ISRAEL JOURNAL OF ENTOMOLOGY VOL. XI 1976

# MORPHOGENETIC EFFECTS OF THE POLYCHLORINATED BIPHENYL, AROCLOR 1254 AND PHENOBARBITAL ON *AEDES AEGYPTI* LARVAE

Shoshana Akov

Department of Entomology Israel
Institute for Biological Research
Ness Ziona

## ABSTRACT

Metamorphosis and adult emergence of *Aedes aegypti* larvae was inhibited by the polychlorinated biphenyl, Aroclor 1254 and the drug phenobarbital. The morphogenetic effects of Aroclor 1254 and phenobarbital were similar to those obtained with low dosages of methoprene and TH-6040. The possible mechanisms for obtaining a typical juvenile hormone-like effect by various unrelated chemicals were discussed.

## INTRODUCTION

Polychlorinated biphenyls (PCBs) are industrial chemicals which have been in use or forty years before they were recognized as enduring environmental contaminants. For a long time PCBs were considered to be relatively non-toxic, because their acute toxicity to animals is low compared to that of most organochlorine pesticides. However, poisoning of humans by PCBs (the "Yusho" disease in Japan in 1968, caused by contamination of rice oil by PCBs) which affected more than 1,000 people, caused severe clinical symptoms. The chemical inertness and low flammability, which makes the PCBs very useful for many applications, also makes them extremely persistent in the environment, more so than the chlorinated pesticides. Pollution of the environment by PCBs is considered a serious threat to fish and fish-eating birds (Ahmed, 1976). PCB residues were found at all trophic levels of the food web in all parts of

the world, including human food, human milk and human adipose tissue, and it is not unanimously agreed whether current PCB levels are hazardous to human health (Highland, 1976). The picture is complicated by the fact that PCBs are mixtures of compounds containing 12-68% chlorine; theoretically 210 different isomers are possible from the 10 positions on biphenyl which can be chlorinated. PCBs are manufactured in several countries and are known under various trade names, i.e. Aroclor (U.S.A.), Kanechlor (Japan), Clophen (Germany), Phenoclor (France), and Fenclor (Italy),. For the Aroclor series (manufactured by Monsanto, U.S.A.) the last two digits indicate the percentage of chlorine; Aroclor 1254 contains 54% chlorine.

The biological impact of PCBs was recently reviewed by Peakall (1975). Many physiological effects of PCBs resemble those of DDT: Impaired reproduction in mammals, decreased eggshell thickness and reduced hatchability in birds, disruption of osmoregulation in fish, and the induction of hepatic microsomal foreign-compound-metabolizing enzymes.

The acute toxicity of PCBs to insects is low compared to that of organochlorine insecticides. Aroclors 1248, 1254 and 1260 were nontoxic at 0.1 ppm to 4th instar mosquito larvae after 48 hours (Deonier *et al.*, 1946). Data on the physiological effects of sublethal doses of PCBs in insects are scarce. Houseflies tolerated large doses of Aroclor 1254, but treatment with non-toxic dosages of PCBs increased the mortality due to other insecticides (Lichtenstein *et al.*, 1969; Fuhreman and Lichtenstein, 1972). Treatment of houseflies with non-toxic dosages of Aroclor 1254 increased the toxicity of the carbamate insecticide carbaryl 14-82 times (Plapp, 1972). The degree of synergism of Aroclor was similar to that of piperonyl butoxide. On the other hand, Aroclor 1254 acted as an inducer of aldrin epoxidase (Rhee and Plapp, 1973). Such a dual effect, namely that a compound which inhibits microsomal oxidations also acts as an inducer of this enzyme system, clearly indicates an interaction with microsomal mixed function oxidases (Hogdson and Philpot, 1974).

The principal component of Aroclor 1254 is pentachlorobiphenyl. When studied in a laboratory ecosystem, the bioaccumulation and biodegradation of radiolabelled pentachlorobiphenyl was found to be similar to that of DDE (Metcalf *et al.*, 1975). In ***Culex*** larvae the biodegradability index (ppm polar degradation products/ppm nonpolar products) for pentachlorobiphenyl was very low (0.0134), and the ecological magnification (ppm in mosquito/ppm in water) very high (17, 345).

The mosquito larvae in the laboratory ecosystem contained an equivalent of 170 ppm of pentachlorobiphenyl. Metcalf *et al.* (1975) did not report on any toxic effects of the PCBs on the mosquito larvae. Since the larvae were added to the laboratory ecosystem on day 26 and removed for tissue analysis on day 30 (Metcalf *et al.*, 1971), effects on metamorphosis could not have been observed.

