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# Increase in the Toxicity of Organophosphorus Insecticides to House Flies Due to Polychlorinated Biphenyl Compounds[1–3]

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Increase in the Toxicity of Organophosphorus Insecticides to House Flies Due to Polychlorinated Biphenyl Compounds. FUHREMANN, T. W. and LICHTENSTEIN, E. P. (1972). *Toxicol. Appl. Pharmacol.* 22, 628–640. The polychlorinated biphenyl (PCB) Aroclor® 1248—although nontoxic by itself to house flies at doses of 10 μg/fly—significantly increased the toxicity of the oxygen analogs of 5 organophosphorus insecticides after simultaneous topical application to houseflies. Both *p,p'*-DDT and *p,p'*-DDE, applied at sublethal doses, also increased the toxicity of paraoxon, as measured by an increase in the mortality of house flies from 2 to 60 and 73%, respectively. The PCB, applied simultaneously with parathion, however, decreased the toxicity of parathion to house flies, but increased mortalities when applied to flies 0.5 hr after the application of the insecticide. Aroclor® 1248 apparently inhibited the conversion of parathion to its toxic activation product, paraoxon, and the breakdown of paraoxon into nontoxic compounds. This was also indicated by the recovery of larger amounts of $^{14}$C-paraoxon and smaller amounts of water soluble $^{14}$C-products from flies treated simultaneously with Aroclor® 1248 plus paraoxon in comparison to those which had been treated with $^{14}$C-paraoxon only. Utilizing the 10,000 *g* supernatant from house fly homogenates, the addition of Aroclor® 1248 with $^{14}$C-paraoxon in vitro also resulted—after 2 hr incubation at 30°C—in higher recoveries of $^{14}$C-paraoxon and in a reduction of the appearance of water soluble $^{14}$C-products. Identical results were obtained with subcellular fractions (10,000 *g* supernatant) from flies treated with Aroclor® 1248 18 hr before homogenate preparation: no difference was observed in the degradation of paraoxon or the appearance of water soluble products with Aroclor® applied to the living flies or to the 10,000 *g* supernatant of homogenates from untreated flies. Since PCBs are in the environment, their potential effects on biological systems, especially in combination with other synthetic chemicals, should not be disregarded.

Polychlorinated biphenyl compounds (PCBs) produced in the U.S. by the Monsanto Chemical Co. under the name Aroclor®, are currently receiving the attention of ecologists, toxicologists and pesticide analysts because of their apparent ubiquity and coincidental occurrence with chlorinated insecticide residues in the environment

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(Holmes *et al.*, 1967; Widmark, 1967; Risebrough *et al.*, 1968; Koeman *et al.*, 1969; Peakall and Lincer, 1970). Many PCB compounds can interfere with the analysis of a number of chlorinated hydrocarbon insecticides (Widmark, 1967; Lichtenstein *et al.*, 1969; Reynolds, 1969, 1971). Litterst and Lichtenstein (1971) reported that the PCB, Aroclor® 1254, exhibited a toxicity similar to that of DDT after human HeLa cells and skin fibroblasts in tissue culture had been exposed to this compound.

Utilizing insects as test organisms, Deonier *et al.* (1946) reported that several Aroclor® compounds were toxic to the larvae of *Anopheles quadrimaculatus* (Say) at 1 ppm in water. Tsao *et al.* (1953) reported that Aroclor® 5460 greatly increased the residual toxicity of lindane, but had no toxic effect by itself to *Musca domestica* (L.). They also reported "some indication that this chlorinated polyphenyl may have a synergistic effect with lindane." "Chlorinated polyphenyls" were also recommended in 1953 for "improving lindane residues" (Hornstein and Sullivan, 1953). The authors stated that "lindane residues can be made to last longer and look better" if applied with chlorinated polyphenyls. Aroclor® 5460 was later reported to exhibit toxicity to the elm leaf beetle *Galerucella xanthomelaena* (Schr.) (Duda, 1957).

The biological interaction and synergistic effects of PCBs on DDT and dieldrin in insects was shown by Lichtenstein *et al.* (1969). These authors reported that the toxicity of PCBs to house flies and fruit flies (*Drosophila*) decreased with an increasing degree of chlorination and appeared to be less toxic than dieldrin by a factor of at least 1000. It was found, however, that sublethal doses of several PCBs increased the toxicity of DDT and dieldrin. As a continuation of this study, the effects and interactions of selected PCB compounds with some organophosphorus insecticides on and in house flies were investigated.

