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Biological Interaction Between Plasticizers and Insecticides[1]

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ABSTRACT

Because of the structural similarity to DDT-related compounds, 11 plasticizers were tested for their toxicological interaction with dieldrin and DDT. Many of these polychlorinated biphenyl compounds were toxic to *Drosophila melanogaster* Meigen and house flies, *Musca domestica* L., but to a lesser extent than dieldrin or DDT. Their toxicity increased with a decrease in their chlorine content. Moreover, sublethal dosages of several of the plasticizers increased the toxicity of dieldrin and DDT. Since plasticizers are in the environment, their potential effects on biological systems, especially in combination with other synthetic chemicals, should not be disregarded.

Plasticizers such as polychlorinated biphenyls are widely distributed in the environment (Risebrough et al. 1968). Wikmark (1967a) reported that extracts of sea eagles, pike, and salmon contained polychlorinated biphenyl plasticizers (PCB). Holmes et al. (1967) reported that polychlorinated biphenyl compounds had been detected in British wildlife. They also stated that *"in birds' livers and eggs they are often in greater* quantities than organochlorine pesticide residues." Koeman et al. (1969) found chlorinated biphenyls in fish, mussels, and birds from the River Rhine and the Netherlands coastal areas and stated that the PCB compounds, "besides being a hazard in their own right" also "interfere with the detection of organochlorine insecticides such as DDT."

Polychlorinated biphenyls are produced by various manufacturers in different countries. Some of them are marketed under the name of Aroclor® by the Monsanto Chemical Co., St. Louis. They represent "a series of inert, chemically-resistant, fire-retarding plasticizers compatible with a wide variety of resins. They vary from mobile, oily liquids to white crystals and hard transparent resins" (Monsanto 1968). They are used in synthetic resins, synthetic and natural rubbers, cellulose resins, paint, varnish, wax, asphalt, and in allyl starch. They also find applications for dust prevention, moisture proofing, sealing, impregnation, and vapor suppression to prolong the residual life of insecticides. "Chlorinated polyphenyls" were recommended in 1955 for "improving lindane residues" (*Hornstein and Sullivan 1955*). The authors stated that "lindane residues can be made to last longer and look better" if applied with chlorinated polyphenyls.

The toxicity of polychlorinated biphenyl compounds was briefly reviewed by Von Oettingen (1955) who reported that "feeding rats about 0.5 gm daily was highly toxic when given over a period of six days and that even 0.05 gm per rat was definitely toxic, causing injury to the liver." According to the same source, the toxicity to rats of chlorodiphenyl by inhalation was already studied in 1937 and some hepatoxic action was found. The threshold limits (maximum allowable concentration for an 8-hr working day) was set by the American Conference of Government Hygienists (1953) as 1.0 to 0.5 mg of polychlorinated biphenyl compounds/m³ of air. The present study was conducted to investigate the effects of some plasticizers on insects, both singly and in combination with dieldrin or DDT.

Materials and Methods.—The insecticides and insecticide metabolites used in this study were analytical grade lindane, heptachlor, heptachlor epoxide, aldrin, dieldrin, *o,p'*-DDT, *p,p'*-DDT, *p,p'*-DDE, and *p,p'*-TDE. Eleven polychlorinated bi- or triphenyl standards were obtained through the courtesy of Dr. R. F. Thomas, USDA, Beltsville, Md. They are referred to in this study as plasticizers A through K and are identified in Table 3 with various Aroclors according to their Monsanto identification number. The plasticizers A through H contain 2 phenyl rings, and their chlorine content increases from A to H. Plasticizer I is a mixture of chlorinated biphenyls and chlorinated triphenyls (60:40), and J and K are chlorinated triphenyls.

Bioassay Procedures.—The effects of the 11 plasticizers, dieldrin, and DDT on insects were tested with *Drosophila melanogaster* Meigen and with house flies, *Musca domestica* L., DDT-susceptible, CSMA-1948 strain.

(a) In 1 series of experiments, the effects of each chemical on insect mortalities were investigated. When *D. melanogaster* were used, 8 ml of hexane containing either 0.1 μg of dieldrin, 3.0 μg of DDT, 200 or 800 μg of plasticizers A, B, C, and D, and 2000 μg of plasticizers E through K were pipetted into 4-oz

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bioassay jars (Edwards et al. 1957). Solvents were then evaporated and fifty 3-day-old *D. melanogaster* were introduced into each of duplicated test jars that now contained the dry residue of the various chemicals. Mortality counts were performed after 24- and 48-hr exposure times.