This paper reports the effect of Aroclor 1254 on the metamorphosis and ecdysis of *Aedes aegypti* L. larvae. The effect of the PCB was compared with that of two insect growth regulators which are most effective in disrupting metamorphosis in mosquito larvae (Mulla and Darwazeh, 1975) and with the drug sodium phenobarbital, which was found to interfere with metamorphosis in houseflies (Yu and Terriere, 1974).

## MATERIALS AND METHODS

*Aedes aegypti* larvae ("Ness–Ziona" strain) were from a colony maintained in this laboratory for more than 20 years and reared at 27°C. Prior to the experiments the larvae were stranded on filter papers and examined under a dissecting microscope. Developmental stages of 4th instar larvae were determined according to the size of the pigmented adult eyes which are externally visible, as described by Spielman and Skaff (1967); A – early larva, adult eyes invisible; B – eyes linear; C – eyes crescentic; D – mature larve, eyes with truncate apex. In the pharate pupa (PP) the thoracic air trumpets were clearly visible. Stage D was the most prolonged stage (about 2 days). The duration of the PP stage was about 1–3 hours and insects of this stage were difficult to obtain in sufficient numbers. We therefore classified late stage D larvae (having a very wide thorax, but before the air trumpets became visibile) as stage E larvae.

Later stages of metamorphosis were: P – colorless pupa, abdomen extended after ecdysis; $PA_1$ – early pharate adult showing beginning of pigmentation; $PA_2$ – late pharate adult, black within pupal exuvium; $A_1$ – emerging adult in process of ecdysis from pupal exuvium; $A_2$ – freely flying adult. Stage $A_1$ extended from the splitting of the pupal exuvium prior to emergence, until the freeing of the tarsi from the pupal skin.

Mortality that manifested itself quickly and occurred at the larval stage was considered a general toxic, rather than a morphogenetic effect (Patterson, 1974). The stage of the insect at time of death was

specified, and any abnormal development described. The stages of larvae treated, the duration of treatment, the number of larvae and the volume of test solutions, and whether food (pellets of rabbit food) was present differed in various experiments. At the end of each experiment larvae were removed from the treated water, washed with tap water, placed singly in test tubes with fresh water, (with or without food)and examined daily until adult emergence.

Adult females which had emerged successfuly from the pupal skin were discarded. Males were kept for two additional days in small cages and fed on sugar solution, in order to examine the 180° rotation of their terminalia (Spielman and Skaff, 1967).

Because of the extremely low solubility of Aroclor 1254 in water (Metcalf *et al.*, 1975), it was applied in the form of a suspension. The appropriate amount of Aroclor was dissolved in ethanol, 1 part of ethanol added to 9 parts of Tween 80 solution (0.5 mg/ml), and the resulting suspension was added to water (4 ml per 100 ml). Since mosquito larvae feed on small particles, Aroclor was also adsorbed to suspended solids, simulated by kaolin. Kaolin (Bolus, Riedal de Haen Ag., Germany) 50 g was suspended in 159 ml hexane, 50 mg Aroclor in 10 ml hexane was added, the mixture shaken and evaporated. The controls contained the appropriate amounts of ethanol. Tween 80, and hexane-washed kaolin, respectively.

Phenobarbital was given in the form of Phenobarbital Sodium solutions, freshly prepared and changed daily, because of their instability. TH-6040 (Dimilin$^{R}$; 1-(4-chlorophenyl)-3-(2,6-diflouobenzoyl)-urea) and methoprene (Altosid$^{R}$; ZR-515; isopropyl 11-methixy-3,7,11-trimethyl-2,4,dodecadienoate 52,5% A. I. obtained from Zoecon) were dissolved in ethanol and directly added to water (0.5% ethanol final concentration).

## RESULTS AND DISCUSSION

Arocol 1254, adsorbed onto kaolin (1 mg/g), had no immediate toxicity for 4th instar larvae and young (colorless) pupae; this concentration of PCBs adsorbed on suspended solid was found to be non-toxic to juvenile Atlantic salmon treated for several weeks (Zitko, 1974). Five g of kaolin was used per 250 ml of tap water containing 25 insects; controls were given hexane-washed kaolin (Table 1a). Mosquito larvae ingest the kaolin, and it has no adverse effect on their development. Stages which require food were fed rabbit pellets (50 mg per 250 ml);

no food was added to non-feeding stages in order to prevent bacterial contamination. Preliminary experiments with young (colorless) pupae showed that Aroclor 1254 adsorbed onto kaolin had no effect on this non-feeding stage - there was no mortality and all insects emerged normally, but there was some toxicity towards fourth instar larvae. When fourth instar larvae were grouped according to their developmental stages, (see Materials and Methods) the younger, still feeding IV D stage larvae were found to be more sensitive than older, IV E larvae, which already had stopped feeding (Table 1a). In both groups there was no larval mortality and no mortality during the pupal stage; all insects died at the time of ecdysis.