## METHODS

### *Chemicals*

Insecticides used in this study were analytical grade oxygen analogs of azinphosmethyl, of Diazinon® (diazoxon), of Dyfonate® (dyfoxon), of malathion (malaoxon), and of parathion (paraoxon). Parathion, *p,p'*-DDE and *p,p'*-DDT were also used. $^{14}$C-Parathion, labeled in the 2,6 ring positions (specific activity 0.58 mCi/mmole) was purchased from the Tracerlab Co., Waltham, Massachusetts. $^{14}$C-paraoxon was prepared by oxidation of $^{14}$C-parathion with bromine water. For this purpose, 5 mg $^{14}$C-parathion (11.3 $\mu$Ci) was placed in a 15 ml, glass-stoppered test tube with 10 ml distilled water and 5 drops of reagent grade bromine. The contents of the tube were shaken for 1 min. Nitrogen was bubbled through the solution until it become completely colorless. The solution was then extracted 5 times with 10 ml portions of chloroform, which were pooled and dried over anhydrous sodium sulfate. To verify the presence of $^{14}$C-paraoxon, the chloroform solution was subjected to thin layer chromatography (TLC) and autoradiography as described below.

Isolates from the thin layer plate that had the same $R_f$ value as reference grade paraoxon were eluted with acetone and analyzed by gas–liquid chromatography (GLC) and liquid scintillation counting (LSC). Results by GLC and LSC were in close agreement and indicated an 80% yield of $^{14}$C-paraoxon.

The PCB compounds used in this study were Aroclor® 1248 and Aroclor® 1268, containing 48 and 68% chlorine by weight, respectively. They had been obtained through

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the courtesy of Dr. R. F. Thomas, USDA, Beltsville, Maryland. NADPH was purchased from Nutritional Biochemical Co., Cleveland, Ohio.

Solvents used were redistilled acetone, chloroform, *n*-hexane and reagent grade, absolute; ethanol, methanol and diethyl ether.

*Insects and the Application of Chemicals*

Two strains of house flies, *Musca domestica* (L.) were used in this study: CSMA, 1948 strain (susceptible to DDT) was obtained from the Wisconsin Alumni Research Foundation, Madison, Wisconsin, and a second strain ($P_3$/sel), resistant to DDT and naphthalene vapors was obtained from Dr. M. Agosin, University of Georgia, Athens.

Two microliters of acetone containing an appropriate amount of the test compound were topically applied with an Isco model M-420 microapplicator onto the abdominal sternites of 2–3 day old female house flies anesthetized with $CO_2$. After treatment, 3 replicates each consisting of 15 flies were held in glass jars for various time periods indicated for each experiment. Flies which had not revived after 30 min were disregarded in subsequent mortality counts. Mortality counts or further experimentation were then performed as outlined below.

*Extraction and Analysis*

Several extraction procedures, described separately with each experiment, were used in these studies.

*Gas–liquid chromatography* was employed for quantitative and qualitative analyses of insecticides and their potential degradation products in the hexane or ether extraction phases. A Packard Model 7834 gas chromatograph equipped with a 1.22 m × 3 mm i.d. Pyrex glass column containing 5% Dow Corning 11 silicone grease coated on 60/80 mesh, acid washed, dimethyldichlorosilane treated, chromosorb W was maintained at a temperature of 180°C and a helium carrier flow rate of 60 ml/min. A potassium chloride thermionic detector, especially sensitive to phosphorus, was modified and operated as described by Giuffrida *et al.* (1966).

*Thin layer chromatography* was performed to evaluate qualitatively the hexane or ether phases of extracts obtained in these studies. These phases were concentrated to approximately 0.05 ml and analyzed by TLC and autoradiography as described by Lichtenstein and Fuhremann (1971).

*Liquid scintillation counting* was performed to determine the radiocarbon content of the various samples by utilizing a Packard model 3320 liquid scintillation spectrometer. One milliliter aliquots of hexane extracts of samples were placed in 14 ml of a toluene-based counting solution while 1 ml aliquots of water phases and ether or acetone extracts were counted in 14 ml of a dioxane-naphthalene based counting solution. Data were corrected for background, counter efficiency and dilutions and expressed as total dpm per extraction phase.

## EXPERIMENTAL

*Effects of PCBs on the Toxicity of Insecticidal Compounds*

To determine the effect of a PCB compound on the toxicity of organophosphorus insecticides, CSMA strain *Musca domestica* were treated as described with an insecticide,

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with Aroclor® or simultaneously with both. For this purpose 3 replicates, each consisting of 15 flies, were topically treated with either 0.02 μg of parathion or paraoxon, 0.04 μg of diazoxon, 0.08 μg of dyfoxon or azinphosmethyl oxygen analog or 0.30 μg of malaoxon per fly. In addition 45 flies were treated with 10 μg Aroclor® 1248 per fly. Finally 45 flies were simultaneously treated with either one of the insecticides at the above mentioned concentrations, plus Aroclor® 1248 (10 μg/fly). Mortality counts were performed after a holding period of 24 hr. These experiments, repeated on different dates, were conducted with paraoxon 6 times, with parathion 4 times and twice with each of the other compounds. The percent mortality of the insects was expressed as averages of the mortality counts from different tests with SD or deviations from the mean.