In addition to these tests, 2-day-old adult female house flies were treated topically on the ventral portion of the abdomen with 2 μliter of acetone containing either 0.02 μg of dieldrin, 0.5 μg of DDT, or 10 and 20 μg of each of the plasticizers. Controls were treated with 2 μliter of acetone only. After 30 house flies had been treated with each of the chemicals as described, they were held in 2 groups of 15 within 2 bioassay jars for 24 hr, then mortality counts were made.

(b) The 2nd series of bioassays was designed to test the combined, and possibly synergistic, effects of the plasticizers with either dieldrin or DDT. For this purpose, *D. melanogaster* flies were exposed to either 200 μg each of the plasticizers, 0.1 μg of dieldrin, or 5.0 μg of DDT. When insecticides only were used (controls), 0.1 ml of hexane containing 250 μg of corn oil was added to the insecticide solution in the bioassay jars to eliminate loss of dieldrin or DDT from the glass surfaces.

In addition, flies were exposed to the combined dry residue of 0.1 μg of dieldrin and 200 μg of either 1 of the plasticizers, or to the combined residue of 5.0 μg of DDT and 200 μg of 1 of the 11 plasticizers. These experiments were replicated 4 times, each replicate consisting of 50 *D. melanogaster* flies.

With house flies, 2 μliter of acetone containing either 0.015 μg of dieldrin, 0.5 μg of DDT or 10 μg of the plasticizers A, B, C, D, and E were applied topically to the ventral portion of 45 insects which were kept in groups of 15 within 3 bioassay jars. In addition, 2 μliter of acetone containing both dieldrin (0.015 μg) and 1 of the plasticizers (10 μg), or DDT (0.5 μg) and 1 of the plasticizers (10 μg) were applied as described. The treated house flies were then held for 24 hr when mortality counts were made. Statistical analyses of the data were performed with the t-test.

Chromatographic Behavior of Plasticizers.—To obtain some knowledge about the chromatographic behavior of the 11 plasticizers, they were dissolved in redistilled hexane and analyzed by gas-liquid chromatography (GLC) and thin layer chromatography (TLC). For comparison purposes the above-mentioned insecticides and metabolites were handled the same way.

For tests by GLC, 2 instruments with 2 different columns were employed in these investigations. One instrument was a Packard gas chromatograph, Model 7834, equipped with a 150-mCi tritium electron affinity ionization detector operated at 50 v. A 1.85-m glass column (4.0 mm ID) containing 5% SE 30 on 60/80 Chromosorb W AW-DMCS was conditioned for 7 days at 250°C before use. A column pressure of 25 psi of nitrogen resulted in a flow rate of 125 ml/min. The injector temperature was maintained at 240°C, the detector cell at 215°C, and the oven temperature at 190°C.

The 2nd instrument was a Jarrell-Ash gas chromatograph, Model 28-700 equipped with a 100-mCi tritium electron affinity ionization detector and operated at 20 v. A 1.22-m glass column (4.0 mm ID) containing a 1:1 mixture of 5% QF 1 and 5% DC 200 coated on Anakrom AS, 80- to 90-mesh, was conditioned for 7 days at 250°C before use. A column pressure of 16 psi of nitrogen gave a flow rate of 100 ml/min. The injector temperature was maintained at 250°C, the detector cell at 210°C, and the oven temperature at 190°C.

One μliter of hexane containing a mixture of insecticides (5×10^{-3} μg of lindane, 5×10^{-3} μg of heptachlor and aldrin, 1×10^{-1} μg of heptachlor epoxide, DDE, and dieldrin, 2×10^{-1} μg of DDT and TDE) was injected onto the column in each of the 2 gas chromatographs. After all of the insecticide peaks had been recorded, 2 μliter of hexane containing 1 of the 11 plasticizers (2×10^{-2} μg of A, B, C, D, E, or F, 5×10^{-2} μg of G or H, 5×10^{-2} μg of I, J, or K) were injected and tested in the same way. To compare the resulting chromatograms, retention times of the various peaks were calculated in reference to the retention time observed with aldrin.

For tests by TLC, 10 μg of each of the 11 plasticizers were spotted on aluminum oxide G-coated glass plates (20×20 cm), 2.5 cm above the lower edge. In addition, 10 μg of each of the insecticides and some of their metabolites were also spotted for reference. The chromatograms were then developed with 1% acetone in *n*-heptane, sprayed with reagent as described by Mitchell (1957) and exposed to UV light for 30 min.

