the general population. DDE, however, does not undergo further breakdown to DDA and this stability, in the main, accounts for the higher human tissue store of DDE than of DDT in the general population.

Aspects of the absorption, storage, and metabolic conversion of ingested DDT and DDT metabolites in man were described by Morgan and Roan[163]. Human subjects ingested daily 5- to 20-mg doses of technical DDT, *p,p'*-DDE, or *p,p'*-DDD for 81–183 days. The serum and adipose concentrations of DDT and DDT metabolites in response to these drugs indicated that the initial dechlorination of DDT was of critical importance to its metabolic fate. Conversion to the saturated *p,p'*-DDD permitted further degradation to the readily excreted *p,p'*-DDA. In contrast, dehydrochlorination yielded *p,p'*-DDE, a stable metabolite that was widely retained in adipose storage. Tissue stores of *p,p'*-DDE in the general population is suggested to originate mainly from preformed dietary *p,p'*-DDE in the diet, rather than from *p,p'*-DDT. Of the DDT related materials studied, propensity for human adipose tissue storage increased in the order *p,p'*-DDD $\leq$ *o,p'*-DDT $<$ *p,p'*-DDT $<$ *p,p'*-DDE. The same relationship of the chemicals existed with respect to stability of the adipose store, once dosage was terminated. Electron capture GLC analysis for pesticide was carried out using 6 ft. × $^1/_4$ in. I.D. columns containing 1.5% OV-17 and 2% QF-1 coated on 100–120 mesh HMDS treated support. The operating conditions were: inlet temperature, 230°; detector temperature, 210°; column temperature, 205°; nitrogen carrier gas flow-rate 20 ml/min; and polarizing voltage, 10–40 V, d.c.

Epidemiologic surveys have shown that the presence of *p,p'*-DDE in serum is indicative of chronic DDT exposure while *p,p'*-DDT levels are measures of recent exposure[164,165].

Aspects of the transport of DDT, DDE and dieldrin in human blood have been recently described by Morgan *et al.*[166]. Blood specimens were studied from workers long exposed to chlorinated hydrocarbon pesticides, and also subjects who had ingested measured quantities of technical DDT about two years prior to these tests[167]. Blood samples were collected in heparinized tubes, erythrocytes separated by centrifugation were washed three times in saline: samples of packed red cells were extracted by a 1:1:1 mixture of ethanol, diethyl ether and hexane and the extract then cleaned-up by the Mills procedure[167]. These extracts, as well as hexane extracts of plasma were then analyzed by electron capture GLC for *p,p'*-DDT, *p,p'*-DDE and dieldrin. Electrophoretic separation of the proteins contained in high-level DDT serum (DDT = 145 ppb, DDE = 110 ppb) was accomplished with the Beckman Continuous-flow paper electrophoresis apparatus employing veronal buffer at pH 8.6, 93 mA current and a heavy-duty cooling system[168]. Clean separation of proteins in approx. 3.6 ml of serum was accomplished in approx. 18 h. It was found that less than 18% of the *p,p'*-DDT and *p,p'*-DDE found in human blood was carried in the erythrocytes while dieldrin was distributed between red blood cells and plasma roughly in proportion to volume. In non-lactescent sera, chylomicra carried less than 1% of the total pesticide; protein-free serum was virtually devoid of pesticide. *p,p'*-DDT and *p,p'*-DDE were found in relation to lipoproteins of various densities, but principally in the triglyceride-rich low-density and very-low-density lipoproteins. The plasma albumin and secondarily the smaller globulins are the principal plasma protein constituents associated with blood-borne *p,p'*-DDT and *p,p'*-DDE.

Dyment *et al.*[169] investigated the relationship between levels of chlorinated insecticides in human milk and serum. Simultaneous chlorinated analyses were performed on milk and serum from 28 lactating women in Texas. No correlation could be established between milk and serum levels of *p,p'*-DDE, *p,p'*-DDT, dieldrin or BHC and it was concluded that breast milk insecticide levels do not have the epidemiologic significance that the serum levels suggest. Blood serum and milk were initially extracted with hexane: the blood extract was then concentrated and analyzed by GLC whereas the milk extract required Florisil clean-up prior to concentration and GLC analysis. A Micro-Tek 220 gas chromatograph equipped with a ^{3}H foil detector was used with 1.5% OV-17 and 1.95% QF-1 on 100–200 mesh Chromosorb W, and 4% SE-30 and 6% QF-1 on 80–100 mesh Chromosorb W; operating temperatures were: column, 200°, inlet, 225°; detector, 205°.

Problems of prevalence of pesticide residues in humans have been elaborated by Davies *et al.*[170]. Two developments which have recently occurred which may profoundly affect the magnitude of sampling surveys are: (1) a description of significant demographic differences within respective populations[14,171,172], thus emphasizing the importance not only of size but also stratification of the community; and (2) studies presenting comparisons of human pesticide residue levels in health and disease, also emphasizing the requirements of precision data or prevalence levels in the healthy population using DDT and DDE as a model[173]. Data were presented by Davies *et al.*[170] from 159 autopsy fat specimens from persons accidentally or violently killed in Dade County, Florida. Significant differences in levels of DDT-derived materials were observed to be associated with race and other demographic variables, and data obtained by necropsy adipose sampling were compared with whole blood studies on a living general population group.

Gas chromatography using the Radomski method[174] was employed to measure residues of chlorinated hydrocarbon pesticides in tissue. These were carried out using a Micro-Tek 220 gas chromatograph with ^{3}H or ^{63}Ni electron capture detectors and 6 ft. × 1/4 in. glass columns packed with high-performance acid-washed DCMS-treated Chromosorb G coated with (1) a mixture of 1.6% OV-1 and 2.75% QF-1 and (2) 2.75% QF-1. The operating conditions were: inlet temperature, 220°; detector temperature, 200° for ^{3}H and 270° for ^{63}Ni; transfer line temperature, 225°; nitrogen input pressure: 70 p.s.i.g.; flow-rate, 110 ml/min for OV-1 + QF-1 and 90 ml/min for QF-1.

Whole blood was analyzed for chlorinated hydrocarbon pesticide residues by a modification of the method of Dale *et al.*[175]. This modification[176] gave results for DDT and DDE in whole blood close comparable (coefficient of correlation, $r = 0.94$ for DDT and DDE, $p < 0.001$) to those given by Dale's original method and was preferred in the interest of rapid testing. After extraction of whole blood with hexane, 5 μl of the extract was analyzed by GLC using the maximum practical sensitivity of the detector. A Micro-Tek 220 with ^{63}Ni electron capture detector in the d.c. mode was used with the following operating parameters: inlet temperature, 220°; column temperature, 185°; detector temperature, 250°; and transfer line temperature, 240°; operating voltage, 35–40 V; nitrogen input pressure, 40 p.s.i.g.; flow-rate, 90 ml/min for QF-1 and 100 ml/min for Dow-200 + OV-1. Two columns were routinely used for confirmation of peaks: (1) 1.6% OV-1 and 2.75% QF-1 on DMCS-treated 80–100 mesh Chromosorb W and (2) 2.75% QF-1 on 60–80 mesh Chromport XXX (Dow). Recovery studies using this modified method[177] have been done at levels of 10 ppb of DDT and 20 ppb of DDE with recovery of 90 and 110% of DDT and DDE, respectively; reproducibility was obtained within 1 ppb on repeated testing at the levels of < 2 and 20 ppb of DDT and 9 and 20 ppb of DDE.

The determination of chlorinated hydrocarbon pesticides in human blood is increasingly used as a method for the estimation of exposure to pesticides[178–183].

In the frequently used procedure of Dale *et al.*[175] blood serum is separated from the clot by centrifugation at 2000 rpm for 10 min, followed by extraction of the serum with 5.0 ml of hexane. A 1.6-ml sample of the hexane extract is concentrated and analyzed by electron capture GLC. Bonderman *et al.*[181] described a statistical examination of the laboratory parameters involved in the recovery of DDE human blood serum by the above method of Dale *et al.*[175]. The age of serum, amount of hexane used for extraction, duration of mixing time and temperature were intercorrelated and all had a statistically significant effect upon the results of the analytical quantitation performed by GLC. The optimum conditions established from this study for the analysis of *p,p'*-DDE residues were: aging time, immediately; hexane volume, 2.0 ml; mixing time, 4 min; and water bath temperature, 40°.

A Micro-Tek MT-220 gas chromatograph with a 130 μCi tritium electron capture detector and a 6 ft. × 1/4 in. O.D. glass U-tube containing 6% QF-1 + 4% SE-30 on 60–90 mesh Anakrom ABS was employed for all analyses. A 3% stabilized DEGS on 80–100 mesh Gas Chrom P column was used for confirmation. The number of theoretical plates calculated for *p,p'*-DDE was 2500. The operating parameters were: oven temperature, 190°; detector temperature, 200° and flow-rate of nitrogen carrier gas, 60 ml/min. The retention time for *p,p'*-DDE under these conditions was 12 min.

A quantitative method for the extraction and determination of DDT and its metabolites in serum was described by Dale *et al.*[182]. When rats were given a single oral dose of ^{14}C-labeled DDT, it was demonstrated by parallel GLC and radiometric measurements that *in vivo* introduced DDT and related materials could not be removed quantitatively from serum by simple hexane extraction. Pretreatment of the serum with equal volumes of formic acid released the binding of DDT and its metabolites permitting their extraction with *n*-hexane resulting in 95% recoveries. When applied to sera of men from the general population and of men who had been exposed to DDT for more than 10 years in a manufacturing plant, this method gave consistently higher results than the simple hexane extraction method. A Varian Aerograph Model 200 chromatograph was equipped with an 8-mCi ^{63}Ni source electron capture detector and a 4.8 ft. × 1/4 in O.D. aluminum tube packed with a mixture of equal parts of 3% OV-17 on 100–120 mesh and 5% OV-210 on 100–120 mesh acid-washed DMCS-treated Chromosorb W. The operating conditions were: detector temperature, 265°, inlet and outlet block temperatures, 240°; and column oven temperature, 205°; nitrogen carrier gas flow-rate, 35 ml/min at 70 p.s.i.; and standing current 1×10^{-9} A. A typical chromatogram of a serum extract (obtained from the formic acid–hexane method) from an occupationally exposed individual and a chromatogram of DDT and related compounds are shown in Fig. 14. Peaks corresponding to *p,p'*-DDE, *o,p'*-DDE and *p,p'*-DDT were found in all samples analyzed. Weak peaks corresponding to *o,p'*-DDE and *p,p'*-DDD were observed occasionally in occupationally exposed individuals while no peak corresponding to *o,p'*-DDD was observed in any of the samples.