Because the susceptibility of flies to toxicants varies over a certain period, differences in mortalities observed due to the application of parathion or paraoxon only, or due to the simultaneous application of one of these insecticides with Aroclor® 1248, were evaluated with the paired *t* test (Steel and Torrie, 1960). In this way data obtained on different days between a control and one variable could be compared.

Utilizing DDT resistant flies ($P_1$/sel strain), identical tests as described above were conducted except that paraoxon was applied at 0.04 μg/fly and Aroclor® 1248 at 10 μg/fly.

To test the effects of a different PCB compound on CSMA flies, a similar test was conducted with Aroclor® 1268 (10 μg/fly) and with paraoxon (0.05 μg/fly).

To determine the LD50 of paraoxon with house flies as affected by Aroclor® 1248, CSMA flies were treated as described with serial dilutions of paraoxon to which none or a standard amount (10 μg/fly) of Aroclor® 1248 was added. Flies were observed over a 10 hr period. Mortality data obtained after 4 hr were used to plot eye-fitted dose mortality curves.

To determine whether PCBs have a residual effect on the toxicity of paraoxon, CSMA flies were treated as described with Aroclor® 1268 (10 μg/fly) and held for 2 days in 12 × 12 in. screen cages. Control flies were treated with acetone only. After this holding period both groups of flies were treated with paraoxon (0.05 μg/fly). Mortalities were recorded after a 24 hr holding period.

*The Effects of Various Quantities of Aroclor® 1248, DDT or DDE on the Toxicity of Paraoxon to House Flies*

To determine the effect of various levels of Aroclor® 1248 on the toxicity of paraoxon to house flies, CSMA strain flies were treated simultaneously with 0.02 μg paraoxon plus 0.1, 0.5, 2, 4, 6, 8 or 10 μg of Aroclor® 1248 per fly. These flies were held for 24 hr, when mortalities were recorded.

To determine whether DDT and DDE, which are structurally similar to the PCBs would also affect the toxicity of paraoxon to CSMA strain *Musca domestica*, 3 groups of flies were treated with various amounts of either *p,p'*-DDT, *p,p'*-DDE or Aroclor® 1248. Another group of flies was treated with 0.02 μg paraoxon per fly, and 3 additional groups of flies were treated with 0.02 μg paraoxon per fly plus either Aroclor® 1248, *p,p'*-DDT or *p,p'*-DDE (Table 2). These flies were then held for 24 hr when mortality counts were performed.

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*Mode of Action of Aroclor® 1248*

Since the toxic effects of parathion on insects is primarily due to the formation of paraoxon in the insect body (Metcalf and March, 1953), experiments were conducted to investigate the effects of Aroclor® 1248 on this process.

*The effect of Aroclor® 1248 on house fly mortalities after in vivo conversion of parathion to paraoxon* was determined by treating flies with parathion and—at intervals thereafter—with Aroclor® 1248. If PCB would primarily effect paraoxon, this effect would only be noticeable at some interval after fly treatment with parathion, during which time paraoxon could have been formed in vivo. Tests were therefore conducted with CSMA flies which were treated with 0.02 $\mu$g of parathion per fly. Immediately thereafter, and also $\frac{1}{2}$, 2 or 4 hr later, groups of 45 flies were treated with 10 $\mu$g of Aroclor® 1248/fly or with acetone only (solvent control). Each group of 45 flies (15 flies per bioassay jar) was then held for 24 hr at which time mortality counts were performed.

*The distribution and persistence of paraoxon in house flies* as affected by Aroclor® 1248 was determined in another series of 5 separate experiments conducted at different dates. For this purpose, 1 group of 500 female CSMA house flies was topically treated with 0.02 $\mu$g ($4.5 \times 10^{-3}$ $\mu$Ci) $^{14}$C-paraoxon per fly and another group of 500 flies with 0.02 $\mu$g $^{14}$C-paraoxon plus 10 $\mu$g Aroclor® 1248 per fly. Each group was then held for 2 hr in 1000 ml cheesecloth-covered Pyrex beakers. At the end of the holding period the radiocarbon content and the amount of paraoxon on the outer surfaces of the insect, the inside of the insect and on the inner glass surfaces of the beakers were determined.