RESULTS AND DISCUSSION.—*The Effect of Plasticizers on Insects.*—Since the polychlorinated biphenyls are of toxicological interest, both their toxicity to insects and their interaction with insecticides were studied in 2 series of experiments, as previously described.

(a) Dieldrin, DDT, or any one of the less chlorinated and more volatile plasticizers (A, B, C, D, E) were toxic to both test insects (Table 1). While these polychlorinated biphenyls contain increasing amounts of chlorine, their toxicity towards the insects decreased (A>B>C>D>E). Dieldrin was the most toxic of

Table 1.—Effect of plasticizers or insecticides on insect mortalities.

	D. melanogaster			House flies	
	Applied[a]	% mortality		Applied[b]	% mortality
Material	(μg)	24 hr	48 hr	(μg/g fly)	24 hr
None[c]		0	0		0
Dieldrin	0.1	31	84	1.0	73
p,p'-DDT	5.0	24	58	25.0	57
Plasticizers[d]					
A	200	0	0	500	17
	800	57	92	1000	43
B	200	0	0	500	10
	800	35	64	1000	30
C	200	0	4	500	17
	800	40	75	1000	20
D	200	0	0	500	13
	800	15	45	1000	13
E	2000	0	0	1000	10
F–K	2000	0	0	1000	0

[a] Applied in 8 ml hexane to 4-oz glass jars. *D. melanogaster* exposed to dry residue.
[b] Applied topically to house flies in 2 μliter of acetone. Weight of 1 fly = 20 mg.
[c] Controls were treated with solvents only.
[d] Letters A to K refer to Aroclors as listed in Table 3.

the compounds, as measured by the percent mortality of the insects within a 24- or 48-hr exposure period. Approximately 25 and 30 times more DDT than dieldrin, and 8000 and 1000 times more plasticizers than dieldrin had to be used to obtain similar mortalities of *D. melanogaster* or of house flies, respectively, within a given time of exposure to these chemicals. The more highly chlorinated and less volatile plasticizers were nontoxic even when used at dosages of 2000 μg with *D. melanogaster* and 20 μg/fly (1000 μg/g fly) with house flies.

(b) Table 2 summarizes the results obtained after the insects had been exposed to sublethal dosages (200 μg for *D. melanogaster*, 10 μg/house fly) of either 1 of the plasticizers A, B, C, D, or E and in combination with dieldrin or DDT. Though no appreciable insect mortalities were observed with plasticizers alone, they significantly increased the toxic effects of both dieldrin and DDT. This effect was most noticeable with DDT on house flies and differences observed were highly significant. After a 24-hr exposure period, 88% of the house flies had died after only DDT had been applied. However, all the insects were killed when DDT was applied in combination with any one of these plasticizers. The polychlorinated biphenyls also increased the toxic effect of DDT with *D. melanogaster*, but to a lesser extent. The most striking effect was observed with compound A. Plasticizers F, G, H, I, J, and K applied with dieldrin or DDT to glass surfaces to which *D. melanogaster* were exposed reduced the toxicity of the insecticide, resulting in mortalities of 30% to none within 48 hr.

In the absence of additional data, no conclusions should be drawn concerning the combined effects of plasticizers and insecticides in tissues of higher animals. However, plasticizers are in the environment (Holmes et al. 1967, Risebrough et al. 1968, Koeman et al. 1969) and their potential hazards, especially in combination with other synthetic chemicals, should not be disregarded.

Chromatographic Behavior of Plasticizers.—Results obtained after solutions of the 11 plasticizers had been analyzed with 2 different columns in separate gas chromatographs are summarized in Table 3. The observed retention times were compared as described with those obtained after the injection of lindane, heptachlor, heptachlor epoxide, aldrin, dieldrin, DDT, DDE, and TDE. The injection of nearly all of the plasticizers resulted in chromatograms with a multitude of peaks, sometimes as many as 15. Photographs of thin layer plates, prepared as previously described, are presented in Fig. 1 and 2. Since analyses of most plasticizers by GLC resulted in chromatograms with many peaks, it is conceivable that each of these plasticizers represents a multitude of compounds that were not separated by TLC as described, thus resulting in 1 or 2 spots only.

Table 2.—Combined effects of plasticizers and insecticides on insects.

