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PRELIMINARY FIELD EVALUATION OF BIODEGRADABLE ANALOGUES OF DDT

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ABSTRACT

Approximately 20 DDT analogues are being evaluated for utility in vector control programs and for other pest control uses as persistent yet biodegradable substitutes for DDT. From residual activity tests and evaluation in plywood huts two compounds OMS-1406, $CH_3C_6H_4CHCCl_3C_6H_4OCH_3$ and OMS-1476, $CH_3C_6H_4CHCCl_3C_6H_4OC_2H_5$ appear to have the most favorable combination of properties. These compounds are much less acutely toxic to mammals and fish than DDT, persist for long periods on plywood, are highly biodegradable and of low environmental persistence, and are residually effective against *Anopheles* and *Culex* mosquitoes. Preliminary data suggests they can be manufactured at costs approaching that of DDT. Additional studies are being conducted on the biodegradability of DDT analogues with altered aliphatic groups.

I. INTRODUCTION

It has now been almost exactly a hundred years since Othmar Ziedler (1874) first synthesized DDT in the laboratory of Adolph von Baeyer, University of Strassburg. In the interim, the pleasant smelling greasy white powder has had an influence on human ecology perhaps unmatched by any chemical discovery including gunpowder, sulfonilamide, penicillin, and plutonium. The history of DDT has been reviewed by Metcalf (1973a). It use in public health programs for the control of the insect vectors of typhus, plague, yellow fever, and malaria has introduced "death control" on a global scale. It has been estimated that DDT has saved approximately 50 million human lives and avertdd more than 1 billion human illnesses (Metcalf 1973b).

The total use of DDT since 1946 has aggregated about 4 billion pounds, with about 20% applied in public health programs and 80% in agriculture. This enormous rate of application of the very persistent, lipid soluble (100,000 ppm), water insoluble (0.0012 ppm) insecticide has distributed DDT and its primary degradation products DDE and DDD everywhere throughout the global ecosystem. These compounds are present in human fat in amounts ranging from about 3 ppm in Eskimos to 26 ppm in inhabitants of India (Durham 1969). DDT and DDE are concentrated in organisms of trophic webs, increasing roughly ten-fold in amount stored in lipids and in the percentage of DDE, at each trophic level (Woodwell et al 1967). In Lake Michigan there is an overall concentration of DDT from water (0.000002 ppm) to coho Salma (ca 10 ppm) to herring gulls (99 ppm) of about 1 x 107 (Harrison et al 1970). Fish in particular have little protection from the physical chemistry of adsorption and Youngs et al (1972) have shown that there is a highly significant correlation between age of lake trout <u>Salvelinus namaycush</u> and total body residues of DDT, DDE, and DDD which ranged from about 1 ppm at 1 year to as

much as 28 ppm at 11 to 12 years of age. In animal livers and other tissues, both DDT and DDE have been shown to be efficient inducers of microsomal oxidase enzymes (Street 1969). Altered microsomal enzyme levels appear to play significant roles in the matabolism of not only exogenous drugs but also in endogenous steroids. The much discussed effects of DDT and DDE on egg shell thinning of birds is an example.

As a result of the long continued controversy about the environmental effects of DDT, this substance was banned in the United States effective January 1, 1973. In addition to the environmental problems from the use of DDT, widespread resistance to its action has slowly developed since 1947. There are presently 14 species of Anopheles vectors of malaria, 18 species of culicine mosquitoes including Culex fatigans vector of filariasis, Culex tarsalis vector of western equine encephalitis, Aedes aegypti vector of yellow fever, the oriental rat flea Xenopsylla cheopis vector of plague and four other fleas, the human body louse Pediculus humanus vector of typhus, Simulium blackflies, and the bedbugs Cimex lectularius and C. hemipterus; which are resistant to the insecticidal action of DDT. (Brown and Pal 1971) Another 27 important agricultural pests also show pronounced DDT resistance (Brown 1971).