Cranmer *et al.*[183] described the determination of DDT and metabolites (including DDA) in human urine by GLC. The procedure included joint electron capture–gas chromatographic determination of DDT, DDE, DDD, and DDA-methyl ester, as well as other extractable chlorinated hydrocarbons such as lindane, dieldrin, and dichloran. Detection of these compounds was possible at levels as low as 1.0 ppb by the combination of the Coulson conductivity and parallel plate electron capture detectors. Identification was facilitated by comparison of the relative responses of

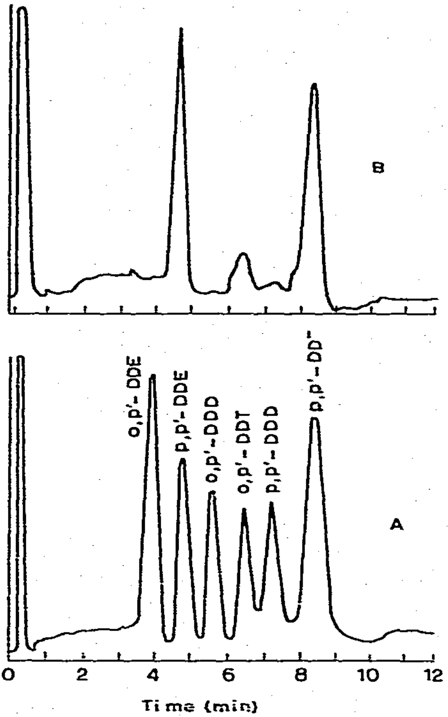


Fig. 14. Gas chromatograms of standard DDT-type compounds (A) and of a serum extract from a person occupationally exposed to DDT (B).

different detection systems and relative retention times on columns with different retention characteristics. Urine samples were also cleaned up following extraction with 2% acetic acid in hexane and methylation by passage over a micro Florisil column according to the procedure of Enos *et al.*[184]. A Micro-Tek Model MT-220 Gas Chromatograph was equipped with the above detectors. A Dohrmann microcoulometer detection could be used in place of the Coulson conductivity system. These systems are roughly equal in their sensitivity to chlorinated hydrocarbons. The most useful column type was a 6 ft. × 1/4 in. O.D. Pyrex glass U-tube packed with 5% QF-1 on 80–100 mesh Gas Chrom Q. The oven temperature was 170–175° and the nitrogen carrier gas flow-rate was 80–100 ml/min. Other successful column packings were 5% QF-1 + 7.5% DC-200 and 3% SE-30 on 80–100 mesh Gas Chrom Q. Fig. 15 illustrates an electron capture chromatogram of a general population urine extract and Fig. 16 is a Coulson conductivity chromatogram obtained by concentrating the extract shown in Fig. 15. Fig. 17 is an example of an electron capture chromatogram of fraction 2 from the micro Florisil clean-up of the extract shown in Fig. 16. The simplest most straightforward method of analysis was found to be concentration of the urine extract to an appropriate volume (*ca.* 0.20 ml) with subsequent conductivity or microcoulometric detection (Fig. 16). However, if electron capture detection is to be used, clean-up by a micro-Florisil column is necessary (Fig. 17).

Datta[185] investigated the *in vivo* detoxification of *p,p'*-DDT via *p,p'*-DDE to *p,p'*-DDA in rats. DDE pretreated animals were used in addition to controls and the

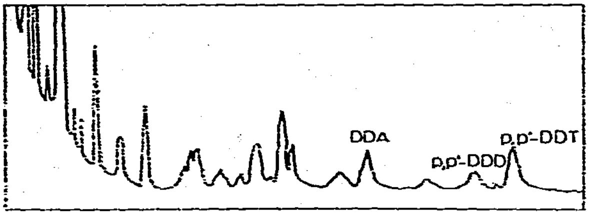


Fig. 15. Example of poor electron capture chromatogram of a general population urine extract.

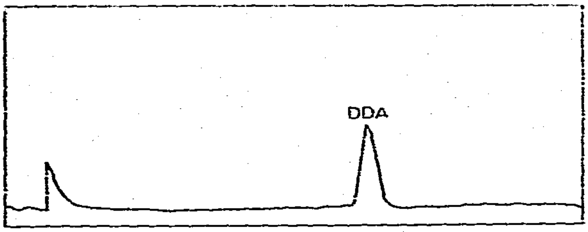


Fig. 16. Example of coulson conductivity chromatogram obtained by concentrating extract shown in Fig. 15.

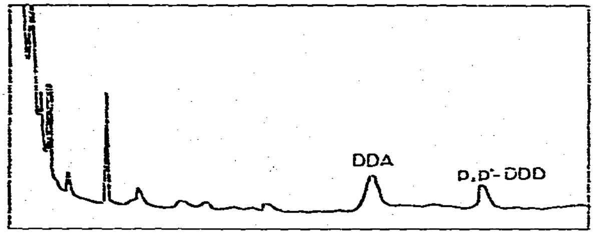


Fig. 17. Example of an electron capture chromatogram of fraction 2 from the micro Florisil clean-up of the extract shown in Fig. 16.

results indicated that two different pathways exist and may be operated simultaneously in intact animals. The predominance of one or the other may depend on physiologic response or the amount of toxicant used. DDE, DDOH, and DDA (from tissue extracts) were identified by comparison with authentic reference compounds by both paper chromatography and micro-infrared spectroscopy. The developing solvent systems used were: (1) immobile phase, 8% 2-phenoxyethanol in ether and mobile phase, 2,2,4-trimethylpentane; and (2) immobile phase, 7% olive oil in acetone and mobile phase, 2% conc. NH_4OH in absolute ethanol. The complete pathway of detoxification of DDT via DDE to DDA was suggested by Datta in this study to be as indicated in Fig. 18. This pathway indicates that DDE detoxification, or enzymatic degradation to DDNU; occurs by a two-step process, apparently an aliphatic chlorine is replaced by hydrogen in an oxidation–reduction process similar to that reported by Castro[155] for the conversion of DDT to DDD by porphyria complexes. Such a detoxification mechanism may be attributed to hematin-type enzymes. The scheme reported by Peterson and Robison[161] for the conversion of DDT via DDD to DDA in rats indicates that DDMS is an intermediate compound between DDMU and DDNU in that pathway. Since DDMS was absent in the DDT via DDE to DDA pathway reported in

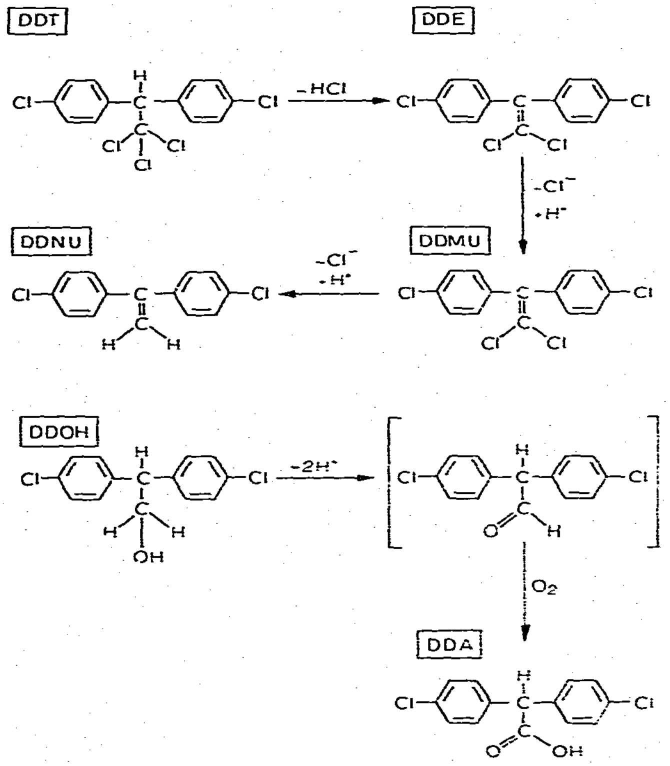


Fig. 18. Metabolic sequence for the conversion of *p,p'*-DDT via *p,p'*-DDE to *p,p'*-DDA in the rat.

the study of Datta[185], it appears that DDT, after a one-step conversion to either DDE or DDD, detoxifies by two different pathways in rats.

Wright *et al.*[187] described studies of residues in chickens fed DDT. Adult chickens were dosed with 10 mg of *p,p'*-DDT per kg of body weight for 90 days and then sacrificed. The residues of *p,p'*-DDT and related compounds were higher in the tissues of treated adult males than in tissues of treated hens. The highest residue levels were found in the fats and preen gland, and the lowest levels were found in the breast muscle and brain. The procedure used to extract the tissues for DDT and metabolites was a modified procedure of Radomski and Fiserova-Bergerova[177]. In general, 0.250 g of tissue was ground in a Thomas tissue grinder with redistilled petroleum ether. The extract was dried with sodium sulfate, concentrated, brought to volume with hexane and 2-μl portions were analyzed using a Micro-Tek 220 gas chromatograph equipped with a ^{63}Ni electron capture detector and a 6 ft. × $^1/_4$ in. stainless-steel column packed with 5% QF-1 coated on 80–100 mesh Chromosorb W.

A rapid method for the determination of *p,p'*-DDT to *p,p'*-DDE ratios in fish was described by Fort[188]. Tissue samples ranging from 0.005–0.1 g were digested in 2–4 ml of formic acid and maintained at 60° for 1.5–2 h followed by extraction four times with hexane. The combined extracts were evaporated to dryness and the residue was washed with three 2-ml portions of acetonitrile. A small amount of alumina (20 mg, 80–200 mesh) was added and the sample centrifuged. After evaporating the supernatant to dryness, the residue was dissolved in a small amount of ethylacetate and spotted on a plate coated with aluminium oxide using the procedure of Kovacs[189]. This above procedure of Fort[188] was suggested to be a more rapid method of analyzing for the presence of DDT in fish than the more commonly employed method of Mills *et al.*[190].

A method for the analysis of fish, animal and poultry tissue for chlorinated pesticide residues was described by Porter *et al.*[191]. Residues of the above tissues were extracted by blending with petroleum ether and then employing the usual petroleum ether–acetonitrile partitioning and Florisil column chromatographic procedures for clean-up. Levels of metabolically incorporated chlorinated pesticide residues extracted from fish, meat and poultry tissue by the petroleum ether method were equivalent to residue quantities extracted by Soxhlet with chloroform–methanol (1:1). Recoveries of representative chlorinated pesticides added to samples of various tissues at levels of 0.1–2.0 ppm ranged from 90–102%. Gas chromatography with electron capture detection according to the Official Methods of Analysis[192] (sec. 29.001) was used for all determinations.

Mestres *et al.*[193] described the analysis of DDT, aldrin, and dieldrin residues in milk and butter. Samples of milk and butter were hydrolyzed with 2 *N* ethanolic KOH and 5% aq. sodium oxalate, prior to extraction with petroleum ether. The extract was then purified on a Florisil column in a manner that resulted in DDE and aldrin being selectively eluted by petroleum alone (b.p. 45–60°); dieldrin was eluted later with a mixture of diethyl ether–petroleum ether. The concentrated purified extracts were analyzed by electron capture of microcoulometric gas chromatography using both DC-200 and QF-1 columns. The sensitivity of the method was limited for routine determinations at 0.01 ppm for aldrin and 0.02 ppm for DDT and dieldrin in milk and quantities ten times higher in butter. An IR method was used to confirm the presence of residues of DDT in milk higher than 0.5 ppm.

Heeschen[194] described procedures for the determination of chlorinated hydrocarbons in milk and dairy products. Table 22 lists the steps involved in the clean-up of milk and milk products for GLC analysis. Fig. 19 and 20 illustrate the column chromatographic equipment utilized for the extraction and clean-up of milk samples for detection of pesticides.