Plasticizer[a]	% mortalities of insects after exposure to: No insecticide	Dieldrin	*p,p'*-DDT
	D. melanogaster after 48 hr[b]		
None[c]	0	66± 6	59±6
A	0	87± 4[d]	93±3[e]
B	0	81± 3[e]	84±6[e]
C	5±2	77± 9	83±6[e]
D	0	72± 4	77±3[e]
E	0	10± 3[e]	47±3[f]
	House flies after 24 hr		
None	0	27± 5	38±8
A	4	49± 3[e]	100[e]
B	4	27± 5	100[e]
C	0	47± 5[f]	98±3[e]
D	6	58±11[f]	100[e]
E	0	24± 6	100[e]

[a] Refers to Aroclors (A to E) as listed in Table 3.
[b] *D. melanogaster* were exposed in 4-oz glass jars to the dry surface deposit of the chemicals: plasticizers, 200 μg; dieldrin, 0.1 μg; DDT, 3.0 μg.
[c] Bioassay jars without plasticizers contained 250 μg of corn oil each.
[d, e, f] Differences observed between treatments with the insecticide only and those with insecticide plus plasticizer are significant at the d = 1%, e = 0.1%, or f = 5% level.
[g] Chemicals were applied topically in 2 μliter of acetone to house flies: plasticizers, 500 μg/g fly; dieldrin, 0.75 μg/g fly; DDT, 15 μg/g fly. Control: only acetone was applied. Weight of 1 fly = 20 mg.

Table 3.—Comparison of plasticizers and insecticides by GLC.

Plasticizer Aroclor		% weight chlorine	GLC I[a] No. of peaks	GLC I[a] Identical with[c]	GLC II[b] No. of peaks	GLC II[b] Identical with[c]
1221-2[d]	A	21	1	None	5	LI
1232-2	B	32	15	LI, HE, AL, DE, DI, TD, DT	15	LI, HE, HO, DE
1242-2	C	42	14	LI, HE, AL, DE, DI, TD,	14	LI, HE, HO, DE
1248-2	D	48	15	LI, HE, AL, DE, DI, TD, DT	15	LI, HE, HO, DE, DT
1254-2	E	54	11	AL, DE, DI, TD, DT	10	HO, DE, TD, DT
1260-2	F	60	15	TD, DT	13	DI, TD, DT
1262-2	G	62	12	TD	13	DI, TD, DT
1268-2	H	68	7	None	8	None
4465-2, 3	I	65	11	TD	11	DI
5442-3	J	42	3	None	7	LI, HE, HO
5460-3	K	60	3	None	0	None

[a] Packard gas chromatograph, column 5% SE 30 on 60/80 Chromosorb W. Oven temp = 190°C.
[b] Varrell-Ash gas chromatograph, column 5% QF-1, 5% DC 200 (1:1) on 80/90 Anakrom AS. Oven temp = 190°C.
[c] LI = Lindane, HE = Heptachlor, HO = Heptachlor epoxide, AL = Aldrin, DI = Dieldrin, DT = *p,p'*-DDT, DE = DDE, TD = TDE.
[d] P = chlorinated biphenyls, 3 = chlorinated triphenyls.

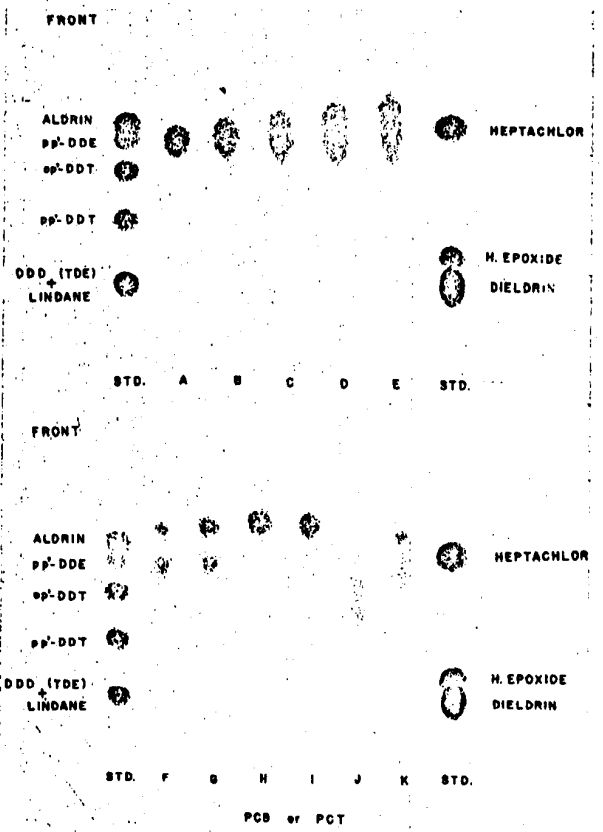

FIG. 1 (above).—Thin layer chromatogram of plasticizers A, B, C, D, and E on aluminum oxide G. Solvent: 1% acetone in n-heptane. STD = insecticide standards for comparison.