The combination of environmental pollution and insect resistance has greatly prejudiced the continued use of DDT for public health programs and in agriculture. Existing commercial substitute insecticides are generally too expensive, less persistent on a variety of building surfaces, and/or more toxic to humans. Thus there is currently an urgent need to find alternative persistent yet biodegradable insecticides who might be more effective than DDT against resistant insects in order to permit the continued conquest and control of insect vector borne human and animal diseases.

Progress in the Development of "Biodegradable DDT Analogues.

During the past seven years, working under grants from the Herman Frasch Foundation of the American Chemical Society and more recently The Rockefeller Foundation, this laboratory has been investigating the principles of biodegradability of pesticides" and the "development of selective non-persistent pesticidal chemicals". Accomplishments from these programs include the development of a laboratory model ecosystem which provides quanitative information on the ecological magnification, biodegradability index, and degradative pathways of candidate compounds (Metcalf et al 1971), and investigations of the degrative pathways of a variety of DDT analogues using this system (Kapoor et al 1970, 1971, 1973). As a result of these investigations a considerable group of new asymmetrical analogues of DDT have been studied which appear to be effective insecticides, substantially more effective than DDT against resistant insects, and which are much more degradable than DDT in the model ecosystem (Metcalf et al 1971, Kapoor et al 1973). These DDT analogues have the very real advantage of being synthesized from cheap raw materials including chloral, toluene, and other substituted benzenes. The AID Contract on which progress is reported here, was consummated to gain additional information about the possible utility of these compounds in vector control programs and their effects on environmental quality.

II. PROJECT OBJECTIVES

The AID Project was developed to meet the following objectives in the further development of the persistent biodegradable analogues of DDT.

1. Further refinement of knowledge regarding biological properties of biodegradable DDT analogues, so that the most promising compounds may be selected for intensified study.

In this laboratory we have synthesized nearly 200 analogues of DDT under the Rockefeller Foundation Program. Most of these are produced by the Bayer Condensation between various substituted benzenes and chloral. Nearly all of them have some degree of insecticidal activity as revealed by submission to Stage I of the World Health Organization "Programme for the Evaluation and Testing of New Insecticides". However, the relative activity for resistant vs. susceptible insects varies substantially, the individual compounds have different spectra of activity to mosquito larvae and adults and to other insect pests, and the compounds have widely different degradative pathways, environmental persistences, and toxicities to warm-blooded animals. Major effort has been expended on consideration of these factors in order to determine the most suitable compounds for various uses.

2. Large-scale laboratory synthesis of biodegradable DDT analogues.

The projected hut and pond tests to be carried out in this program and the substantial and continuing demands for experimental samples make it important to have additional quantities of specific analogues for field evaluation. The production of these larger quantities have enabled us to make chemical studies of the nature of the technical products produced using GLC etc. From these investigations and in consultation with representatives of several large commercial firms, we have reached some reasonable conclusions about cost of manufacture.

3. Hut tests of the biodegradable DDT analogues to determine their effects on adult mosquitoes.

A series of 6 plywood huts were constructed, complete with window exit traps, to evaluate the persistence of the biodegradable DDT analogues in killing Anopheles albimanus and Culex fatigans. The huts and the window traps for measuring excitorepellancy and mortality of escaping mosquitoes are shown in Figure I. Studies have been made over nearly a one year period on the durability of initial deposits from emulsion concentrates of 5 compounds including DDT.

The hut tests have been supported and amplified by investigations of the residual activity of various DDT analogues on ¼ m.^{2} plywood sheets, to the mosquito species, and by detailed laboratory investigation of the effects of chemical structure on toxicity and absorption of radiolabeled DDT analogues by mosquito larvae.

4. Pond tests of biodegradable DDT analogues.

The toxicity of DDT to fish and its accumulation in fish tissues are major problems in its use directly in aquatic systems, e.g. for control of Simulium or mosquito larvae, and wherever DDT residues enter the aquatic environment from manufacturer or field use. Problems with DDT residues in fish are particularly acute in rice paddies and pond fish culture, in newly developed reservoirs, in large fresh water lakes, and in estuaries. We have evaluated the duration of toxic action and rate of degradation in the aquatic environment of about 25 DDT analogues. These initial studies were carried out in the plastic covered screenhouse with large aluminum "ponds" containing green sunfish Lepomis cyanellus, and associated plankton and other organisms as shown in Figure I.