TABLE 22

CLEAN-UP OF MILK AND MILK PRODUCTS FOR GAS CHROMATOGRAPHIC ANALYSIS

1 Extraction of butter oil using hexane–acetone or deep-freezing, warming up to 60°, and centrifuging
2 Mixing of 1.0 g of fat and 1.5 g of Celite 545 in beaker
3 Filling of chromatographic column with butter oil–Celite mixture
4 Selective extraction of the chlorinated hydrocarbons with 3.75 ml of DMSO under pressure
5 Clean-up of the insecticide containing DMSO extract on Florisil aluminiumhydroxide using hexane as eluant
6 Reduction of the eluate to 5 or 1 ml
7 Application of 5 or 10 μl on the gas chromatograph

Miller *et al.*[195] described a collaborative study of the accuracy and reliability of the FDA modification[196] of the Mills method[190] for the determination of organo-

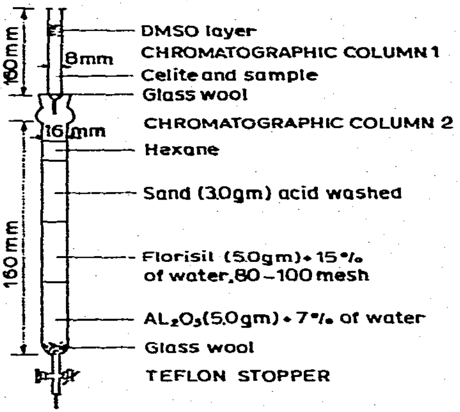


Fig. 19. Scheme of column chromatographic extraction of milk samples for detection of pesticides.

chlorine pesticide residues in animal tissue. TDE, *p,p'*-DDT and total DDT in raw bovine adipose tissue and heat-extracted fat samples including samples spiked with *p,p'*-DDT were analyzed by electron capture GLC on 6 m × 1.8 mm columns of 10% DC-200 on Anakrom ABS or Gas-Chrom Q operated at 225° with nitrogen or helium as carrier gas. The results showed variations in accuracy between laboratories and between samples.

The determination of organo chlorine pesticide residue levels in human milk was described by Newton and Greene[197]. Samples of human milk were collected in 1970 from 39 rural and 28 urban donors in Victoria (Australia) and analyzed by electron capture gas chromatography. All samples contained DDT, DDE, and hexachlorobenzene (HCB). Twenty-nine samples contained dieldrin (mean 0.006 ppm). 12 contained DDD (mean 0.007 ppm) and 3 contained both dieldrin and DDD. Total DDT averaged 0.139 ppm for rural and 0.145 ppm for urban donors and HCB averaged 0.042 ppm and 0.063 ppm, respectively.

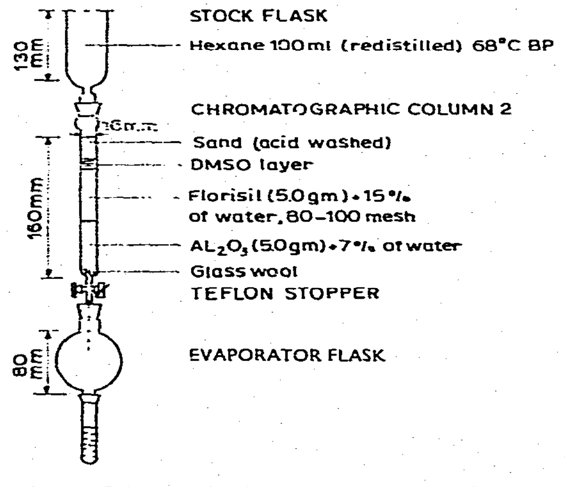


Fig. 20. Scheme of column chromatographic clean-up of milk samples for detection of pesticides.

A Varian Aerograph Model 1200 Chromatograph was used equipped with a 250 mCi tritium electron capture detector and a 5 ft. × $\frac{1}{8}$ in. Pyrex glass column packed with an equal mixture of 10% DC-200 and 15% QF-1 on individually coated 80–100 mesh Gas Chrom Q. A glass liner was used in the injection port. The oven, detector and inlet temperatures were 180°, 185° and 210°, respectively, and the carrier gas was nitrogen at 35 ml/min. Fat was determined by the Gerber method[198], and chlorinated hydrocarbon residues by modified procedures of Giuffrida *et al.*[199] for separating the fat and Mills *et al.*[190] for the cleanup. TLC with $AgNO_3$–UV visualization as described by Abbott *et al.*[200] was also used to confirm about one-half the samples. Additional confirmation of HCB was carried out on four samples by scraping the developed and UV-exposed HCB spot from the plate, extracting the alumina with hexane, and injecting the concentrated extract onto the first column.

To account for all samples tested having HCB, it was suggested that HCB could have entered the food chain of Victorians from the improper channeling of HCB-treated seed wheat into the local poultry and stock food industries following a series of severe reductions in wheat acreage during the period of world-wide wheat over-production in the past decade.

The comparative metabolism of DDT, methylchlor and ethoxychlor in mice, insects and in a model ecosystem has been described by Kapoor *et al.*[201]. Ethoxychlor (2,2-bis(*p*-ethoxyphenyl)-1,1,1-trichloroethane) like methoxychlor was metabolized by O-dealkylation to yield 2-(*p*-hydroxyphenyl)-2-(*p*-ethoxyphenyl)-1,1,1-trichloroethane and subsequently to 2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane. In the model ecosystem ethoxychlor was found in fish at the top of the food chain at 1500 times the amount in water, together with larger amounts of polar and dealkylation products. Methylchlor (2,2-bis(*p*-methylphenyl)-1,1,1-trichloroethane) was metabolized by oxidation of aryl methyl groups to form 2-(*p*-hydroxymethylphenyl)-2-(*p*-methylphenyl)-1,1,1-trichloroethane and by subsequent oxidation to 2,2-bis(*p*-carboxyphenyl)-1,1,1-trichloroethane. In the model ecosystem methylchlor was found in fish at 1400 times the amount in water together with larger amounts of polar metabolites. These two analogs of DDT (ethoxychlor and methylchlor) were much more biodegradable than DDT which was concentrated in fish at 85,000 times the amount in water. ^{3}H-substituted ethoxychlor was prepared by the method of Hilton and O'Brien[202] and purified by column chromatography on silica gel by elution with 2% diethylether in petroleum ether (b.p. 60–68°). [^{14}C]methylchlor was synthesized from [^{14}C]toluene, condensed with chloral in conc. sulfuric acid. The product was purified by column chromatography on silica gel with petroleum ether (b.p. 60–80°).

Tables 23 and 24 list the properties (m.p., TLC and detection) of ethoxychlor and methylchlor, respectively, and their model metabolites. The pathways of metabolism for ethoxychlor and methylchlor in insects, mice, and in the model ecosystem (Figs. 21 and 22) showed that these compounds, like methoxychlor and methiochlor previously investigated[203], are persistent, biodegradable analogs of DDT. It was suggested that the CH_3, CH_3O, C_2H_5O, and CH_3S moieties can serve as degradative handles in the DDT type molecule and that the incorporation of any of these can markedly accelerate the biodegradability of the molecule. Detailed methodology of the model ecosystem has been previously described by Kapoor *et al.*[203] and Metcalf *et al.*[204].

Chopra and coworkers[205–207] described systematic studies on the degradation

TABLE 23

PROPERTIES OF ETHOXYCHLOR AND ITS MODEL METABOLITES

Abbreviations: HDE_1 = hexane–dioxane–diethyl ether (80:20:10); HDE_2 = hexane–dioxane–diethyl ether (90:60:10); UV = exposure to UV light for 5–10 min; D–Z = spraying with 0.5% diphenylamine + zinc chloride (in acetone), heating at 110° for 10 min and exposure to UV light for 5 min.

Compound	*m.p. (°C)*	R_F		*Detection*	
		HDE_1	HDE_2	*UV*	*D–Z*
$C_2H_5OC_6H_4HCCCl_3C_6H_4OC_2H_5$	105	0.49	0.71	None	Steel gray
$C_2H_5OC_6H_4CCCl_2C_6H_4OC_2H_5$	107	0.55	0.71	None	Pink
$C_2H_5OC_6H_4HCCCl_3C_6H_4OCH_2CH_2OH$	117		0.40	None	Steel gray
$C_2H_5OC_6H_4HCCCl_3C_6H_4OH$	65–7		0.49	Light yellow	Black
$HOC_6H_4HCCCl_3C_6H_4OH$	194.0		0.27	Yellow	Black
$HOC_6H_4CCCl_2C_6H_4OH$	212.0		0.30	Yellow	Pink
$HOC_6H_4COC_6H_4OH$	213–215.0		0.18	Yellow	None

TABLE 24

PROPERTIES OF METHYLCHLOR AND ITS MODEL METABOLITES

Abbreviations: P = petroleum ether (b.p. 60–68°); BDA = benzene–dioxane–glacial acetic acid (90:30:1); D–Z = spraying with 0.5% diphenylamine + zinc chloride (in acetone), heating at 110° for 10 min and exposure to UV light for 5 min.

Compound	*m.p. (°C)*	R_F		*Detection (D–Z)*
		P	*BDA*	
$CH_3C_6H_4HCCCl_3C_6H_4CH_3$	83	0.28	0.71	Black
$CH_3C_6H_4CCCl_2C_6H_4CH_3$	82	0.38	0.71	Pink
$CH_3C_6H_4HCCCl_3C_6H_4CH_2OH$	74		0.53	Gray
$CH_3C_6H_4HCCCl_3C_6H_4COOH$	107		0.48	Gray
$HOH_2CC_6H_4HCCCl_3C_6H_4CH_2OH$	Gummy solid		0.30	None
$HOOCC_6H_4HCCCl_3C_6H_4COOH$	274–6		0.33	None

of *p,p′*-DDT in tobacco smokes. *p,p′*-DDT was pyrolyzed at 900° in a nitrogen atmosphere and the pyrolysis products were collected in pentane at −80° and isolated by fractional distillation and chromatography on alumina and Florisil columns. The products isolated were: *p,p′*-DDT, *p,p′*-DDE, *p,p′*-TDE, bis(*p*-chlorophenyl)chloromethane, bis(*p*-chlorophenyl)methane, *p,p′*-dichlorobiphenyl, *α,p*-dichlorotoluene, hexachloroethane, chlorobenzene, tetrachloroethylene, trichloroethylene, carbon tetrachloride, chloroform and dichloromethane. The solid (first eight) pyrolysis products were identified by gas chromatography and IR spectrometry and the liquid pyrolysis products (the last six) were identified by GLC and colorimetric tests. Besides these pyrolysis products, *p,p′*-DDM, and *cis*- and *trans-p,p′*-dichlorostilbenes (present in exceeding small quantity) were also detected in the pyrolyzate and identified by GLC.