For this purpose, the flies of each group were anesthetized with $CO_2$ and then placed into 250 ml glass stoppered Erlenmeyer flasks. To remove external residues with acetone, each group of flies was shaken 3 times with 75 ml portions of acetone. These acetone rinses were quantitatively decanted through a Büchner funnel, pooled and designated "External."

The rinsed flies were then macerated with 50 g of anhydrous sodium sulfate in a Waring Blendor and homogenized 3 times with 100 ml of chloroform. The chloroform extracts of each group were dried over anhydrous sodium sulfate and evaporated on a flash evaporator at 35°C. The residue was then redissolved in *n*-hexane and designated "Internal."

The chloroform extracted fly pulp was then dried overnight at 50°C, its weight was determined and aliquots were combusted in an oxygen filled Schöniger flask (Kelly *et al.*, 1961). The liberated $^{14}CO_2$ was absorbed in 3 ml phenethylamine : methanol (1 : 2). This solution was then dissolved in 14 ml of toluene-based counting solution for determination of the radiocarbon content by LSC. Results obtained from the combusted fly material were then designated as "Bound" radioactivity.

Finally the residue on the inside walls of the beakers in which the treated flies had been held was removed by first scrubbing the beaker walls with 10 ml water (using the cheesecloth beaker covers) and then with five 25-ml portions of acetone. The water and acetone were combined and concentrated in a flash evaporator at 35°C until most of the acetone was eliminated. The remaining water (and acetone) were then adjusted to volume, and aliquots were used for the determination of their radiocarbon content. Results obtained by this procedure were designated as "Excreted" radioactivity.

It had been determined in preliminary experiments that, after adjusting the pH of the water to 1.5 with 2 N HCl, extraction with *n*-hexane or diethyl ether did not remove any

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radioactivity from the water. Paraoxon, added to aliquots of this water, however, was extracted with both hexane and diethyl ether. For additional analyses by TLC and autoradiography, the acidified water was evaporated to dryness on a flash evaporator at 40°C, and the residue was redissolved in methanol.

*Degradation of paraoxon by homogenates of house flies as affected by Aroclor® 1248.* In the experiments described above, Aroclor® 1248 and paraoxon had been applied to the living insect. To further elucidate the mode of action and/or the effects of Aroclor® 1248 on the persistence of paraoxon in house flies, experiments were conducted in vitro. Flies were treated with Aroclor® 1248, held for 18 hr, and paraoxon was added to the 10,000 *g* supernatant prepared from homogenates of these flies. Homogenates were also prepared from acetone treated (solvent control) flies, and both the Aroclor® and paraoxon were added simultaneously to the 10,000 *g* supernatant prior to incubation.

In preliminary experiments 400 female CSMA flies were each topically treated with 10 μg Aroclor® 1248 as described. Control flies were treated with acetone only. The 2 groups of flies were then held for 18 hr at room temperature. After that time the dead flies were discarded, while the living flies were anesthetized with $CO_2$ and frozen over dry ice to facilitate removal of the abdomens from the insect bodies; 300 abdomens from each group were used for enzyme preparation at 0.4°C. The abdomens were homogenized in a 50 ml conical, ground glass tissue grinder (Bellco Glass, Vineland, New Jersey) with 30 ml grinding medium consisting of 0.15 M sodium phosphate buffer plus 0.25 M sucrose and 0.2% bovine serum albumin-fraction V (Grand Island Biological Co., Grand Island, New York). The pH was 7.3. The homogenates (10 abdomens/ml grinding medium) were then centrifuged at 1500 *g* for 2 min. After the floating material and substances adhering to the upper walls of the centrifuge tubes had been resuspended in the supernatant, this supernatant was centrifuged at 10,000 *g* for 20 min in a Beckman model L2-50 ultracentrifuge. The resulting supernatant, along with resuspended floating and adhering substances served as the enzyme source. To demonstrate that the degradation of paraoxon was an enzyme catalyzed reaction, tests were conducted both in the absence of insect homogenate and also with the 10,000 *g* supernatant which previously had been heated for 10 min in boiling water to denature the protein. Replicate incubation mixtures consisted of 3 ml of boiled or unboiled enzyme preparation to which 300 μg NADPH ($1.3 \times 10^{-4}$ M) and 50 μg (0.113 μCi) of $^{14}$C-paraoxon contained in 10 μl ethanol were added. These incubation mixtures, contained in aluminum foil-covered 10 ml Erlenmeyer flasks, were then incubated for 4 hr in the dark in a 30°C water bath rotary shaker (120 rpm). To terminate the reaction 5 ml of acetone was added to each mixture, which was then quantitatively transferred with 2 5-ml portions of water and 5 ml of acetone into a 60 ml separatory funnel. These incubation mixtures were then extracted with three 10-ml portions of *n*-hexane and then with 3 10-ml portions of diethyl ether resulting in a water-acetone phase and an organic solvent phase. The organic solvent phase was dried over anhydrous sodium sulfate and adjusted to volume. All fractions were then analyzed by LSC for their radiocarbon content, while the organic solvent phases were also analyzed by GLC, TLC and autoradiography.