The greater sensitivity to Aroclor 1254 of the IV D larvae might be because they were still actively feeding and thus ingested more PCB than the older larvae, but it is also possible that at this stage the larvae are more susceptible to this chemical,. With Aroclor on kaolin the relative amounts of the chemical taken up by the larvae may vary not only because some stages feed and other do not. The larval feeding activity creates a turbulence, which might affect the relative amounts of Aroclor 1254 in water. In order to minimize these differences, the larvae were grouped according to their age and Aroclor was applied in suspension with Tween 80 (see Materials and Methods). The larvae were kept in the treated water until pupation, and the pupae were transferred into clean tap water. All dead insects were examined. The viability of the emerged free-flying females was not tested. The emerged males were kept alive for two more days in order to examine their terminalia. Results in Table 1b show that all adults in the control group (ethanol and Tween 80 only) and with 0.04 and 0.2 ppm Aroclor 1254 emerged normally, One male (out of 10 males) in the group treated with 0.04 ppm Aroclor had incompletely rotated terminalia. This is considered to be a typical hormono-mimetic symptom (Spielman and Skaff, 1967). More hormone-like effects were obtained by increasing the amounts of Aroclor 1254 to 1.0 and 5.00 ppm. Again, as with PCB absorbed on kaolin, there was no mortality during the larval or pupal stages, but only at larvae-pupae ecdysis or during emergence.

The length of time spent in the Aroclor 1254 solution might be important. None of the adverse effects of Aroclor in suspension appeared earlier than three days after starting the treatment. Insects which pupated relatively early (and were removed to clean water, since it was found previously that the PCB did not affect pupae) emerged normally. All insects which died during the larval-pupal ecdysis were those which pupated relatively late. For this reason, in subsequent experiments

| Developmental stage at beginning of treatment | Aroclor 1254 IV D | Aroclor 1254 IV E | Controls IV D | Controls IV E |
|---|---|---|---|---|
| Number of larvae | 27 | 96 | 49 | 50 |
| % dead as colorless pupa (P) | 37 | 20 | -- | -- |
| % dead as early pharate adult ($PA_1$) | 0 | 0 | -- | -- |
| % dead as late pharate adult ($PA_2$) | 11 | 9 | -- | -- |
| % dead in process of emergence ($A_1$) | 52 | 14 | -- | -- |
| % of normally emerged adults ($A_2$) | 0 | 57 | 98* | 100 |
| Number of males with non-rotated terminalia | none | none | none | none |

* one larva was alive but failed to pupate after 10 days

Table 1a. Effect of Aroclor 1254 adsorbed onto kaolin on fourth instar Aedes aegypti larvae (see details in text).

| | Aroclor 1254 (ppm) None | 0.04 | 0.2 | 1.0 | 5.0 |
|---|---|---|---|---|---|
| Number of larvae | 50 | 25 | 25 | 25 | 27 |
| % dead as colorless pupa (P) | -- | -- | -- | 16 | 18 |
| % dead as pharate adult ($PA_1$,$PA_2$) | -- | -- | -- | -- | -- |
| % dead in process of emergence ($A_1$) | -- | -- | -- | 12 | 30 |
| % of normally emerged adults ($A_2$) | 100 | 100 | 100 | 72 | 52 |
| Number of males with non-rotated terminalia | none | 1 | none | none | none |

Table 1c. Effect of 5 ppm of Aroclor on fourth instar Aedes aegypti larvae (see details in text).

| Developmental stage at beginning of treatment | Aroclor 1254 IV D | Aroclor 1254 IV E | Controls IV D and IV E |
|---|---|---|---|
| Number of larvae | 27 | 58 | 100 |
| % dead as colorless pupa (P) | 7.5 | 3 | 2 |
| % dead as pharate adult ($PA_1$,P | 7.5 | -- | 1 |
| % dead in process of emergence ($A_1$) | 63 | 57 | -- |
| % of normally emerged adults ($A_2$) | 22 | 40 | 97 |
| Number of males with non-rotated terminalia | none | 2 | none |

Table 1b. Effect of graded amounts of Aroclor 1254 in suspension on Stage IV D Aedes aegypti larvae (see details in text).