Fig. 23 illustrates the pyrolysis apparatus which consisted of three sections: a hopper unit, a pyrolysis tube and traps. The hopper unit and traps were made of glass and the pyrolysis tube was made of glass. All the connections in the apparatus were made of glass. *p,p′*-DDT (10 g) was introduced into the tube (T) of the hopper and stoppered. From these it was introduced into the pyrolysis tube (PT) with a glass pis-

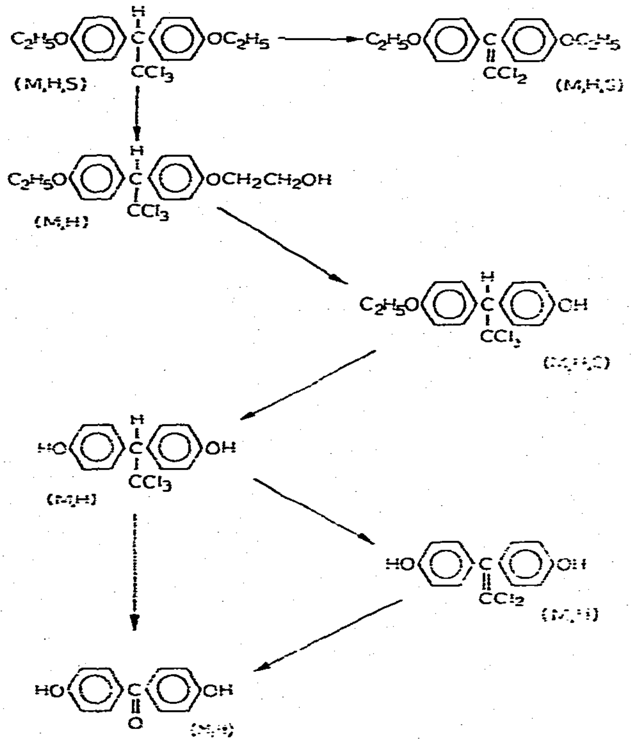


Fig. 21. Pathways of ethoxychlor metabolism in the mouse (M), housefly (H), and salt marsh caterpillar (S).

ton. From the other end of the hopper, nitrogen was introduced into the tube at the rate of 150 ml/min. The pyrolysis tube, 50 cm × 2.5 cm diameter, had an indentation in the middle of which a 2-cm thick bed of broken quartz pieces (BQ) rested. The pyrolysis tube was heated in a muffle furnace to 900°. Small quantities of *p,p'*-DDT were dropped on a red hot broken quartz bed over a period of about 2 h. The escaping pyrolysis products were trapped in five traps (Fig. 23). After the pyrolysis operation was completed, the pentane solutions in the traps were combined and the residue left in each trap was extracted with 20 ml of pentane. The pentane solutions and extracts were combined and filtered and the filtrate (PE) processed as shown in Fig. 24. PE was worked up into three fractions: pentane reflux fraction (PRF), pentane extract distillate (PED) and non-volatile fraction (NVF).

The PE was refluxed with an ice-cold water cooled reflux condenser and the escaping gases trapped in a pentane trap at −80° to give PRF. The PE after the removal of PRF was distilled on a water-bath at 50° to give a viscous yellowish-brown solid residue, NVF (0.155 g) and the distillate PED. The gas chromatograms of the PED and NVF are shown in Figs. 25 and 26.

The colorimetric tests were performed only on the constituents of the PED. These tests consisted of the following: (1) dichloromethane was detected by the method based on that by Gronsberg[208]; (2) chloroform and trichloroethylene were detected by a modification of the Fujiwara test as reported by Feigl[209]; (3) chloroform and tri-

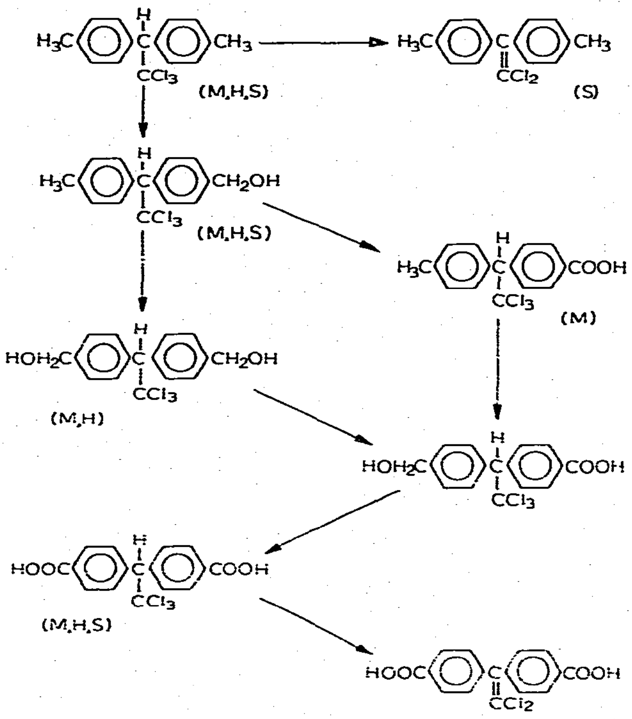


Fig. 22. Pathways of methylchlor metabolism in the mouse (M), housefly (H), and salt marsh caterpillar (S).

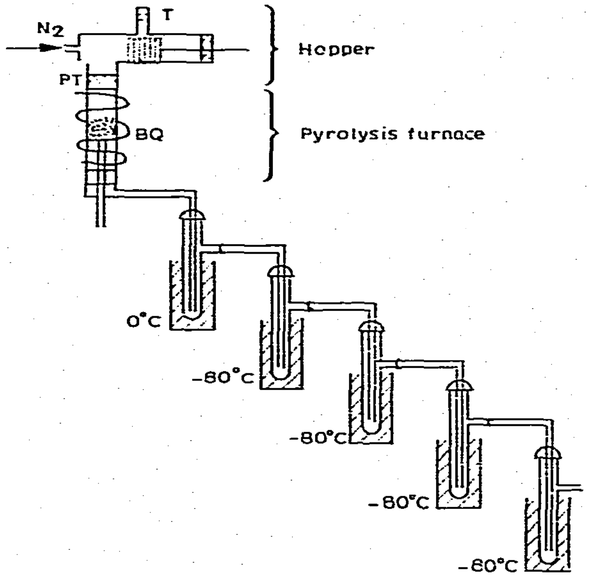


Fig. 23. Pyrolysis apparatus with traps. For abbreviations see text.

TABLE 25

IDENTIFICATION OF VARIOUS PYROLYSIS PRODUCTS IN PED FROM THE PYROLYSIS OF *p,p'*-DDT IN A NITROGEN ATMOSPHERE

Fraction No.	*No. of compounds present*	*Reference compound*	*Results of chromatography*			*Results of colorimetric tests*					*Identification of the unknown compound*
			Column A	*Column B*	*Column C*	*Test 1*	*Test 2*	*Test 3*	*Test 4*	*Test 5*	
F1	One	dichloromethane	n.s.*	n.s.	n.s.	violet SARC**	pink SARC	no color SARC	no color SARC	—	dichloromethane
F2	One	chloroform	n.s.	n.s.	n.s.	no color SARC	violet SARC	yellow orange SARC	yellow orange SARC	—	chloroform
F3	One	carbon tetrachloride	n.s.	n.s.	n.s.	no color SARC	pink SARC	no color SARC	yellow orange SARC	—	carbon tetrachloride
F4	One	trichloroethylene	n.s.	n.s.	n.s.	no color SARC	violet SARC	yellow orange SARC	—	—	trichloro-
F5	One	tetrachloroethylene	n.s.	n.s.	n.s.	no color SARC	no color	yellow orange SARC	—	—	tetrachloroethylene
F6	Two	chlorobenzene	n.s.	n.s.	n.s.	—	—	—	—	orange spots with R_F SARC	chlorobenzene
		hexachloroethane (see F7, Table 26)									

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* n.s. = no separation.

** SARC = same as reference compound.

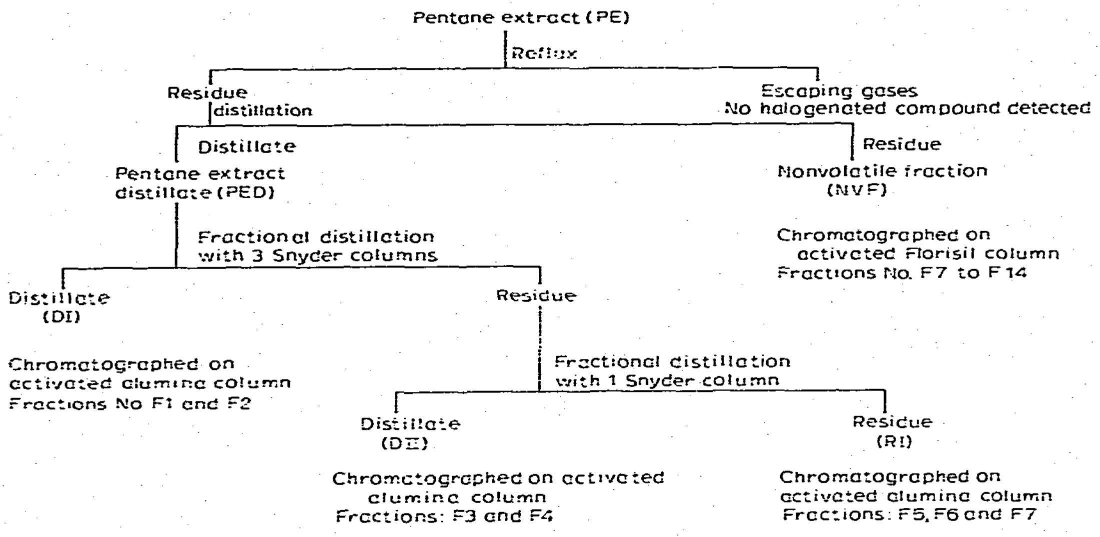

Fig. 24. Procedure for isolation of pyrolysis products of *p,p'*-DDT.

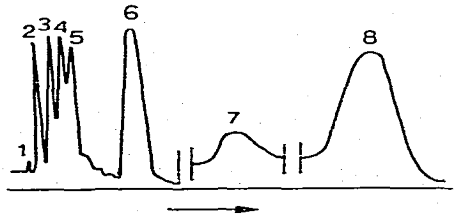

Fig. 25. GLC chromatogram of PED on a 3% SE-30 column. 1 = Pentane; 2 = dichloromethane; 3 = chloroform; 4 = carbon tetrachloride; 5 = trichloroethylene; 6 = tetrachloroethylene; 7 = chlorobenzene; 8 = hexachloroethane.