The final experiments were conducted at a different time with another group of flies. These tests were performed the same way as described above, but in addition 200 μg Aroclor® 1248 in 10 μl ethanol was added to each replicate homogenate of control flies (acetone pretreated) simultaneously with paraoxon just prior to incubation. This was

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done to determine whether a time lapse after the in vivo application of Aroclor® was necessary to affect the degradation of paraoxon, or if the addition of this PCB to the prepared enzyme would cause a similar effect in vitro. Differences due to Aroclor® treatment were evaluated with the *t* test (Steel and Torrie, 1960).

## RESULTS AND DISCUSSION

### *Effects of PCBs on the Toxicity of Insecticidal Compounds*

Data presented in Table 1 indicated that at a dose of 10 μg/fly, Aroclor® 1248 by itself was not toxic to female CSMA house flies. However, mortalities of flies due to paraoxon were increased from 5% (without Aroclor® 1248) to 95% (with Aroclor® 1248). Conversely, this PCB reduced the mortality due to parathion. Differences observed in mortalities due to Aroclor® 1248 applied with paraoxon were significant at the 0.1% level and with parathion at the 2% level. These data might, therefore, indicate that Aroclor® 1248 inhibited the in vivo conversion of parathion to paraoxon and the detoxification of paraoxon itself. Mortalities observed after the application to flies of

TABLE 1

EFFECT OF THE POLYCHLORINATED BIPHENYL, AROCLOR® 1248, ON THE TOXICITY OF INSECTICIDAL COMPOUNDS AFTER TOPICAL APPLICATION TO *Musca Domestica*[a]

| Insecticide[b] | μg/fly | Aroclor® 1248 None (control) (% mortality/24 hr) | Aroclor® 1248 10 μg/fly (% mortality/24 hr) | % Control[c] |
|---|---|---|---|---|
| None | — | — | 0 | — |
| Paraoxon | 0.02 | 5 ± 5 | 95 ± 6[d] | 1900 |
| Parathion | 0.02 | 16 ± 5 | 2 ± 2[e] | 13 |
| Diazoxon | 0.04 | 28 ± 2 | 86 ± 4 | 313 |
| Dyfoxon | 0.08 | 4 ± 3 | 68 ± 16 | 1700 |
| P=O-Azinphosmethyl | 0.08 | 50 ± 12 | 96 ± 2 | 192 |
| Malaoxon | 0.30 | 29 ± 19 | 83 ± 6 | 286 |

[a] Insecticide and/or Aroclor® applied topically to the ventral portion of the abdomen.
[b] Total number of tests (each 3 times replicated—15 flies per replicate) were 6 for the control (None) and with paraoxon, 4 with parathion and 2 with each of the remaining compounds. Results are expressed as mean ± SD.
[c] % Control = Mortality in % of mortality observed with insecticide but no Aroclor® (None—control)
[d,e] Differences in mortality due to Aroclor® treatment are significant at the 0.1% level (d), or the 2% level (e) as determined by the paired *t* test.

the oxygen analogs of other commonly used organophosphorus insecticides were also substantially increased when applied in combination with Aroclor® 1248. Since the effects of this PCB were most pronounced with paraoxon, this compound was selected as a model for further study.

Similar results were obtained with another strain of *Musca domestica* ($P_2$/sel). Aroclor® 1268, a PCB with a chlorine content of 68%, also increased fly mortalities

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(% dead within a 24 hr exposure period) due to paraoxon by a factor of 2.7 as compared to flies that were treated with paraoxon only.

When the effect of Aroclor® 1248 on the LD50 of paraoxon was investigated, it was found that PCB increased the toxicity of paraoxon by a factor of 4 (Fig. 1), resulting in an LD50 of 0.013 μg paraoxon/fly. Mortalities observed after paraoxon only had been applied did not further increase 2 hr after the insecticidal application. However, mortalities observed with the application of comparatively lower levels of paraoxon *plus* Aroclor® continued to increase up to about 10 hr, indicating a possible inhibition of the rapid detoxification of paraoxon.

When Aroclor® 1268 was applied 2 days prior to paraoxon treatment, mortalities were increased from 16% (acetone pretreated) to 81% (Aroclor® pretreated) 24 hr after paraoxon application. This would indicate a residual effect of PCBs on paraoxon toxicity to house flies.