FIG. 2 (below).—Thin layer chromatogram of plasticizers F, G, H, I, J, and K on aluminum oxide G. Solvent: 1% acetone in *n-heptane*. STD = insecticide standards for comparison.

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Effect of Temperature on the Progeny Production and Longevity of *Lespesia archippivora*[1] in the Laboratory[2]

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ABSTRACT

The reproduction and the lifespan of the female tachinid parasite *Lespesia archippivora* (Riley) in the laboratory were directly related to temperature. The largest number of off-spring (425) was obtained from a single female held at 30°C; the means of total production ranged from 18 to 15°C to 225 at 25°C. When reproductive activity was measured as the mean number of puparia produced per oviposition day, activity appeared to peak (15.9) at 30°C. Mean female longevity ranged from 40 to 55 days at 15°C to 12 to 16 days at 35°C depending on the length of the daily oviposition period (4 or 6.5 hr). Most oviposition occurred before the 15th day after eclosion.

Lespesia archippivora (Riley) is a widely distributed indigenous parasite of many common lepidopterous pests of crops in North America, among which the bollworm, *Heliothis zea* (Boddie), and the tobacco budworm, *H. virescens* (F.), are clearly the most important. The prevalence and apparent climatic adaptability of this tachinid seem to give it great potential as a biological control agent if enough flies can be made available at a given time.

In our previous work with *L. archippivora* (Bryan et al. 1968) we described a rearing technique, reported the times required for development at several temperatures, and discussed the possibility of producing the parasite in the laboratory. The present paper describes research on larval production and length of female fly life, 2 factors which are of obvious importance in a mass-rearing program that would be necessary if inundative releases are undertaken.

METHODS AND MATERIALS.—Adult *L. archippivora* were obtained by the rearing technique described by Bryan et al. (1968) with beet armyworms, *Spodoptera exigua* (Hübner), as the laboratory host. Pairs of flies were removed from the rearing cages the day of eclosion and as they were mating. To facilitate determination of female longevity, the males were marked with a fluorescent pigment dissolved in alcohol, and each pair was placed in a separate cage. These cages were the lower halves of 3.8-liter cardboard food containers that had both ends screened with nylon (17× 17 mesh/2.54 cm²). A piece of sponge (moistened daily to provide water) and ½ sugar cube were placed in each cage and fastened to the bottom.

In the 1st test (1967), the pairs were placed in constant-temperature cabinets maintained at 15, 20, 25, 30, or 35±1°C immediately after they were caged. In the 2nd test (1968), each pair was caged, and all pairs were held at room temperature (25.6–27.8°C) for 2 days to permit oögenesis to proceed at the same pace. Then the pairs were placed in constant-temperature cabinets as before. In both tests, 4 replicates were tested simultaneously at each temperature.

The 4th day after the flies became adults and daily throughout the lifespan of the female, 20 4th- and/or 5th-instar beet armyworm larvae were placed on the upper (screened) surface of each cage and confined there for 6.5 hr (1st test) or 4 hr (2nd test) by placing the lid of a 150-mm-diam glass petri dish over them. The female flies oviposited through the nylon screen.

After each daily exposure the beet armyworm larvae were removed and placed in individual ½-oz clear plastic creamers partly filled with lima bean-agar diet (Shorey 1963, Bottger and Patana 1966) that were then covered with paper lids. Records were made daily of the life of the flies, the number of host larvae offered for oviposition, and the number of host larvae recovered. (Occasionally some were lost because of cannibalism or because they escaped.)

The creamers containing the host larvae were held at room temperature for 14 days. Then the degree of parasitization was determined by the presence or absence of fly puparia. The number of puparia produced was used as the measure of fecundity, because counting the eggs as they were laid was impossible, and dissecting each host larva to determine the number of maggots it contained was impractical. This method of assessment probably underestimates the

[1] Diptera: Tachinidae.

[2] In cooperation with the University of Arizona Agricultural Experiment Station, Tucson 85721. Accepted for publication Feb. 12, 1969.