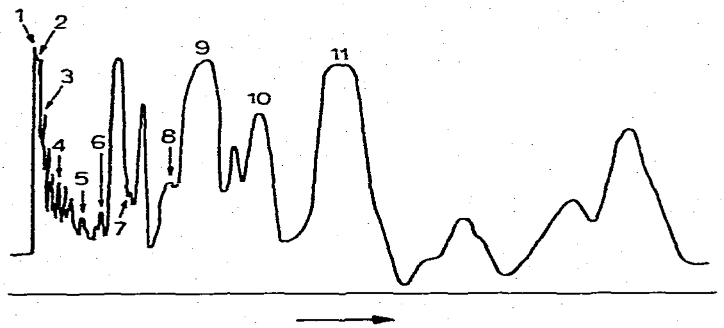

Fig. 26. GLC chromatogram of NVF on a 3% SE-30 column. 1 = Pentane; 2 = hexachloroethane; 3 = *α,p*-dichlorotoluene; 4 = *p,p'*-dichlorobiphenyl; 5 = bis-(*p*-chlorophenyl)methane; 6 = *cis-p,p'*-dichlorostilbene; 7 = bis-(*p*-chlorophenyl)chloromethane; 8 = *p,p'*-DDM; 9 = *p,p'*-DDE and *trans-p,p'*-dichlorostilbene; 10 = *p,p'*-TDE; 11 = *p,p'*-DDT.

chloroethylene were also tested by a modification of Brumbaugh and Stallard's method[210]; (4) carbon tetrachloride and chloroform were detected by a modification of Belyakov's method[211]; and (5) chlorobenzene was detected by its conversion to aniline with potassium amide in liquid ammonia. A Micro-Tek MT-200 gas chromatograph equipped with a ^{63}Ni electron capture detector and a Dohrmann Model C-200 microcoulometer with a Model S-100 combustion unit was employed. The glass columns were 6 ft. × 1/4 in. with (A) 3% SE-30 on 80–90 mesh Chromoport XXX, (B) 5% SE-30 on 80–90 mesh Chromoport XXX, and (C) 20% Carbowax 20M on 80–100 mesh Chromoport XXX. The identification of the various pyrolysis products in the PED and the NVF from the pyrolysis of *p,p'*-DDT in a nitrogen atmosphere is illustrated in Tables 25 and 26. As a result of the above studies the mechanisms involving fragmentation of *p,p'*-DDT were suggested to involve the formation of free radicals (Fig. 27) and carbenes (Fig. 28).

TABLE 26

IDENTIFICATION OF VARIOUS PYROLYSIS PRODUCTS IN NVF FROM THE PYROLYSIS OF *p,p'*-DDT IN A NITROGEN ATMOSPHERE

Fraction No.	*No. of compounds present*	*Reference compound*	*Results of chromatography*		*IR spectra*	*Identification of the unknown compound*
			Column A	*Column B*		
F7	One	hexachloroethane	SARC[*]	SARC	SARC	hexachloroethane
F8	One	*α,p*-dichlorotoluene	SARC	SARC	SARC	*α,p*-dichlorotoluene
F9	One	*p,p'*-dichlorobiphenyl	SARC	SARC	SARC	*p,p'*-dichlorobiphenyl
F10	One	bis(*p*-chlorophenyl)methane	SARC	SARC	SARC	bis(*p*-chlorophenyl)-methane
F11	One	bis(*p*-chlorophenyl)chloromethane	SARC	SARC	SARC	bis(*p*-chlorophenyl)-chloromethane
F12	One	*p,p'*-DDE	SARC	SARC	SARC	*p,p'*-DDE
F13	One	*p,p'*-TDE	SARC	SARC	SARC	*p,p'*-TDE
F14	One	*p,p'*-DDT	SARC	SARC	SARC	*p,p'*-DDT

[*] SARC = same as reference compound.

6. LIQUID CHROMATOGRAPHIC AND LIQUID CHROMATOGRAPHIC–MASS SPECTROSCOPIC PROCEDURES

The potential use of high-performance liquid chromatography for the determination of pesticides or pharmaceutically active ingredients in plant or animal tissues was recently evaluated by Eisenbeiss and Sieper[212]. The most important factors influencing the efficiency of a residue method, *e.g.* detection limit, reproducibility and linearity are comparable to those of gas chromatography if suitable column packings and sensitive detectors are used. Liquid chromatography has an additional advantage of being able to detect less volatile substances and substances that decompose rapidly at high temperatures. Eisenbeiss and Sieper[212] suggested that high-performance liquid-chromatography could be useful for confirmatory analysis or as an alternative to conventional gas chromatography. In their studies monochromator detectors for

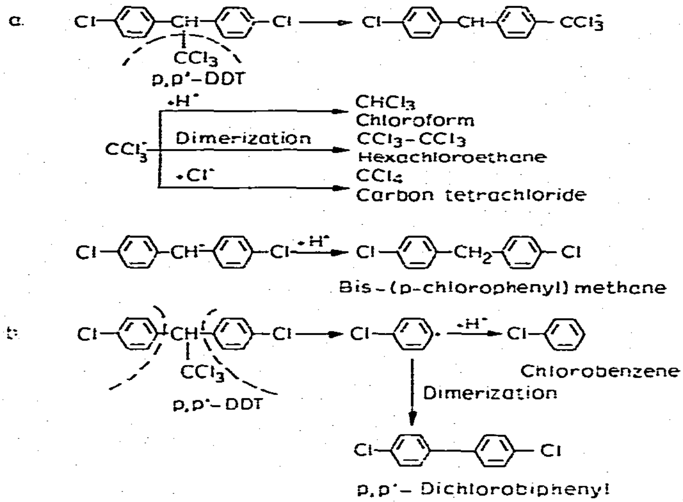

Fig. 27. Mechanisms for the fragmentation of DDT molecule involving the formation of free radicals.

the UV and visible spectral ranges were modified for liquid chromatography and employed with a new adsorbent column packing, Perisorb A, a porous-layer bead (E. Merck) which could be used both in adsorption and partition chromatography. Table 27 shows the minimum detectable levels of *p,p′*-DDT, PCB (Chlophen A40), 2,4-D, MCPA and dithianone. DDT could be determined within a concentration range of

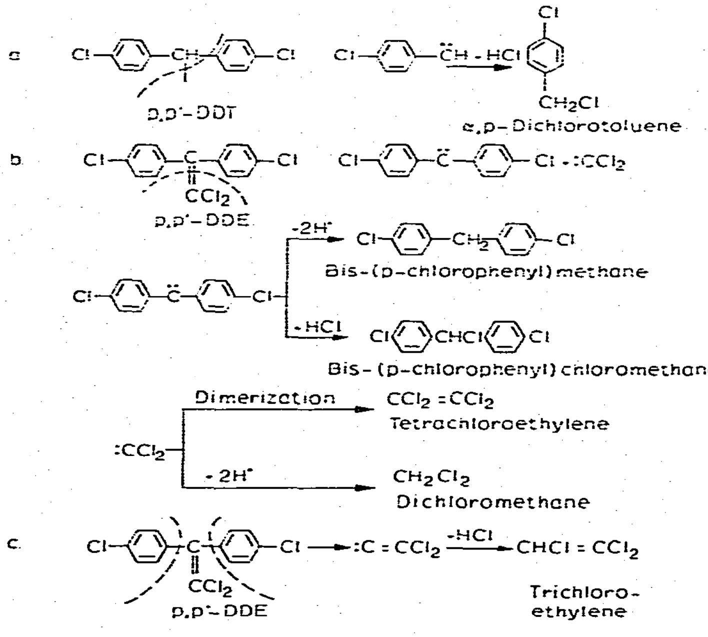

Fig. 28. Mechanisms for the fragmentation of DDT molecule involving the formation of carbenes.

TABLE 27

MINIMUM DETECTABLE SAMPLE FOR SOME PESTICIDES IN LIQUID CHROMATOGRAPHY

Pesticide	*Minimum detectable amount (ng)*	*Detector*	
		Type	*Wavelength (nm)*
p,p'-DDT	~5	Zeiss, PMQ II	210
Dithianone	~5	PM 4-CHR	254
MCPA	~5	PM 4-CHR	278
2,4-D	~5	PM 4-CHR	278
PCB (Chlophen A40)	~1	Zeiss, PMQ II	210

three orders of magnitude without any loss of linearity (Fig. 29). (amounts of 0.01–10 µg of DDT in 10 µl were injected). In general the linearity was limited by the optical density and not by the column.

The utility of liquid chromatography for the confirmatory analysis in the separation of PCBs and DDT is illustrated in Fig. 30.

Lovins *et al.*[213] described the utility of liquid chromatography–mass spectrometry for the analysis of mixtures of DDT, dieldrin, and DDD. A DuPont Model 830 liquid chromatograph equipped with a 254-nm-wavelength UV detector was used with a DuPont Model 21-490 single focusing mass spectrometer equipped with linear scan electrical detection and variable ionizing voltage. Fig. 31 illustrates a scheme of the LC–MS interface showing the major components including the capillary line from LC to pneumatic valve located on the hollow probe. Fig. 32 depicts a scheme of the probe assembly showing the major components. A liquid chromatograph of a mix-

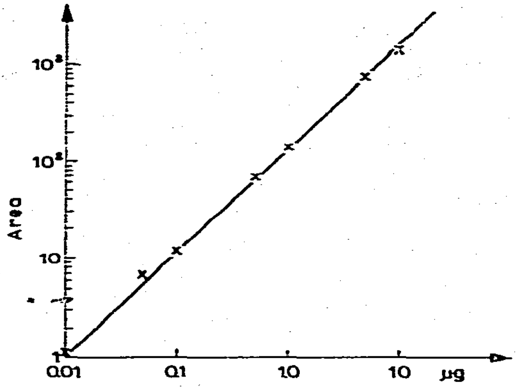

Fig. 29. Linearity with increasing amounts of DDT. Detector: Zeiss PMQ II; 210 nm. Column: Perisorb® A: 500 × 2 mm. Injection volume: 10 µl.

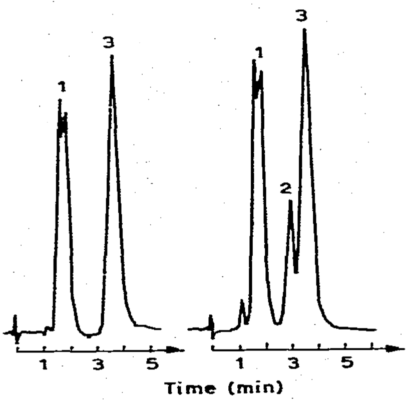

Fig. 30. Separation of PCBs and DDT in liquid chromatography as an example of a confirmatory analysis. Column: Perisorb® A: 500 × 2 mm. Mobile phase: *n*-hexane (anhydrous). Instruments: UFC 1000 with a modified PMQ II (210 nm) as detector. 1 = PCBS; 2 = *o,p'*-DDT; 3 = *p,p'*-DDT.

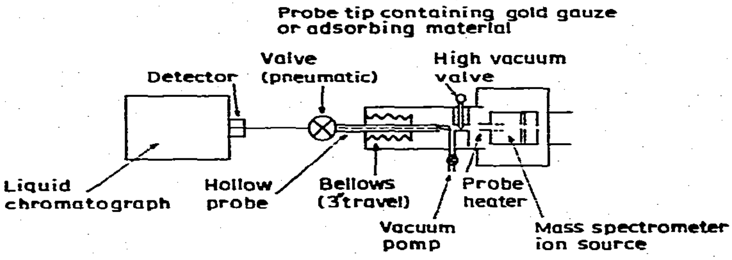

Fig. 31. Scheme of LC–MS interface showing the major components, including the capillary line from LC to pneumatic valve located on the hollow probe, the motor driven hollow probe (I.D. = 0.032 × 12 3/8 in. long) on 3-in. bellows, and the high vacuum inlet valve (motor driven).