5. Supplementary evaluation of the biodegradable DDT analogues against other pest insects. Investigators in other laboratories have supplied evaluation data on a number of other insect species.

From these data it is clear that replacement of the aryl-chlorine groups with degradophores of CH_3, CH_3O, C_2H_5O, and CH_3S substantially decreases the rate of biomagnification of the compounds in fish (E.M.) and improves the rate at which they and their metabolites are eliminated from the animal body. Studies of this nature are providing useful information for the development of new pesticides and are adding to our basic knowledge of biodegradability. This sort of information is presently being obtained with DDT analogues having altered aliphatic groups, i.e. replacement of CCl_3 with $CHCH_3NO_2$ (Prolan series), $C(CH_3)_3$, and dichlorocyclopropyl. Because of the necessity for synthesizing a radiolabeled molecule for each investigation the work is laborious and more details will be reported subsequently. However, as shown in the Table above, the $CH_3OC_6H_4CHC(CH_3)_3C_6H_4OCH_3$ compound (OMS-944) has been completed. (Coats et al 1973) Its environmental properties are nearly identical to methoxychlor strongly suggesting that aliphatic modifications of the trichloromethyl group do not affect biodegradability as much as changes in the aryl portion. Additional information about this point is given by the fish toxicity data for the related $C_2H_4OC_6H_4CHC(CH_3)_3$ $C_6H_4OCH_3$ OMS-1355 in Table 3 , Section III-4.

2. <u>Induction of microsomal oxidase enzymes in mice</u>.

The most serious biological problem with storage of DDT tissue residues in higher animals is the effect of inducing higher levels of the microsomal oxidases or drug metabolizing enzymes. This <u>in vivo</u> process has been credited with the production of the "thin eggshell" syndrome in carnivorous birds. Nigg et al (1973) in this laboratory have injected a variety of DDT analogues into

mice and have measured the effect on cytochrome P-450 and on the microsomal processes of O-dealkylation, N-demethylation, and epoxidation. The data are summarized as:

	enzyme level as percent of control			
analogue	epoxidation	O-demethylation	N-demethylation	cytochrome P-450
DDT	278*	161*	96	191*
methoxychoor	69	79	81	79
ethoxychlor	195*	109	112	149*
methylchlor	160	106	100	121
methiochlor	386*	191*	86	208*
OMS-1395 CH_2O-SCH_3	91	114	111	
OMS-1476 CH_3-OC_2H_5	79	75	117	
OMS-1425 CH_3-Cl	67	87	76	

* significant difference from control at 5% level.

Both ethoxychlor and methiochlor show significant activity as inducers but the three asymmetrical analogues OMS-1395, 1476, and 1425 seemed to be free of inducing activity.

B. Chemical Investigations

The studies reported here and requests for samples from a variety of collaborators have required the repetitive synthesis of quantities of the various analogues aggregating several pounds. A number of new asymmetrical and potentially biodegradable analogues have been synthesized and will be reported on subsequently.

To define the optimum conditions for the synthesis of OMS-1476 (CH_3-C_2H_5O) the following investigations have been made, changing the composition of the acidic condensing agent.

To a cooled and stirred mixture of p-ethoxyphenyl carbinol 0.02 mole (5.2g.) and toluene 0.022 mole (3 ml.) at -20°C, a variable mixture of conc. sulfuric acid and acetic-acid (25 ml.) was added. After stirring for 2 hours at -10°C, the mixture was poured on ice and extracted with ether. The residual oil obtained after drying and removal of ether was evaluated by GLC and the purity determined.

H_2SO_4%	HAc%	yield	% OMS-1476
100	0	2.154g.	0% [1]
90	10	5.77	28.5
80	20	6.125	52.3
70	30	6.4	80
60	40	6.5	3-10
50	50	4.7	0 [2]

1 Product was $C_2H_5C_6H_4CHCCl_3OCHCCl_3C_6H_4OC_2H_5$

2 Product was $C_2H_5OC_6H_4CHCCl_3OC(O)CH_3$

Intensive discussions with Mitsubishi Chemical Co. and American Cyanamid Co. suggest that the ultimate cost of OMS-1476 and OMS-1406 could be between $0.30 and $0.50 per pound. Montrose Chemical Co. has offered to formulate standard WP for future investigation.