(with Aroclor 1254 and other compounds) the developmental stages of the insects were synchronized as far as possible and the period of treatment was uniform.

The results in Table 1c show that stage IV D larvae were somewhat more sensitive to Aroclor 1254 than older, stage IV E larvae. In this experiment Aroclor was applied (5 ppm) in suspension, and all larvae were uniformly treated for 18 hours, then rinsed twice with tap water and transferred to clean water (with food for the younger larvae). The difference between larvae treated in stage IV D and those treated in stage IV E was only in the greater percentage of apparently normally emerging adults; the characteristics of the partially emerged insects were similar in both groups.

All the morphological abnormalities observed after treatment with sublethal doses of Aroclor 1254 were typical of juvenile hormone mimics (Spielman and Skaff, 1967, Mayer *et al.*, 1976). Death occurred mainly at larval-pupal ecdysis, and during adult emergence. The dead pupae maintained many larval characteristics: they remained colorless, their abdomens were extended, they moved abnormally and died after struggling for hours near the surface of the water, apparently from drowning. With lower dosages most insects survived the pupal period (with all compounds at marginally effective doses there was no mortality during the pupal stage). Death occurred at all stages of adult emergence. Some individuals died just after the splitting of the pupal case, but all stages of partially emerged adults could be observed with incompletely extracted proboscis, wings, tip of abdomen, or legs. Some partly emerged adults struggled for hours, (sometimes for days, when only the legs remained in the pupal skin) in order to extricate themselves completely. When such adults were dissected free, their tarsi remained folded, because the cuticle had already hardened. Some adults were able to free themselves from the pupal skin by breaking their legs; these adults were unable to fly, but remained alive on the surface of the water, sometimes for several days. Free-flying males that eclosed normally but failed to complete the 180° rotation of their terminalia were relatively rare, but many of the males which were unable to fly but remained alive on the surface of the water (usually their tarsi were broken and could be seen inside the pupal case) also had incompletely rotated terminalia: 45°, 90° or 135°. No external abnormalities were observed in females which remained alive on the surface of the water and were unable to fly, except for broken tarsi.

The toxicity of Aroclor 1254 to *Aedes aegypti* larvae was somewhat lower than that reported for *Anopheles* larvae (Deonier *et al.*, 1946), who found that 1 ppm Aroclor 1254 caused more than 50% mortality within 48 hours. With water-insoluble compounds comparisons between tests using different procedures are very difficult; moreover, the duration and timing of the sensitive periods are not the same for all species of mosquitoes (Staal, 1975).

Phenobarbital was shown to disrupt housefly development, but the effects observed were rather non-specific (Yu and Terriere, 1974). The effect of phenobarbital on mosquito larvae was therefore tested, at various stages. Young colorless pupae were relatively unaffected and no changes in their swimming activity were observed in pupae placed in 0.1%, 0.2%, 0.5% or 1% phenobarbital solutions for their entire pupal period. They also emerged normally.

In fourth instar larvae two different effects were observed - an earlier, pharmacological effect and a later effect on development. With concentrations ranging from 0.025% to 0.1% phenobarbital the larvae gradually became sluggish, stopped exhibiting the characteristic alarm reaction towards light, and gradually stopped swimming to the surface to breathe and sank to the bottom. These effects occurred after 12-24 hours. The percentage or larvae which were immobilized depended on the concentration of the drug and the period of exposure. The paralysis was reversible and the larvae recovered upon being placed into clean water. The ability to recover was related to the time that the larva had spent at the bottom - death was probably due to lack of oxygen. After 24 hours of exposure, about 50% of the larvae treated with 0.075% phenobarbital were dead. However, larvae were able to recover from paralysis after 0.1%, 0.2% and 0.4% phenobarbital (the highest concentration tested) if placed into clean water in time. There were great variations in the susceptibility of individual larvae-in the highest concentrations of phenobarbital, when more than half of the larvae were dead, a few larvae often remained completely unaffected.

In the experiment given in Table 2 the treated larvae were of stage IV D. Treatment was for 16 hours and there was no immediate mortality due to paralysis with up to 0.08% phenobarbital (but all larvae treated with 0.1% or more for 16 hours were dead). After being transferred into clean water all larvae behaved normally. The effects on metamorphosis and adult emergence were similar to those obtained with Aroclor. There were no deaths during the larvael or pupal stages.