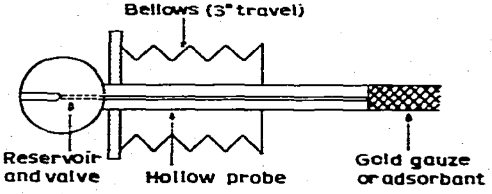

Fig. 32. Scheme of probe assembly showing the major components: pneumatic valve and reservoir (5-ml capacity) coupled to the hollow probe (12 3/8 × 0.16 in.; 0.032 in. I.D.) operating on the bellows (1.8-in. diameter) having a travel of 3 in. The probe tip (1.09 × 0.128 in. I.D.) is removable and can be packed either with gold gauze or adsorbent.

ture of dieldrin, DDT, and DDD separated on a DuPont ETH column using 70% heptane–30% isopropyl alcohol at 1,000 p.s.i. and ambient temperature is shown in Fig. 33 while Fig. 34 shows the mass spectra of the components isolated using the LC–MS interface probe (corresponding to the compounds in peaks 1, 2, and 3 in the liquid chromatogram shown in Fig. 33). The above LC–MS system was found effective in the separation of the effluent peaks from a liquid chromatograph, via the separation of the solute from the solvent by flash evaporation in the probe tip followed by the automatic insertion of the isolated material into the ion source of the mass spectrometer for analysis.

7. NON-CHROMATOGRAPHIC TECHNIQUES

A number of non-chromatographic techniques have been utilized for the quantitation of DDT, its derivatives as well as other organochlorine pesticides. Colorimetric methods of analysis of DDT and derivatives have been reviewed by Miskus[6]. Blinn and Gunter[214], Bruce[215] and Frehse[216] reviewed the utility of IR analysis for the determination of residues of chlorinated hydrocarbons such as DDT, dieldrin, endrin, chlordane, aldrin, endosulfan, lindane, methoxychlor in a range of samples including crops, soils, air, and water. Nuclear magnetic spectroscopy has been utilized by Biros[217] for semiquantitative analysis and confirmation of mixtures of *p,p'*-DDT and *p,p'*-DDE residues isolated from human adipose tissue of an occupationally exposed individual and animal liver tissue dosed with *p,p'*-DDT. The relative error found for the mole fractions of *p,p'*-DDT and *p,p'*-DDE in standard mixtures ranged from 0.5–4.1% de-

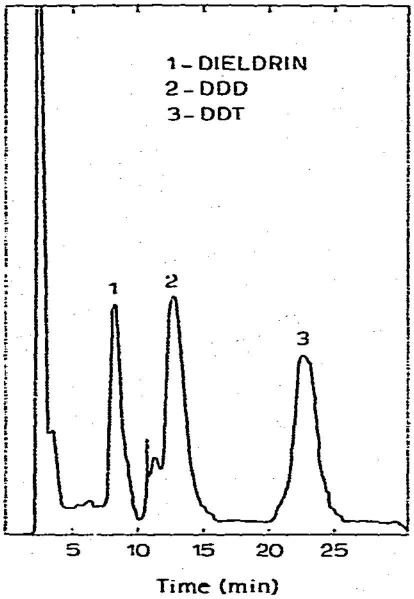

Fig. 33. Liquid chromatogram of a mixture of dieldrin, DDD, and DDT separated on a DuPont ETH column using 70% heptane–30% isopropyl alcohol at 1000 p.s.i., ambient temperature.

pending on the relative concentrations of pesticides present. Gas chromatographic analyses performed on the tissue extracts by electron capture and electrical conductivity detection agreed well with the results obtained by nuclear magnetic resonance (NMR). Spectral data for a number of compounds of the DDT type have been published by Keith *et al.*[218] and the quantitative aspects of the NMR method examined.

Polarographic determinations of DDT and its metabolites have been reported by Davidek and Janicek[219], Fukami *et al.*[220], Keller *et al.*[221], and Kemula[222,223].

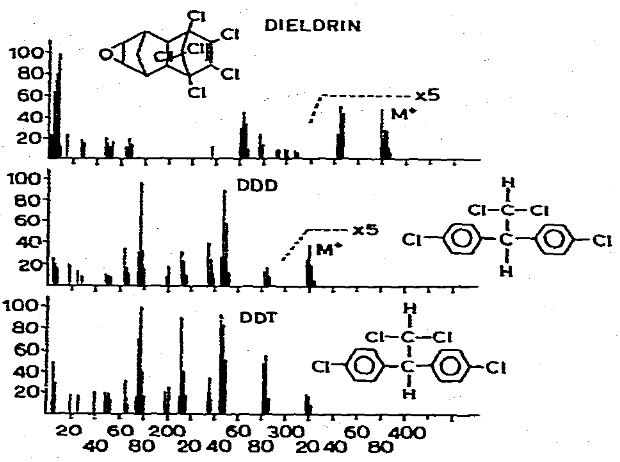

Fig. 34. Mass spectra of the components isolated using the LC–MS interface probe corresponding to the compounds in peaks 1, 2, and 3 in the liquid chromatogram shown in Fig. 33.

The spectrophotometric determination of DDT in solutions after separation by TLC was reported by Feklisova[224]. After separation of DDT by TLC on silica gel with hexane as the mobile phase and location with $AgNO_3$–aq.NH_3–30% aq.H_2O_2 reagent, the plate was exposed to UV radiation for 10 min. The DDT was then extracted with hexane, the extinction measured at 221 or 237 nm, and the amount of DDT obtained from a calibration curve. The Lambert-Beer law was followed in the 5–10 ng per ml range. From 5–30 ng of DDT could be determined with a relative error of ± 5% and down to 1 ng per ml of hexane could be detected.

8. DETERMINATION OF KELTHANE

As has been pointed out earlier technical DDT contains approximately 0.17–4.0% *p,p'*-TDE (*p,p'*-DDD) (rhothane). It has also been shown that *p,p'*-TDE as well as kelthane (dicofol) are metabolic products of DDT. Since both TDE and kelthane also have insecticidal utility *per se*, a consideration of their preparation, properties and analysis is germane.

Kelthane is made by the reaction of 1,1-dichlorophenyl-1,2,2,2-tetrachloroethane with silver acetate and the hydrolysis of the resultant ester. The technical product is a brown viscous oil, about 80% purity. The pure compound is a white solid melting at 78.5–79.5°. The formulations of kelthane include: 18.5% wettable powder, 19.5% and 42% emulsion concentrate. Kelthane is a non-systemic acaricide used for the control of mites on a wide range of crops at concentrations from 0.5–4.0 lb./acre.

The acute oral LD_{50} of kelthane for male rats is 809 ± 33 mg/kg; for female rats 674 ± 16 mg/kg, the acute dermal LD_{50} for rabbits is 1,870 mg/kg. Kelthane has been reported by Anderlan[225] to produce malformations when administered to mice.

Kelthane is a widely employed acaricide for the control of phytophagous mites. The toxicity of keltane was studied by Smith *et al.*[226]. McKinley and Grice[227] suggested that DDE was a metabolite of kelthane. The storage, distribution and metabolism of kelthane in the rat has been elaborated by Brown *et al.*[228]. GLC was used for the determination of kelthane and its metabolites following both oral and i.p. administration of kelthane. Following clean-up of tissue extracts on Florisil by hexane (hexane concentrated following acetonitrile–aqueous partitioning, and chromatographed on Florisil–Celite) concentrated hexane eluate was analyzed by GLC using a glass column packed with 4% SE-30 and 6% QF-1 on 100–120 mesh acid-washed Chromosorb W. The operating parameters were: injector, column and detector temperatures, 200°, 185°, and 200°, respectively; nitrogen carrier gas flow-rate, 32 ml/min. Under these conditions kelthane was decomposed quantitatively to 4,4'-dichlorobenzophenone. In the estimation of kelthane and its metabolites, the parent compound and dichlorobenzophenone were separated by TLC on silica gel G using 4% ethyl acetate in hexane as developing solvent. The results were confirmed by using a 5% QF-1 on Chromosorb W column at 145°. The metabolites found were 4,4'-dichlorobenzophenone, 4,4'-dichlorobenzhydrol, and 1,1-bis(4-chlorophenyl)-2,2-dichloroethylene (DDE).

The determination of *p,p'*-kelthane and its *p,p'*-dichlorobenzophenone degradation product by TLC and oscillopolarography has been reported by Ott *et al.*[229]. TLC was carried out using silica gel GF_{254} (E. Merck) plates developed with 10%

methanol in *n*-pentane. The chromogenic reagent was 10% N,N-dimethyl-*p*-phenylazo-aniline (methyl yellow) in 95% ethanol. The R_F values for kelthane and dichlorobenzophenone were 0.29–0.36 and 0.77–0.86, respectively. Variations in R_F values were not greater than ± 0.03 for either compound in the range 0.5–10 μg whether chromatographed singly or in admixture. Detection of kelthane and dichlorobenzophenone was also accomplished by the use of fluorescent quenching of fluorescent TLC plates under UV light. Oscillopolarography (employing a Davis Southern Analytical Differential Cathode Ray Polarotrace Model A 1660 A and Goyan oscillopolarographic cells (Western Scientific) with an electrolyte solution of 0.2 *M* aq. tetramethyl ammonium bromide) of purified kelthane at 2.5 μg/ml in the analytical solution revealed two reduction potentials: −0.65 and −0.85 V. These reduction potentials became slightly more negative with increasing concentration from 1.3–10.0 μg/ml in the final analytical solution. The single reduction potential for purified dichlorobenzophenone was −1.26 ± 0.01 V over the range 2.5–10 μg/ml.

The separation of kelthane (dicofol) and its degradation product dichlorobenzophenone from a standard Florisil column has been achieved by Morgan[230] using elution mixtures consisting of (1) purified diethyl ether–redistilled petroleum ether (b.p. 30–60°) (1:9) and (2) purified diethyl ether–petroleum ether (1:4). Florisil (60–100 mesh) was first preheated to 165°, then heated 18 h at 130° and held at 130° until ready for use. Column eluates were concentrated and analyzed on a Jarrell-Ash gas chromatograph equipped with dual electron capture detectors. One column was fitted with a 5 ft. × 3/16 in. glass column containing 10% DC-200 on 60–80 mesh Gas Chrom Q. A second 5 ft. × 3/16 in. glass column was packed with equal portions of 10% DC-200 and 10% QF-1 on 60–80 mesh Gas Chrom Q. The instrument was operated at 190° with a nitrogen flow-rate of 200 ml at 20 p.s.i. Recoveries of kelthane from fortified extracts of alfalfa and lettuce in eluting mixture 1 were 96 and 98%, respectively, at the 2.0-ppm level. Recovery of *p,p′*-dichlorobenzophenone from fortified extracts of alfalfa and lettuce including mixture 2 were 95 and 96%, respectively, at the 1.0-ppm level.

The GLC determination of *p,p′*-kelthane (1,1-bis(chlorphenyl)-2,2,2-trichloroethanol) has revealed the presence of seven peaks[231] in the technical grade material and only one[232,233] or two peaks in the purified samples.