C. Hut Tests of Biodegradable DDT Analogues to Determine their Effect on Adult Mosquitoes, with Supplementary Residual Studies.

The possible utility of persistent biodegradable analogues of DDT in residual spray applications for malaria control and eradication is of considerable importance to AID in view of the immense support given to developing countries for malaria suppression. Clearly the length of residual persistence of an insecticidally active deposit on building surfaces is of decisive importance to the concept of cheap and effective malaria suppression by insecticides, with a minimum of environmental pollution.

1. Residual insecticide evaluation on plywood in laboratory.

Plywood sections, 0.25 meter square, were sprayed with emulsions of DDT and selected analogues from 25% EC formulations in xylene with 2% Trilon X-100 emulsifier. These were stored in the laboratory at approximately 25°C. At regular intervals as shown in Figure 2, 20 adult 2-3 day old Culex fatigans (C. pipiens quinquefasciatus) mosquitoes were exposed, under a half petri dish, to the treated surface for 60 minutes, then transferred to screened cages, supplied with sugar water and observed for mortality over the subsequent 7 days. Observations were made on the rate of knockdown during the 60 minute exposure period. The data from these experiments, now covering almost exactly one year, are shown in Figure 2.

The DDT analogues investigated and their LC_{50} values to adult mosquitoes from the standard WHO Stage I investigation (Metcalf et al 1971) were:

		LC_{50} µg/cm²	
		Culex fatigans	Anopheles albimanus
DDT	OMS-15	6.9	0.23
methoxychlor	OMS-44	16	13.0
$CH_3C_6H_4CHCCl_3C_6H_4Cl$	OMS-1425	5.2	1.5
$CH_3C_6H_4CHCCl_3C_6H_4OC_2H_5$	OMS-1476	8.0	0.75
$CH_3SC_6H_4CHCCl_3C_6H_4OCH_3$	OMS-1395	160	160
$C_2H_5OC_6H_4CCl_3NHC_6H_4OC_2H_5$	OMS-1445	160	160
$CH_3C_6H_4CHCCl_3C_6H_4OCH_3$	OMS-1406	10	3.3

OMS 1395 and 1445 although not performing well in the WHO test on filter paper had shown considerable activity in other screening tests and it was thought that they might be more effective on plywood surfaces.

As shown in Figure 2 and by the data of Table 1, however, these two analogues OMS 1395 and 1445 were of low activity in the plywood tests as well. Over the initial 3 weeks of evaluation, the rates of knockdown and mortality were as shown in Table 1 .

Figure 2 shows that effective residue persistence was obtained only with DDT, methoxychlor, OMS-1425, OMS-1476, and apparently with OMS-1406 which has been evaluated only for about 10 weeks. OMS-1495 was initially quite effective but lost activity much more rapidly than DDT, methoxychlor and OMS - 1476. DDT appeared somewhat less effective than either methoxychlor or OMS-1476 as might be expected from its generally poorer performance against Culex fatigans. There was little if anything to choose between the behavior of methoxychlor and OMS-1476 which demonstrate a residual insecticidal life under these conditions of at least one year. It is too early to predict the effective life of OMS-1406 but it is a promising compound.

2. Hut Tests

Detailed evaluation of residual spray insecticides for anopheline control has been carried out in small houses or huts equipped with window traps, since the original investigations of this type (Metcalf et al 1945). For comparative analysis of the behavior of the DDT analogues under study here, 6 huts approximately 4'x4'x8' with a door and two windows were constructed of 3/8" plywood as shown in Figure 1. The windows were equipped with exit traps of gallon paper cartons with a cloth sleeve and a screen wire end. (Figure 1) The huts were sprayed with the test compound from a 25% emulsion concentrate, with 2% Triton X-100 diluted 1:4 with water and applied with continuous agitation at a rate of approximately 2 g. per m^2. (25g. of compound were sprayed on the total interior surface of each hut). At the intervals shown in Figures 3 and 4, 100 Anopheles albimanus adult mosquitoes 2-4 days old, were liberated in each hut. The progress of the experiment was followed over the next 3 hours and the mosquito- "knockdown" on the hut floor was determined together with the 24-hour mortality of the mosquitoes in the window-exit traps (returned to the laboratory and kept at constant temperature). At the completion of each 3 hour experiment, the hut was very lightly fogged with a pyrethrins aerosol to determine the total number of mosquitoes. The experiments were run from August 30 until October 9 when cold weather intervened, were resumed on May 11, and are still being continued. A control (unsprayed) hut was evaluated each time the sprayed huts were assayed. Control mortalities varied between 8 and 25% over the total experiment.