Similar results were obtained with phenobarbital treatments lasting for 24-48 hours (treatments shorter than 16 hours were not tested). With the longer treatments there was some larval mortality, and only surviving free-swimming larvae were taken. In general there was little or no mortality after treatment for 24 hours with 0.05% phenobarbital or less.

| Amount of phenobarbital | .03% | .04% | .05% | .06% | .08% |
|---|---|---|---|---|---|
| No. of larvae | 20 | 19 | 10 | 17 | 11 |
| No. dead as colorless pupa (P) | 6 | 9 | 7 | 14 | 8 |
| No. dead as pharate adult ($PA_1$,$PA_2$) | 0 | 0 | 0 | 0 | 0 |
| No. dead in process of emergence ($A_1$) | 6 | 7 | 3 | 2 | 3 |
| No. of normally emerged adults ($A_2$) | 8 | 3 | 0 | 1 | 0 |
| No. of males with non-rotated terminalia | 1 | 1 | 0 | 0 | 0 |

Table 2. Effect of phenobarbital on stage IV D *Aedes aegypti* larvae

The morphogenetic effects were greatest in IV D larvae (in younger larvae the paralyzing effect and mortality due to the drug were somewhat higher). With IV E larvae a higher proportion of emerging adults was obtained.

Since the effects of phenobarbital closely resembled those of a juvenile hormone (JH) mimic, we compared them to the effects of methoprene, the most potent JH analog for mosquito larvae. The LC 50 of methoprene for late fourth instar ***Aedes aegypti*** larvae under laboratory conditions is about 0.5 ppb (Staal, 1975). The difficulty in determining effective dosages of methoprene solutions is due to their relative instability. In our experiments the larvae were left in the same solution until death or adult emergence. The relative sensitivity of the younger larval stages (IV C or less) could not be assessed because the younger larvae reached the period of their maximal susceptibility one or two days later than the IV D larvae and the amount of the active compound remaining in solution in the presence of larvae and microorganisms was unknown (Spielman and St. Onge, 1974).

The aim of our experiments was to observe the morphogenetic effects of methoprene, rather than finding the concentrations most appropriate for effective control. Methoprene was used at six dosages ranging from 0.6 ppb to 12 ppb. When IV D and IV E larvae were treated with the higher dosages death occurred at all pupal stages, including the early and late pharate adults ($PA_1$ and $PA_2$). No deaths at these stages were observed with the less toxic Aroclor and phenobarbital. With lower dosages all stages of imperfectly emerged adults ($A_1$) could be found. Treatment of the younger (IV B and IV C) larvae with all concentrations of methoprene also yielded imperfectly emerged adults. The time of death with lower dosages was typical of the hormono-mimetic effect - no deaths in the larval or pupal stages, only at ecdysis.

TH-6040 is known to interfere with the synthesis of insect cuticle (Ishaaya and Casida, 1974); it is not yet clear whether this effect is hormonally mediated. It was therefore interesting to find that the morphogenetic effects of TH-6040, at lethal and sublethal dosages were exactly the same as those of Aroclor, phenobarbital and methoprene. The larvae were left in the TH-6040 solutions until death. Since TH-6040 is a relatively stable compound, it may be assumed that the concentrations remained unchanged during the experiment. When larvae of various stages were treated with lethal dosages of TH-6040 the time of death and the developmental stage at death varied with the dosage and with the stages treated. At all concentrations tested (4,6,9,12 and 18 ppb of TH-6040) all stage IV C and younger larvae died during the larval-pupal molt, as pharate pupae (PP) or colorless pupae (P) with extended abdomen. Differences due to concentration of TH-6040 and the developmental stage of the larva at the beginning of the treatment could be detected only with stage IV D and stage IV E larvae (Table 3). It seems that the younger larvae in all the TH-6040 concentrations had time to accumulate enough TH-6040 to completely inhibit their first mold. Stage IV D larvae which stayed longer in the TH-6040 solution were more sensitive than IV E larvae. In both age groups death occurred earlier in the higher concentrations and therefore the insects died at an earlier stage of their development. The developmental stage at death is related to the time of death (Table 3). Deaths were not restricted to molts - although no larvae died, some insects died during the pupal stage as pharate adults ($PA_1$ and $PA_2$).