Morgan[234] described the identification and relative retention times of *p,p′*-kelthane and its breakdown product, *p,p′*-dichlorobenzophenone using GLC. Purified kelthane yielded two major peaks which were obtained as shown in Fig. 35. The

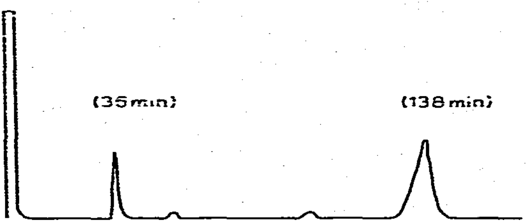

Fig. 35. Chromatogram of 300 μg of *p,p′*-kelthane injected into a Varian Autoprep Model 700-A GLC with a ¼″ by 6′ SS column packed with 5% Dow-11, 60–80 mesh, DMCS treated Chromosorb W. Injection temperature, 230°; Column temperature, 220°, detector temperature, 260°; helium flow-rate, 60 ml/min.

amount of breakdown of *p,p′*-kelthane to *p,p′*-dichlorobenzophenone varied with the carrier gas used and the operating parameters of the GLC. This is apparent in comparing Fig. 35, where a 300-μg sample of *p,p′*-kelthane was analyzed on a Varian Autoprep, with Fig. 36 illustrating a chromatogram obtained using 5 μg of the same compound analyzed on a Dohrmann Model 100 gas chromatograph using nitrogen carrier gas. In this latter figure, the first peak with a retention time of 4.5 min. was *p,p′*-dichlorobenzophenone and the last peak is *p,p′*-kelthane with a retention time of 16.6 min. (both columns were packed with 5% Dow-11 on 60–80 mesh DMCS-treated Chromosorb W and the relative retention times on both instruments were identical.

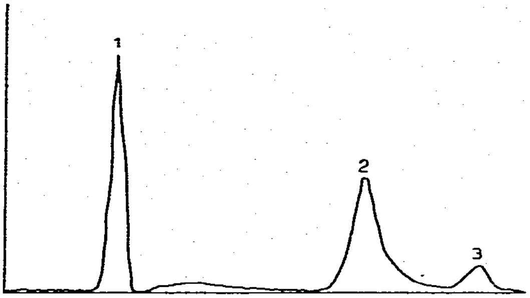

Fig. 36. Chromatogram of 5 μg of *p,p′*-kelthane injected into a Dohrmann Model 100 GLC with a ¼″ by 5′ glass column packed with 5% Dow-11, 60–80 mesh, DMCS treated Chromosorb W. Injection temperature, 240°; column temperature, 218°; nitrogen flow-rate, 100 ml/min. 1 = *p,p′*-dichlorobenzophenone; 2 = unidentified; 3 = *p,p′*-kelthane.

Fig. 37 shows a chromatogram of 1 ng of *p,p′*-kelthane injected into a Jarrell-Ash electron capture gas chromatograph, using nitrogen carrier gas. The difference in the peak heights of the intermediate breakdown products was believed to be due to the sensitivities of the two detectors. Table 28 lists the relative retention times of *p,p′*-dichlorobenzophenone and *p,p′*-kelthane on three commonly used GLC columns, *e.g.*, Dow-11, DC-200 and QF-1/DC-200.

Black *et al.*[235] described a GLC analysis of kelthane (technical) and its minor impurities. An Aerograph Model 1200 gas chromatograph was used with FID and a

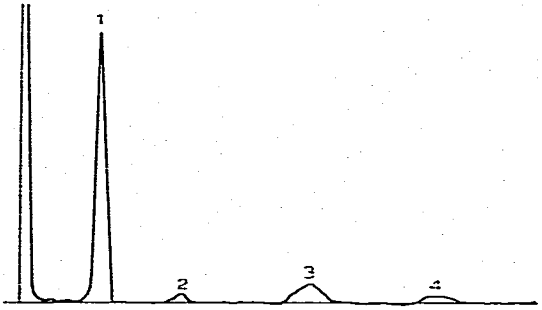

Fig. 37. Chromatogram of 1 ng of *p,p′*-kelthane injected into a Jarrell-Ash electron capture GLC with a ¼″ by 5′ glass column packed with 10% DC-200, 60–80 mesh Gas-Chrom Q. Injection temperature, 220°; column temperature, 190°; nitrogen flow-rate, 250 ml/min. 1 = *p,p′*-dichlorobenzophenone; 2 = unidentified; 3 = unidentified; 4 = *p,p′*-kelthane.

TABLE 28

RELATIVE RETENTION TIMES OF DICHLOROBENZOPHENONE AND KELTHANE ON THREE COMMONLY USED GLC COLUMNS

Compound	*Time relative to aldrin*		
	Dow-11	*DC-200*	*QF-1 + DC-200*
Aldrin	1.0	1.0	1.0
p,p'-Dichlorobenzophenone	1.0	1.0	1.2
p,p'-DDT	2.9	3.0	3.2
p,p'-Kelthane	3.8	4.6	4.8

6 ft. × 1/8 in. I.D. glass column packed with 3% Oronite polybutene 128 (Analabs) on 60–80 mesh Gas Chrom Q using temperature programming from 135–205° at 4°/min. The flow-rates (ml/min) were: hydrogen, 30; helium carrier gas, 35; and air, 300. Table 29 lists the relative retention time data of components of technical kelthane. The identification of the impurities listed in Table 29 was based on the chemistry of the process, IR and mass spectrographic data. A typical chromatogram of technical kelthane is shown in Fig. 38. Early attempts by Black *et al.*[235] to establish a reproducible gas chromatographic system using the above column resulted in the formation of a sizeable thermal decomposition peak at relative retention time (RRT) 0.71. This

TABLE 29

RELATIVE RETENTION TIME (RRT) DATA OF COMPONENTS OF TECHNICAL KELTHANE

No. in Fig. 38	*Component*	*RRT**
1	Unknown	0.15
2	Internal standard	0.22
3	Unknown	0.30
4	Unknown	0.32
5	Unknown	0.34
6	*o,p'*-Dichlorobenzophenone	0.37
7	Unknown	0.43
8	*p,p'*-Dichlorobenzophenone	0.46
9	*o,p'*-Dichlorobenzil 1-(2-Chlorophenyl)-1-(4-chlorophenyl)-2,2-dichloroethylene	0.53
10	*p,p'*-Dichlorobenzil	0.59
11	1,1-Bis(4-chlorophenyl)-2,2-dichloroethylene	0.62
12	Unknown	0.66
13	1-(2-Chlorophenyl)-1-(4-chlorophenyl)-2,2,2-trichloroethane	0.68
14	1,2-(4-Chlorophenyl)-1-keto-2,2-dichloroethane**	0.71
15	Unknown	0.76
16	1,1-Bis(4-chlorophenyl)-2,2,2-trichloroethane	0.78
17	1-(2-Chlorophenyl)-1-(4-chlorophenyl)-2,2,2-trichloroethanol	0.86
18	1,1-Bis(4-chlorophenyl)-2,2,2-trichloroethanol 1-(2-Chlorophenyl)-1-(4-chlorophenyl)-1,2,2,2-tetrachloroethane	1.00
19	1,1-Bis(4-chlorophenyl)-1,2,2,2-tetrachloroethane	1.07

* Relative retention time: *p,p'*-kelthane = 1.00.

** A thermal decomposition product, not present in technical kelthane.

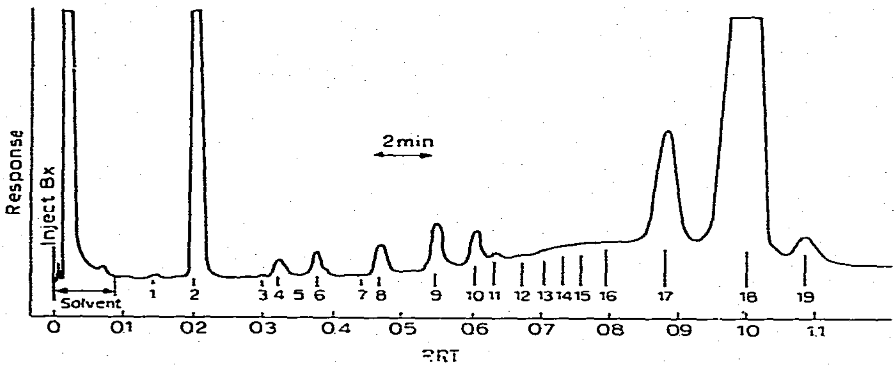

Fig. 38. Chromatogram of technical kelthane; see Table 29 for peak identification.

column decomposition product eluted between *o,p'*-DDT (RRT 0.68) and *p,p'*-DDT (RRT 0.78) and could be mistaken for the DDT isomer. By carefully controlling column packing density and by adhering to the specified operating parameters, this artifact (subsequently synthesized, isolated and identified as 1,2-(4-chlorophenyl)-1-keto-2,2-dichloroethane) was virtually eliminated.

Gas and thin-layer chromatograms of Valencia and Navel orange rind stripping solutions from mature fruit treated commercially in the trees early in the season with the acaricide kelthane had revealed the presence of an unknown compound not present in control samples. The unknown compound had a TLC R_F value and GLC retention time almost identical with those of heptachlor. Westlake *et al.*[236] proved conclusively that this compound was not heptachlor and that its retention time and other characteristics were those of *o,p'*-kelthane, a compound known to be present in small amounts in technical grade kelthane.

Gas chromatographic analysis of aliquots of Florisil column fractions obtained by elution with 6% diethyl ether in hexane were performed using an Aerograph Pestilyzer Model 680 equipped with an electron capture detector and a 2 ft. × 1/8 in. O.D. borosilicate column packed with 100–200 mesh acid-washed HMDS Chromosorb W support coated with 5% SF-96 at 150°. Verifications were carried out using a 29 cm × 1.134 in. I.D. Teflon column packed with 60–80 mesh Teflon coated with 1% Apiezon L at 150°. A nitrogen carrier gas flow-rate of 20 ml/min was maintained through both columns. Further aliquots were analyzed using an Aerograph Autoprep 705 GLC unit with an electron capture detector and a 2 ft. × 1/8 in. stainless-steel column packed with 3.4% GF-1 and 6.2% DC-200 on gas Chromatosorb Q at 180° with a nitrogen flow-rate of 75 ml/min. TLC was performed on fluorescent silica gel plates developed with diethyl ether–benzene (5:1) (R_F *o,p'*-kelthane 0.65–0.72). The similarity of the retention times of heptachlor and *o,p'*-kelthane was shown by the formation of a distinct doublet peak when a mixture of the two compounds was chromatographed on the SF-96 and Apiezon L columns. A mixed QF-1/DC-200 column completely resolved the two peaks despite the close relative retention times (0.77 and 0.94, respectively, when aldrin = 1.00). Table 30 gives the retention times, relative to aldrin for the three gas chromatographic columns used. The most notable feature was the efficiency of the GF-1/DC-200 column in separating heptachlor and *o,p'*-kelthane (*o,p'*-dichlorobenzophenone) despite the very small difference in reten-

TABLE 30

EFFECT OF COLUMN PACKING ON RETENTION TIMES RELATIVE TO ALDRIN

Compound	*Time relative to aldrin*		
	SF-96	*Apiezon L*	*QF-1 + DC-200*
Heptachlor	0.83	0.73	0.77
o,p'-Kelthane	0.89	1.10	0.94
o,p'-Dichlorobenzophenone	0.89	1.10	0.94
Aldrin	1.00	1.00	1.00
p,p'-Kelthane	1.20	1.80	1.16
p,p'-Dichlorobenzophenone	1.20	1.80	1.16

tion times. This column, however, could not separate aldrin and *o,p'*-dichlorobenzophenone and it was cautioned that an analyst might easily be led to believe that aldrin was present in a routine examination of kelthane treated sample. This observation emphasized the necessity for confirming gas chromatographic analyses by a more specific method.