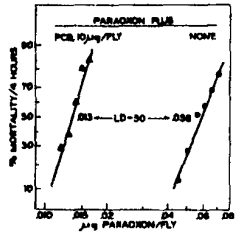


FIG. 1. Dose-mortality curves obtained 4 hr after application of paraoxon only or of paraoxon plus PCB (Aroclor® 1248) to *Musca domestica*.

*The Effects of Various Quantities of Aroclor® 1248, DDT or DDE on the Toxicity of Paraoxon to House Flies*

Results obtained after paraoxon or paraoxon plus increasing amounts of Aroclor® 1248 had been applied to house flies are presented in Fig. 2. Topical application of 0.02 μg paraoxon caused only a 6% mortality of house flies over a 24 hr period while the

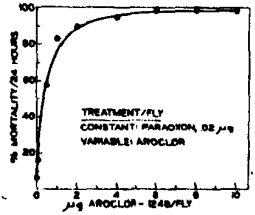


FIG. 2. Effect of increasing amounts of Aroclor® 1248 on the toxicity of a constant amount of paraoxon (0.02 μg/fly) simultaneously applied to female *Musca domestica*.

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addition of increasing amounts of Aroclor® 1248 with a constant amount of paraoxon (0.02 μg/fly) increased the toxicity of paraoxon in a nearly linear mode. Two μg of this PCB were sufficient to increase fly mortality due to paraoxon to 90%.

The effect of Aroclor® compounds in increasing the toxicity of paraoxon to house flies also occurs with structurally related chlorinated aromatics (Table 2). *p,p'*-DDT, which is essentially nontoxic to house flies at 0.05 μg/fly, increased the mortalities due to paraoxon. *p,p'*-DDE, which was nontoxic to flies even at a level of 5 μg/fly, increased the fly mortality due to 0.02 μg of paraoxon when applied at a level of 0.1 μg DDE per fly. These data illustrate the potential effects and interactions of environmental chemicals in biological systems. DDE, a widely distributed metabolite of DDT in the environment was at least as effective as its parent compound in increasing the toxicity of paraoxon to flies.

TABLE 2

EFFECTS OF THE CHLORINATED AROMATICS, AROCLOR® 1248, *p,p'*-DDT OR *p,p'*-DDE, ON THE TOXICITY OF PARAOXON TO *Musca Domestica*[a]

| Paraoxon (μg/fly) | Chlorinated aromatic (μg/fly) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | None | Aroclor® | | | *p,p'*-DDT | | *p,p'*-DDE | | |
| | — | 0.1 | 1.0 | 5.0 | 0.05 | 0.1 | 0.1 | 1.0 | 5.0 |
| | % Mortality of house flies/24 hr[b] | | | | | | | | |
| None | — | 0 | 0 | 0 | 2 | 17 | 2 | 2 | 0 |
| 0.02 | 6 | 16 | 88 | 100 | 60 | 55 | 73 | 86 | 90 |

[a] Paraoxon and/or chlorinated aromatics applied topically to the ventral portion of the abdomen.
[b] Average of 3 replicates, each consisting of 15 flies.

*Mode of Action of Aroclor® 1248*

The role of Aroclor® in decreasing the toxicity of parathion and in increasing that of paraoxon (Table 1) indicated possible effects on enzymatic processes. If PCBs inhibit enzymatic degradation of these insecticides, it could explain the divergent effects with parathion and paraoxon. It is generally accepted that the toxicity of parathion is due to its in vivo conversion to paraoxon (Metcalf and March, 1953). Assuming that the PCB inhibited both the enzymatic conversion of parathion to paraoxon and the degradation of paraoxon itself, this would explain the above mentioned findings.

*The effect of Aroclor® 1248 on house fly mortalities after in vivo conversion of parathion to paraoxon.* Results obtained in experiments where flies had been treated with parathion (0.02 μg/fly) and simultaneously with Aroclor® 1248 (10 μg/fly) demonstrated that the PCB reduced the toxicity of parathion as indicated by a decrease in mortality from 18 to 0%. However, when flies were treated with Aroclor® 30 min after the parathion application, fly mortalities were 4 times greater than with control flies treated with acetone (solvent controls) 30 min after the application of parathion. No further substantial increase in fly mortalities was observed when Aroclor® was applied 2 or 4 hr after fly treatment with parathion. Since parathion is converted to paraoxon during the holding period, the subsequent treatment with Aroclor® possibly inhibited the

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degradation of paraoxon and thereby increased its toxic action, resulting in increased mortalities.