The overall results of the hut tests are shown in Figure 3 which indicates the total mortality of released mosquitoes knocked down in the hut or escaping into the window traps, after 24 hours; and in Figure 4 which shows the percentage of the released mosquitoes in the window-exit traps.

Evaluation of compound OMS-1406, the CH_3-OCH_3 analogue, was begun 9 months later than the first 5 compounds due to the limited number of huts available.

Compound OMS-1395, the CH_3O-SCH_3 analogue of DDT showed some initial effectiveness but after one month was essentially inactive. The longer effectiveness in the hut than on plywood board in the laboratoary (Figure2) probably due to better protection from light in the relatively dark huts, suggesting that oxidation of the CH_3S- to CH_3SO moiety is photocatalytic.

Compound OMS-1425, the CH_3Cl analogue was much more residually effective in the hut tests (Figure 3) compared to the effectiveness on the plywood board (Figure 2) and appeared to approach DDT, methoxychlor, and OMS-1476, the CH_3-OC_2H_5 analogue, in total effectiveness over the year exposure. It is evident that these latter three compounds retain their insecticidal activity under these conditions for at least a year and both methoxychlor and OMS-1476 closely approach DDT in total effectiveness. Differential susceptibility of Anopheles albimanus and Culex fatigans may also be a factor.

The measure of irritability or excitorepellency shown in Figure 4 is obviously variable. It seems that all 5 of the highly effective compounds produce a rather similar irritability reaction and that this physiological effect is a result of insect nervous instability in the early stages of DDT intoxication. Therefore, it should occur with any active DDT analogue in the pre-knockdown stage. Amplification of this point is obtained from the data in Table 2 which shows in detail the results of mosquito releases over the first 40 days for the 5 most effective compounds. The average percentage of the total number of Anopheles albimanus released and subsequently finding their way into the window-exit traps was: DDT $46 \pm 4.0\%$, methoxychlor $32 \pm 4.7\%$, CH_3-OC_2H_5 $37 \pm 3.5\%$, CH_3-Cl $29 \pm 1.2\%$, and CH_3-OCH_3 $29 \pm 10\%$. From the values for standard error of the mean it appears that DDT is significantly more active in producing symptoms of excito-repellency than the other compounds.

3. Radiotracer Experiments on Rate of Absorption of Compounds by Tarsi of Mosquitoes.

The insecticidal effectiveness of a residual deposit against adult mosquitoes is dependent both on rate of absorption by the lipids of the tarsi, and intrinsic activity at the site of action. Interesting data on the rate of absorption of adulticidal compounds was obtained by using radio-labeled compounds applied to filter paper at 1 150 $\mu g/cm^2$ and exposing groups of 20 adult ♀ Anopheles albimanus to the surfaces for varying amounts of time up to 60 minutes. Each group of insects was analyzed for total radioactivity absorbed by total combustion analysis by the Schöniger oxygen flask technique. The results are shown in Figure 5. Surprisingly regular plots were obtained by this technique and demonstrate nearly a 100-fold difference in rates of absorption of the test compounds. Carbaryl which is a very poor adulticide was absorbed very slowly while propoxur which is an excellent adulticide was absorbed the most rapidly. In connection with the DDT analogues of the present study OMS-1476, (CH_3-OC_2H_5) was clearly absorbed at a more rapid rate than methoxychlor, and much more rapidly than DDT. From a large amount of data available from Stage I evaluation of DDT analogues in the WHO Programme, it is apparent that analogues having a methyl group on the aryl ring are relatively more effective against adult mosquitoes than alkoxy substituents. Typical data (Metcalf et al 1971) include:

p,p,-substituents		LC_{50} ug/Cm^2	
		Culex fatigans	Anopheles albimanus
Cl	Cl	6.9	0.23
CH_3	CH_3	1.7	1.0
CH_3O	CH_3O	16.0	13
Cl	CH_3	5.2	1.5
CH_3	CH_3O	3.3	10
CH_3	C_2H_5O	8.0	0.75
CH_3O	C_2H_5O	160	13

These data suggest that the typical hydrocarbon analogue (toluene) is more soluble in the lipids of the mosquito tarsus than the aryloxy derivatives (anisole, phenetol). This interesting point is being evaluated further for additional leads on the design of DDT analogues for residual spray applications.

In summary, the residual toxicity evaluations demonstrate that methoxychlor, OMS-1425 (CH_3-Cl), and OMS-1476 (CH_3-OC_2H_5) sprayed on plywood surface will kill both Culex and Anopheles adults for at least one year. Hut tests completed on July 27, exactly 11 months after spraying produced the following mortalities: DDT 86%, methoxychlor 86%, CH_3-Cl 88%, and CH_3-OC_2H_5 94%. These tests will be continued through the present season. Other promising compounds will be evaluated as soon as hut space permits.

D. Pond Tests of Biodegradability

Pond evaluation of DDT analogues was organized to seek compounds free from the problem of biological concentration from trace contamination in water to relatively large amounts in fish and other aquatic organisms, which may exceed FDA tolerances and threaten reproductive success of the organisms. Because of the large number of compounds to be evaluated, the initial tests were made in large aluminum pools 5 feet in diameter, holding 1300 liters (Figure I). These were used both outdoors in summertime at 16-35°C., and in winter in a polyvinyl covered screenhouse, gas heated to 1-15°C. The DDT analogues were added, dissolved in acetone, to the water at a final concentration of 0.1 ppm using 130 mg. per pool. Each pool contained 5 green sunfish, Lepomis cyanellus. Whenever fish were killed by the test compound, the tank was restocked weekly. The data in Table 3 show the number of weeks that the various DDT analogues were toxic to the fish. At appropriate intervals one-liter water samples were removed from the tanks, extracted with diethylether and evaluated by gas chromatography, GLC. Fish were also removed from the tanks, homogenized, and extracted with acetonitrile for GLC assay. Results are shown in Table 4.

1. Toxicity of DDT Analogues to Green Sunfish, Lepomis cyanellus
   Toxicity of the various compounds studied

Fish toxicity data is summarized in Table 3 for DDT and 17 analogues. The figures in the Table 3 indicate the duration of toxicity in days, i.e. the period over which the compound was lethal to the green sunfish. Because of the limitations on the number of tanks available and those imposed by winter, two sets of investigations were made (1) out-of-doors under summer sun (average temperature 16-35°C) and (2) under transparent polyvinyl plastic (average temperature 1-15°C). The two sets of tests were very useful in demonstrating the importance of environmental temperature in controlling the rate at which the poikilothermic fish degrade xenobiotic compounds. Sunlight

may also play a role into photochemical degradation although the polyvinyl chloride plastic is light transparent. Thus methoxychlor was toxic for only 2 days at the higher temperature vs. 41 days at the lower. OMS-1476, one of the most promising analogues, was effectively non-toxic at high temperature and much less toxic than methoxychlor at the lower temperature. This compound is degraded in vivo both by O-deethylation and CH_3-oxidation. It is clear that fish perform this latter process more efficiently than O-dealkylation as is shown by the much higher fish toxicity of OMS-1473, 1355, and 1356. The extreme toxicity of OMS 1355 and 1356 was very surprising as these two compounds were selected by Holan (1971) as especially promising DDT analogues and have been patented for commercial development as DDT-substitutes. The data on these two compounds together with the studies we have previously made on the degradative fate of ethoxychlor (Kapoor et al 1971) suggest that the diethoxydiphenyl-methane moiety is not sufficiently degradable to be incorporated in a fully satisfactory DDT-substitute.