The breakdown of purified *p,p'*-kelthane to *p,p'*-dichlorobenzophenone in 15–85% yields in gas chromatographic systems has been originally reported by Gunther *et al.*[237]. Both *p,p'*- and *o,p'*-kelthane were converted in good yields to their corresponding dichlorobenzophenone in the three gas chromatographic systems used in the above study of Westlake *et al.*[236]. Technical grade kelthane could not be gas chromatographed reproducibly until it has been passed through the Florisil column clean-up procedure. The fraction eluted by 6% ether in hexane contained compounds with the same GLC retention times as those from purified *o,p'*- and *p,p'*-kelthane.

Kelthane has been analyzed by colorimetry (the basic procedure is that used by Rosenthal *et al.*[238] involving hydrolysis to chloroform which is determined colorimetrically by the Fujiwara reaction). Kelthane has also been analyzed via hydrolysis to 4,4'-dichlorobenzophenene which is measured by UV spectroscopy according to the procedure of Gunther and Blinn[239]. A colorimetric determination of kelthane residues in fruit and vegetables has been reported by Gunderson[240]. The procedure involved initial extraction and clean-up as described by Mills *et al.*[190] with modification of the Florisil clean-up step. The determinative step was a modified Fujiwara alkaline-pyridine reaction and the product of this reaction was treated with benzidine in formic acid and the resulting solution analyzed spectrophotometrically at 530 nm. The method could detect as little as 5 ng of kelthane with recoveries of 93–100% at levels of 1.2 and 5 ppm. Other spectrophotometric procedures for the determination of kelthane have been reported by Gordon *et al.*[241] and Hughes[242].

TDE is prepared via the chlorination of ethanol (below 35°; until the lower layer has a density of 1.29 at 20°) and this is then condensed with monochlorobenzene. The technical product has a setting point of about 86° and contains the *o,p'*-isomer as the main impurity. Pure TDE has a melting point of 112°. (It is of interest to note that the *o,p'*-isomer has shown promising results in the treatment of malignant tumors of the adrenal gland.) Pure TDE has a melting point of 112°.

TDE is a non-systemic contact and stomach insecticide which, although is not of the general high potency of DDT, has equal or greater potency against certain insects, *e.g.*, hornworms, mosquito larvae, and leaf-rollers. The acute LD$_{50}$ for rats is

3.4 g/kg and the chronic oral toxicity for rats is about one-third that of DDT. By skin absorption TDE is about one-fourth as toxic as DDT, by inhalation it is also less toxic than DDT. TDE is available as technical grade or as a 50% wettable powder.

Ott and Gunther[243] reviewed aspects of the decomposition of DDT to DDD during gas chromatography as well as in metabolic systems. It has been earlier reported that gas chromatography of DDT resulted in its degradation to DDE and DDD[244-245]. Ott and Gunther[243] presented unequivocal evidence of the formation of DDD from the recycling of a starting sample consisting of two 1.0-mg portions of pure DDT through a commercial gas chromatograph. GC-2A and MCGC instruments were used with a 2 ft. × 1/8 in. O.D. stainless-steel column packed with 2% SF-96 on 100–120 mesh acid washed and twice hexamethyl disilazane treated[246] Chromosorb P.

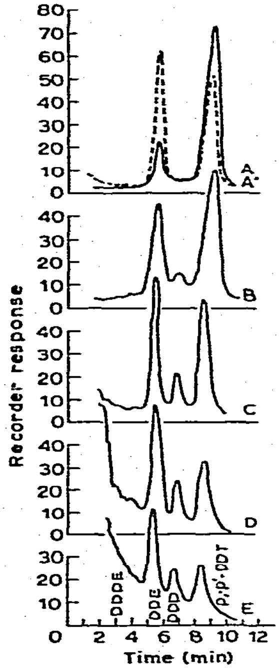

Fig. 39. Gas chromatograms of recycled *p,p′*-DDT through a GC-2A chromatograph: A plus A′ represent the first pass and were consecutive 1.0-mg injections each of *p,p′*-DDT; B, the third pass, resulted from an injection of total trapped effluents from the second pass (not shown); C, D, and E are the sixth, ninth, and tenth passes, respectively. The chromatograms were run at GC-2A attenuation of 10 for A, A′, and B; 2 for C; and 1 for D and E. In each tracing the solvent peak has been eliminated for clarity and convenience. The peaks have been labelled on the basis of relative retention time data.

The operating parameters were: injection block temperature, 205°; column temperature, 170°; nitrogen carrier gas at 20 p.s.i. and flow-rate, 39 ml/min; and a net resistance on the range switch of the coulometer of 51.2 Ω with connection to a one-millivolt recorder. Fig. 39 illustrates gas chromatograms of recycled *p,p′*-DDT through a GC-2A chromatograph. Immediate breakdown of DDT to considerable amounts of DDE is noted in each of the two injections making up the first pass, followed by the latter passes which show a progressive decrease in the relative area of DDE and of DDD. Retention time data from MCGC determinations also supported DDE and DDD as the major gas chromatographic degradation products of DDT. Fig. 40 illustrates analytical data from MCGC determination based on relative integrated peak areas of the components in each aliquot from the ten passes of DDT through the GC-2A. The two major breakdown products and one minor product were measurably detected by MCGC. The study of Ott and Gunther[243] cautions the exceptional experimentation and interpretation required in the analysis of DDT in residue and metabolic studies.

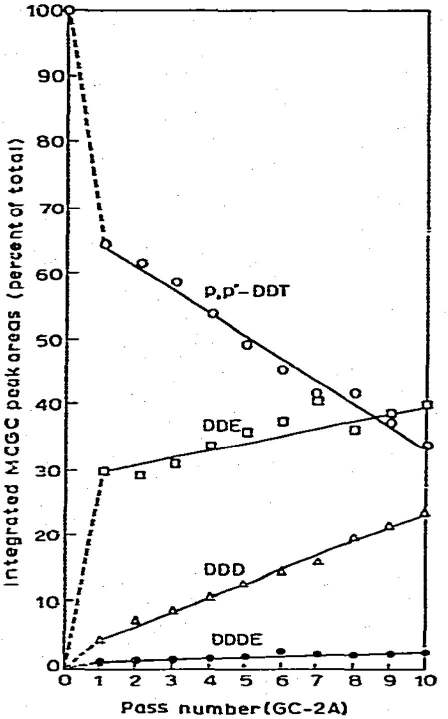

Fig. 40. Graph of data from MCGC analysis of aliquots from each of the ten passes of *p,p'*-DDT through the GC-2A. Breakdown of *p,p'*-DDT and accumulation of breakdown products by the GC-2A is shown. Products DDE, DDD, and DDDE as labelled have been identified by comparative gas chromatographic retention time and peak area data, by R_F values in TLC and by IR spectra.

The synthesis of ^{14}C-labeled isomers of dichlorodiphenyl dichloroethanes (DDD) was described by Counsell and Willette[247]. *o,p'*-[^{14}C]DDD, *m,p'*-[^{14}C]DDD and *p,p'*-[^{14}C]DDD were synthesized by acid catalyzed condensation of [^{14}C]chlorobenzene with excess 2,2-dichloro-1-(*o*-, *m*-, and *p*-chlorophenyl)ethanols. The carbinols were prepared in good yields by reverse addition of chlorophenyl Griguard reagent to dichloroacetaldehyde. The isomers of DDD have been shown to have a predilection for adrenal tissue and in the case of *o,p'*-DDD, has been shown to produce tumor regression in metastatic adrenal cortical carcinoma and to cause remission of symptoms in patients with Cushing's syndrome[248–250].

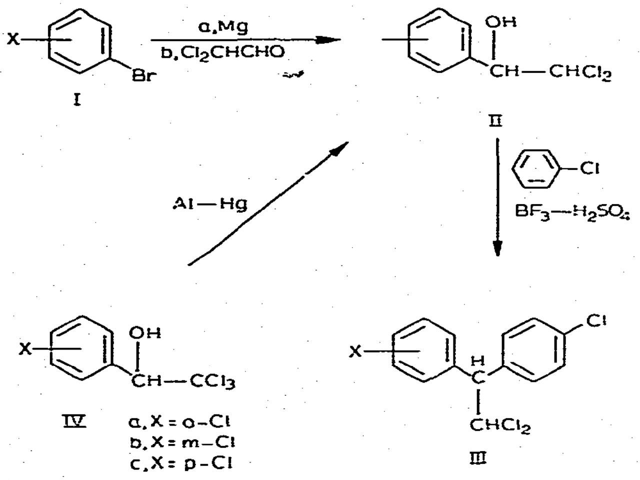

Fig. 41. Preparative scheme for [^{14}C]DDD isomers.

o,p'-DDD has also been found to have an effect on steroid metabolism. For example, it has been shown to stimulate cortisol metabolism[251] to alter the extra-adrenal metabolism or cortisol[252] and to inhibit specific enzymes[253]. Fig. 41 illustrates the preparative scheme for the isomeric [^{14}C]DDD derivatives. The purity of the labeled compounds was determined by TLC and GLC. Eastman chromagrams, type K301R with fluorescence indicator were developed with benzene, and spots detected with UV light and iodine vapor. Chromatograms of ^{14}C-labeled compounds were scanned with an RCS-363 Radiochromatogram Scanner (Atomic Associates). GLC was carried out with an F&M Model 400 chromatograph equipped with an electron capture detector and a 122 cm × 3 mm I.D. glass column containing 3.8% SE-30 on 80–100 mesh Diatoport S prepared according to Horning *et al.*[254]. The column, detec-

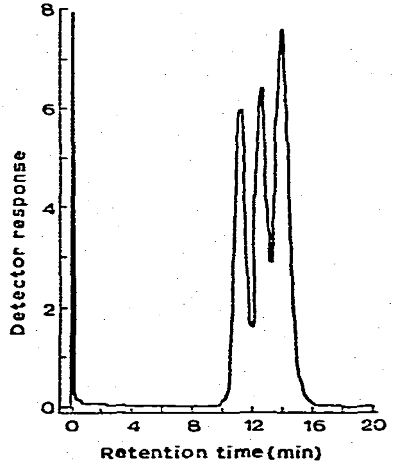

Fig. 42. GLC tracing of 2.5 ng each of ^{14}C-labeled *o,p'*-, *m,p'*-, and *p,p'*-DDD, injected in 6 μl of benzene.

tor, and flash heater temperatures were 185°, 210°, and 230°; inlet pressure 40 p.s.i., flow-rate 70 ml/min. Fig. 42 shows a chromatogram of the separation of 2.5 ng each of the ^{14}C-labeled isomeric DDD compounds under the above operating conditions.

9. SUMMARY

The column, thin-layer, gas–liquid and liquid chromatographic, the combined liquid and gas–liquid chromatographic–mass spectroscopic techniques, as well as the biological and toxicological aspects of DDT and its metabolites have been reviewed. Primary focus was placed on their utility, ecological distribution, and toxicity, as well as diverse techniques for their separation from polychlorinated biphenyls and other chlorinated pesticides and subsequent analysis.

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