*The distribution and persistence of* $^{14}$*C-paraoxon on and in house flies*, due to the presence of Aroclor® 1248, is presented in Table 3. In the presence of Aroclor® 1248, paraoxon was more persistent. This was demonstrated by GLC analyses which showed that in the absence of Aroclor® (None–Table 3) only 7 and 6% of the applied paraoxon were recovered from the outside ("External") and the inside ("Internal") of the flies, respectively. In the presence of Aroclor® 1248, however, 18% of the applied paraoxon was recovered from the fly rinsings ("External") and 16% was recovered from fly extracts ("Internal"). Data based on radiocarbon content also indicated similar results, although $^{14}$C-compounds other than paraoxon are included in these values.

TABLE 3

EFFECTS OF AROCLOR® 1248 ON THE FATE OF $^{14}$C-PARAOXON IN *Musca domestica* (IN VIVO)

| Applied[a] paraoxon plus | Recovered[b] in percent of applied from housefly fractions | | | | | |
|---|---|---|---|---|---|---|
| | | External[c] | Internal[c] | Bound[c] | Excreted[c] | Total |
| None | $^{14}$C-DPM | 15 ± 6 | 17 ± 5 | 3 ± 1 | 36 ± 6 | 71 |
| | Paraoxon | 7 ± 2 | 6 ± 4 | — | ND[g] | 13 |
| Aroclor® | $^{14}$C-DPM | 21 ± 8 | 26 ± 5[e] | 5 ± 2 | 18 ± 5[e] | 70 |
| | Paraoxon | 18 ± 9[f] | 16 ± 8[f] | — | ND[g] | 34 |

[a] Topically applied $^{14}$C-paraoxon (0.02 μg = 4.5 × $10^{-4}$ μCi/fly) or $^{14}$C-paraoxon plus 10 μg Aroclor® 1248 per fly.

[b] Data were obtained 2 hr after house fly treatment, 500 flies/treatment per test. Results are averages of 4–5 tests ± SD. Paraoxon determined in μg by GLC.

[c] External = Acetone rinses of whole flies; Internal = chloroform extracts of homogenized flies after external rinses; Bound = nonextractable $^{14}$C determined by combustion of extracted flies; Excreted = water-acetone rinses of fly holding jars after the 2 hr holding period.

[e,f] Differences in recoveries due to Aroclor® treatment are significant at the 1% (d), 0.1% (e) or 5% (f) level as determined by paired *t* test.

[g] No paraoxon was detected.

Water soluble products of the originally applied $^{14}$C-paraoxon were obtained from the inside surface of the holding jars as described. These $^{14}$C-products were not extractable with *n*-hexane or diethyl ether at a pH of 1.5, although added amounts of $^{14}$C-paraoxon could be fully extracted by this method. This indicated that the "excreted" materials were not paraoxon. In addition, analyses by TLC and autoradiography of the water soluble products indicated a radioactive spot with the same $R_f$ value as that of authentic amino paraoxon. This, however, could not be confirmed by spraying the plate with color reagents specific for amino compounds (Lichtenstein and Fuhremann, 1971). Results obtained by LSC showed that the amount of water soluble $^{14}$C-products which had been "excreted" by flies treated with paraoxon only was twice the amount "excreted" by those flies treated with $^{14}$C-paraoxon plus Aroclor® 1248. The total amount of paraoxon (measured by GLC) recovered from the "External" and "Internal" fractions was 13% of the applied dosage in the absence of Aroclor® 1248, but amounted to 38% in its presence (Table 3). These data demonstrate that the presence of Aroclor®

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1248 on or in house flies prevented the breakdown of $^{14}$C-paraoxon into unidentified water soluble products, thereby causing greater insect mortalities.

The total recoveries of $^{14}$C-compounds from control and Aroclor® treated flies were identical (70 and 71%) (Table 3). The unaccountable radioactivity may have volatilized from the surface of the treated flies or been lost during the process of individual treatment of 500 flies and subsequent experimental procedures.

*Degradation of paraoxon by homogenates of house flies as affected by Aroclor® 1248.* Preliminary studies with $^{14}$C-paraoxon treated house fly homogenates indicated that no degradation of paraoxon occurred in the absence of a fly homogenate or with boiled fly homogenates. In both cases, all the paraoxon and radiocarbon added to the incubation mixtures was recovered as determined by GLC and LSC, respectively. With unboiled homogenates from flies treated 18 hr previously with acetone (controls), however, only $17 \pm 1$% of the applied paraoxon and $31 \pm 3$% of the applied radiocarbon were recovered from the organic extraction phases of the incubation mixtures. This indicated that degradation of paraoxon to water soluble compounds had occurred with non-boiled homogenates. When homogenates from Aroclor® treated flies were used, more paraoxon ($27 \pm 5$% of applied) and more radiocarbon ($41 \pm 0$% of applied) were recovered from the organic solvent phases, indicating that Aroclor® inhibited the degradation of paraoxon.