The dosage of TH-6040 allowing 50% of the adults to emerge normally was 3.6 ppb. With lower doses of TH-6040 (0.18 ppb to 3.0 ppb) there was no pupal mortality in any age group of fourth instar larvae treated. Most of the adults emerged normally; those who did not died during adult emergence. Among the emerged males derived from larvae treated with the lowest concentration of TH-6040, about

Table 3. Effect of TH-6040 on fourth instar Aedes aegypti larvae (10 larvae in each concentration of TH-6040)

| Stage Treated | Amount of TH-6040 (ppb) | Number of larvae dead at each stage of development (see Table 1 and 2) | | | | | Number of larvae dead after starting treatment | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | P | $PA_1$ & $PA_2$ | $A_1$ | $A_2$ | normal adult | 1d | 2d | 3d | 4d or later |
| IV E | 18 | 6 | 3 | 1 | - | | 4 | 2 | 3 | 1 |
| | 12 | 5 | 2 | 2 | 1 | | 1 | 3 | 4 | 1 |
| | 9 | 2 | 6 | 1 | 1 | | 1 | 1 | 3 | 4 |
| | 6 | 1 | 5 | 2 | 2 | | 1 | 1 | 3 | 3 |
| | 4 | - | 2 | 4 | 4 | | - | - | 3 | 3 |
| IV D | 18 | 10 | - | - | - | | - | 2 | 5 | 3 |
| | 12 | 10 | - | - | - | | - | 1 | 5 | 4 |
| | 9 | 10 | - | - | - | | - | 3 | 3 | 4 |
| | 6 | 7 | 3 | - | - | | - | 4 | 3 | 3 |
| | 4 | - | 5 | 4 | 1 | | - | - | 3 | 6 |

10% had incompletely rotated terminalia. This was a higher percentage than observed with Aroclor, phenobarbital or methoprene. Imperfectly rotation of terminalia in the male mosquito was interpreted as indicating precocious hardening of the cuticle (Spielman and Skaff, 1967). It is therefore interesting that TH-6040, which is known to interfere with cuticle synthesis and hardening causes this typical hormonomimetic effect in mosquitoes when applied at marginally effective doses. (A male with imperfectly rotated terminalia is unable to mate).

The morphogenetic effects of the environmental pollutant, Aroclor 1254 and the drug phenobarbital are typical of the effects of an insect growth regulator (IGR) with juvenile hormone (JH) activity. Staal (1975) used the term IGR in order to include compounds which are not related to natural JHs, but have a similar effect, though their mode of action might be different. IGRs lack immediate toxicity; the period of

greatest sensitivity is generally the last larval instar and the most readily observed effect is abnormal morphogenesis.

In mosquitoes the effect of IGRs with JH activity is essentially non-specific: nonemergence of the adult from the pupal skin. The first description of the effect of a synthetic JH mimic on *Aedes aegypti* larvae was by Spielman and Skaff (1967). Patterson (1974) showed similar effects with additional JH analogs. Natural JH, methoprene and a new fluorescent IGR (a hybrid containing a methylenedioxyphenyl group and a fluorescent moiety) also inhibited metamorphosis and adult emergence in *Aedes aegypti* (Mayer *et al.*, 1976). Staal (1975) observed no clear-cut juvenoid effects in adult mosquitoes which failed to emerge normally after treatment with some of the newer, more potent IGR compounds. The recognition of the nonemergence syndrome in mosquitoes as a typical JH effect is based on indirect evidence. Compounds such as natural JH and active analogs that produce clear-cut JH effects to other insects usually evoke the nonemergence syndrome in mosquitoes, while inactive analogs do not.

The morphogenetic effects of Aroclor 1254 and phenobarbital indicate that JH-like effects in mosquito larvae must be interpreted cautiously. These effects might be entirely non-specific toxic effects, rather than typical JH-like effects as defined by Staal (1975). In the past such effects might have been overlooked since bioassays involving mosquitoes were primarily concerned with larvicides and were usually discontinued after 24 or 48 hours. Mosquito larvae can bioaccumulate water-insoluble compounds (Metcalf *et al.*, 1975) and it is probable that additional chemicals, particularly lipophilic compounds of relatively low toxicity, will be found to cause hormone-mimetic effects in mosquito larvae. The new fluorescent IGR, which is a methylenedioxyphenyl derivative (Mayer *et al.*, 1976), might therefore not be a suitable tool for the study of the mode of action of insect hormones.