The water phase, however, contained less radiocarbon when incubation mixtures from Aroclor® treated flies were used, as compared to incubation mixtures from acetone treated flies (controls). This appears to be additional evidence of reduced activity of homogenates from Aroclor® treated house flies.

In the final series of experiments, Aroclor® 1248 had been applied either to houseflies ("in vivo," Table 4) which were held for 18 hr prior to the preparation of homogenates, or to homogenates ("in vitro", Table 4) prepared from acetone (control) treated house flies. Results in Table 4 demonstrate that in the absence of an enzyme preparation or with the boiled 10,000 *g* supernatant from the fly homogenate nearly all the paraoxon and radiocarbon applied to the incubation mixtures was recovered in the hexane: ether extracts. However, with homogenates from untreated flies (controls), only 33% of the applied paraoxon and 36% of the applied radiocarbon were recovered from the organic extraction phases of incubation mixtures. When either Aroclor® was added with paraoxon to homogenates from untreated flies, or when homogenates were prepared from Aroclor® treated flies, results were similar (Table 4) to each other. The amount of paraoxon recovered from homogenates containing Aroclor® 1248 was 1.6 times greater than amounts recovered from homogenates without Aroclor®. The water phases derived from samples where no homogenate was present or from boiled homogenates did not contain appreciable radiocarbon; however, the water phases derived from unboiled homogenates of untreated flies contained 56% of the totally applied radiocarbon (Table 4). When Aroclor® was present in the homogenates, the radiocarbon content of the water phases was only 40–43% of the total application. These data indicate that Aroclor® 1248 increased the persistence of paraoxon and decreased the formation of water soluble metabolites. However, no difference was observed in the degradation of paraoxon or the appearance of water soluble products when either Aroclor® was applied to the living house flies or when Aroclor® was added to the 10,000 g supernatant of homogenates from untreated flies.

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TABLE 4

EFFECT OF AROCLOR® 1248 ON THE IN VITRO DEGRADATION OF $^{14}$C-PARAOXON WITH HOUSE FLY HOMOGENATES

| Enzyme source | Recovered as % of applied from extraction phases: Hexane:ether $^{14}$C[a] | Hexane:ether P=O[b] | Hexane:ether %CK[c] | Water $^{14}$C | Water %CK[d] | Total $^{14}$C |
|---|---|---|---|---|---|---|
| None | 99 ± 2 | 95 ± 2 | 288 | 3 ± 0 | 5 | 102 ± 2 |
| Untreated flies,[e] boiled | 99 ± 1 | 97 ± 0 | 294 | 3 ± 0 | 5 | 102 ± 1 |
| Untreated flies[e] (CK) | 36 ± 2 | 33 ± 3 → | 100 | 56 ± 1 → | 100 | 93 ± 3 |
| Aroclor® treated (in vivo) flies[f] | 55 ± 2[g] | 54 ± 1[g] | 164 | 40 ± 2[g] | 71 | 94 ± 1 |
| Aroclor® in vitro[h] | 52 ± 2[g] | 52 ± 1[g] | 158 | 43 ± 0[g] | 76 | 95 ± 2 |

[a] $^{14}$C-dpm determined by LSC. Results are averages of replicated tests.
[b] Paraoxon determined by GLC. Results are averages of replicated tests.
[c] Paraoxon in % of the amount recovered from homogenates of controls: untreated flies (CK = 100%).
[d] $^{14}$C-dpm in % of radiocarbon recovered from the water phase of homogenates of controls: untreated flies (CK = 100%).
[e] Control flies, topically treated with acetone only 18 hr before preparation of the 10,000 *g* supernatant from fly homogenates.
[f] Aroclor® 1248 was topically applied to house flies 18 hr before the preparation of the 10,000 *g* supernatant from fly homogenates.
[g] Differences due to Aroclor® treatment are significant at the 1 or 5% level (*t* test).
[h] Aroclor® 1248 was added directly to the 10,000 *g* supernatant from fly homogenates.

After analyses of the organic extracts by TLC and autoradiography, radioactive spots were observed that corresponded to paraoxon and to aminoparaoxon. Color confirmation using reagents specific for amino compounds gave a positive reaction for the "aminoparaoxon" spot. Because of interfering substances in the water phase after the final ether extraction, no TLC could be performed on these samples although they contained substantial radioactivity.

Since flies—used for the preparation of the 10,000 *g* supernatant—had been treated 18 hr previously with Aroclor® 1248 in acetone (2 μl/fly) or with acetone only (controls), solvent effects on these processes in living house fly populations are currently being investigated.

Preliminary *in vitro* tests with subcellular components of rat liver did not show any appreciable inhibitory effects of Aroclor® 1248 on the degradation of paraoxon (Lichtenstein *et al.*, unpublished).

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