The mode of action of IGRs with JH activity is not known. Some JH analogs might replace the natural hormone, others, particularly compounds which are not JH analogs, might interfere with the metabolism of the natural hormones. The regulation of insect hormone titers at certain critical moments during the life cycle probably depends on the breakdown of natural JH; inhibiting the degradation of the JH could result in abnormal metamorphosis. There is some indirect evidence for the hypothesis that the same enzyme systems which are known to metabolize insecticides and other foreign compounds take part in the degradation of insect hormones. Thus, certain insecticide-resistant

strains of insects also show cross resistance toward IGRs (Staal, 1975). Various methylenedioxyphenyl derivatives, including the insecticide synergist piperonyl butoxide, exhibit JH-like activity (Mayer *et al.*, 1976). These compounds are known as inhibitors of microsomal mixed function oxidases; it is possible that inhibiting these enzymes might interfere with the breakdown of natural hormones. However, methylenedioxyphenyl compounds also act as inducers of microsomal enzymes and affect enzymes other than microsomal oxidases (Hodgson and Philpot, 1974).

It would be tempting to speculate if the morphogenetic effects of Aroclor 1254 and phenobarbital on *Aedes aegypti* larvae are in any way related to changes in their microsomal enzymes. This question cannot be answered at present, because the microsomal foreign-compound-metabolizing enzymes have not been studied in *aegypti* larvae. Both compounds are known to interact with microsomal enzymes. Phenobarbital is probably the best-known inducer of microsomal oxidases in many animal species, including insects (Agosin and Perry, 1974). Aroclor 1254 acts both as an inducer and as an inhibitor (Peakall, 1975) and also has a dual effect in houseflies (Plapp, 1972; Rhee and Plapp, 1973).

The relationship between the activity of microsomal oxidases and the regulation of the hormone titre was studied by Terriere and Yu (1973, 1976) and Yu and Terriere (1974, 1975a, 1975b) in houseflies. Piperonyl butoxide and phenobarbital fed to housefly larvae inhibited adult emergence and increased the level of microsomal oxidase activity. Hormone analogs which inhibited development also acted as inducers of microsomal oxidases. These results were interpreted as an indication of a direct connection between microsomal oxidase activity and the regulation of hormone titre by these enzymes. However, the role of microsomal oxidation in the breakdown of natural insect hormones is not clear. Many yet unknown enzyme systems are involved in the inactivation of ecdysones (Svoboda *et al.*, 1975) and the degradation of natural JHs is probably by epoxide hydrases and esterases (Slade *et al.*, 1976).

It is possible that the morphogenetic effect of Aroclor 1254 in mosquito larvae as well as the effects in houseflies (Plapp, 1972; Rhee and Plapp, 1973) are due to a contaminant of the PCB. Many PCBs, including Aroclor 1254, are contaminated by polychlorinated dibenzofuran, including the extremely toxic tetrachlorodibenzofuran (Curley *et al.*, 1975; Bowes *et al.*, 1975). The toxicity and biological activity of tetrachlorobenzofuran is similar to that of tetrachlorobenzodioxin (TCDD, a contaminant of the herbicide 2,4,5–T and the fungicide penta-

chlorophenol). TCDD is a most potent inducer and suppressor of microsomal foreign-compound-metabolizing enzymes (Hook et al., 1975).

## REFERENCES

Agosin, M. and A. S. Perry, 1974. Microsomal mixed-function oxidases, *In* "The Physiology of Insects." (Ed. M. Rockstein), Vol. 5, pp. 537–596, Academic Press, New York and London.

Ahmed, A. K., 1976. PCBs in the environment. ***Environment***, 18: No. 2, 6–11.

Bowes, G. W., M. I. Mulvihill, B. R. T. Simoneit, A. L. Burlingame and R. W. Risebrough, 1975. Identification of chlorinated dibenzofurans in American polychlorinated biphenyls. ***Nature***., 256:305–307.

Curley, A., V. W. Burse, R. W. Jennings, E. C. Villanueva and R. D. Kimbrough, 1975. Evidence of tetrachlorodibenzofuran (TCDF) in Aroclor 1254$^{R}$ and urine of rats following dietary exposure to Aroclor 1254$^{R}$. ***Bull. Environ. Contam. Toxicol.*** 14:151–158.

Donier, C. C., A. Jones and H. H. Incho, 1946. Organic compounds effective against larvae of ***Anopheles quadrimaculatus*** – laboratory tests. ***J. Econ. Entomol.***, 39:459–462.

Fuhremann, T. W. and E. P. Lichtenstein, 1972. Increase in the toxicity of organophosphorus insecticides to houseflies due to polychlorinated biphenyl compounds. ***Toxicol. Appl. Pharmacol.***, 22:628-640.

Highland, J., 1976. PCBs in food. ***Environment***, 18:no.2, 12–16.

Hodgson, E. and R. M. Philpot, 1974. Interaction of methylenedioxyphenyl (1,3–benzodioxole) compounds with enzymes and their effects on mammals. ***Drug Metabol. Rev.***, 3:231–301.

Hook, G. E. R., J. K. Haseman and G. W. Lucier, 1975. Induction and suppression of hepatic and extra hepatic foreign-compound-metabolizing enzyme systems by 2,3,7,8–tetrachlorodibenzo-*p*-dioxin. ***Chem. Biol. Inter.***, 10:199-214.

Lichtenstein, E. P., K. R. Schulz, T. W. Fuhremnn and T. T. Liang, 1969. Biological interaction between plasticizers and insecticides. *J. Econ. Entomol.*, 62:761–765.

Mayer, Richard T., A. C. Bridges, J. Cocke, R. Meola and J. K. Olson. 1976. Fluorescent insect growth regulators (FIGRs): a new tool for insect physiologists. *J. Insect Physiol.*, 22:515–520.

Metcalf, R. L., G. K. Sangha and I.P. Kapoor, 1971. Model ecosystem for the evaluation of pesticide biodegradability and ecological magnification. *Envir. Sci. Technol.*, 5:709–713.

Metcalf, R. L., J. R. Sanborn, P.–Y Lu and D. Nye, 1975. Laboratory model ecosystem studies of the degradation and fate of radiolabeled tri-tetra–and pentachlorobiphenyl compared with DDE, *Arch. Envir. Contam. Toxicol.*, 3:151–165.

Mulla, M.S. and H. A. Darwazeh, 1975. Activity and longevity of insect growth regulators against mosquitoes. *J. Econ. Entomol.*, 68:791–794.

Patterson, J.W., 1974. A comparison of the morphogenetic and sterilizing activities of juvenile hormone mimics on *Aedes aegypti*. *J. Insect Physiol.*, 20:2095–2106.

Peakall, D. B., 1975. PCBs and their environmental effects. *CRC Critical Revs. Envir. Control*, 5:469–508.

Plapp, F.W. Jr., 1972. Polychlorinated biphenyls: an environmental contaminant acts as an insecticide synergist. *Envir. Entomol.*, 1:580–582.

Rhee, K. S. and F. W. Plapp, Jr., 1973. PCBs as inducers of microsomal enzyme activity in the housefly. *Arch. Envir. Contam. Toxicol.*, 1:182–192.

Slade, M., H. K. Hetnarski and C. F. Wilkinson, 1976. Epoxide hydrase activity and its relationship to development in the southern armyworm *Prodenia eridania*. *J. Insect Physiol.*, 22:619–622.

Spielman, A. and V. Skaff, 1967. Inhibition of metamorphosis and of ecdysis in mosquitoes. *J. Insect Physiol.*, 13:1087–1095.

Spielman, A. and E. St. Onge, 1974. Stability of exogenous juvenile hormone: Effect of larval mosquitoes. *Envir. Entomol.*, 3: 259-261.

Staal, G. B., 1975. Insect growth regulators with juvenile hormone activity. *Ann. Rev. Entomol.*, 20:417–460.

Svoboda, J. A., J.N. Kaplanis, W. E. Robbins, and M. J. Thompson, 1975. Recent developments in insect steroid metabolism. *Ann. Rev. Entomol.*, 20:205-220.

Terriere, L. C. and S. J. Yu, 1973. Insect juvenile hormones: Induction of detoxifying enzymes in the housefly and detoxication by housefly enzymes. *Pestic. Biochem. Physiol.*, 3:96–107.

Terriere, L. C. and S. J. Yu, 1976. Interaction between microsomal enzymes of the housefly and the moulting hormones and some of their analogs. *Insect Biochem.*, 6:109–114.

Yu, S. J. and L. C. Terriere, 1974. A possible role of microsomal oxidases in metamorphosis and reproduction in the housefly. *J. Insect Physiol.*, 20:1901–1912.

Yu, S. J. and L.C. Terriere, 1975a. Activities of hormone metabolizing enzymes in houseflies treated with some substituted urea growth regulators, *Life Sci.*, 17:619–625.

Yu, S. J. and L. C. Terriere, 1975b. Microsomal metabolism of juvenile hormone analogs in the house fly, *Musca domestica* L. *Pesticide Biochem. Physiol.*, 5:418–430.

Zitko, V., 1974. Uptake of chlorinated paraffins and PCB from suspended solids and food by juvenile Atlantic salmon. *Bull. Envir. Contam. Toxicol.*, 12:406–412.