A comparison of the toxicity of the a-trichloromethylbenzyl analogues OMS 1461 and 1536 is informative. Both of these compounds have typical DDT-like activity (Hirwe et al 1971). However, the benzylaniline (1461) is very rapidly degraded by sunlight to form an aniline and a benzoic acid moiety. The benzyl phenyl ether (1536) is much more stable and does not cleave readily between the two rings. This is clearly shown by the GLC data of Table 4 .

Fish toxicity data is clearly very important in determining the ultimate environmental impact of these DDT-type compounds. An added indication of the superiority of the asymmetrical biodegradable analogues is given by data from industry (CIBA-Geigy) giving LC_{50} values to carp and trout of: DDT 0.033,0,028; OMS-1395 10,1; and OMS-1476 10, 1.

The presence of a methylenedioxyphenyl group -O-CH_2-O- (CMS-1399) which is known to have autosynergistic properties also greatly retarded the *in vivo* degradation of this compound in fish compared to its close relative methoxychlor.

Comparison of the rates of disappearance of the α-trichloromethyl-benzyl derivatives CMS 1461 and 1536 showed the extreme lability of the former, which is rapidly cleaved to aniline and benzoic acid moieties, and the great persistence of the latter which is obviously not cleaved readily. Approximate half-life values in water for most of the compounds studied are given in Table 4

Effects of piperonyl butoxide. This widely used insecticide synergist is an inhibitor of multi-function (microsomal) oxidase action. Piperonyl butoxide has been shown to retard the *in vivo* O-dealkylation of the methoxy groups of methoxychlor (Kapoor et al 1970). In the pool experiments, 10 parts of piperonyl butoxide to 1 part of methoxychlor increased the length of toxic action of methoxychlor to the green sunfish from 2 weeks to 13 weeks at 15-38°C. The increased duration of toxic action was not due to any protective affect in the water phase but rather was the result of a substantial *in vivo* protective effect on the degradation of methoxychlor as shown by GLC analysis.

	ppm methoxychlor			
	H_2O		fish	
	alone	p.b.	alone	p.b.
initial	.0575	.0464	13.5	24.0
1 week	.029	.0194	--	24.5
10 days	--	--	--	18.0
2 weeks	.0165	.0120	--	11.0
3 weeks	.0112	.0092	--	--
4 weeks	.0030	.0030	1.75	1.78

2. Chemical Studies of Degradation of DDT Analogues in Water and Green Sunfish.

The results of GLC evaluation of the rates of degradation of the various DDT analogues in water and in fish are summarized in Table 4 . In general these results support the fish toxicity values of Table 3 . Compared to methoxychlor (OMS-44) the asymmetrical compound OMS-1395 disappeared very rapidly. The methylthio (CH_3S-) analogue is readily oxidizable to sulfoxide (CH_3SO-) and sulfone (CH_3SO_2-) moieties which promote rapid dehydrochlorination of the trichloroethane to dichloroethylene (DDE analogues). GLC data on extracts of the green sunfish from this tank showed the following rapid loss of parent compound.

% total extractives

	CH_3S-	CH_3SO-	CH_3SO_2-
initial	1.36	63	36
1 week	2.8	62	35.2
2 weeks	0	58	42
6 weeks	+	47	53

This compound has been found to be of very short persistence in the mouse and other organisms (Kapoor et al 1973) and on surfaces (Section III-B).

The very promising asymmetrical analogue OMS-1476 was rapidly degraded, at a rate of about twice that of methoxychlor in water and much faster in fish.

The neopentyl (-$C(CH_3)_3$) analogue (OMS-1355) was amazingly stable in fish tissues and comparision with the -CCl_3 analogue OMS-1473 shows essentially no difference in rate of degradation. Thus the -$C(CH_3)_3$ group is not attacked biologically to any extent and degradability in this molecule proceeds almost entirely through O-dealkylation of the aromatic rings. This conclusion is supported by detailed studies of Coats et al (1973) comparing the degradability of methoxychlor and dianisyl neopentane in a laboratory model ecosystem where the two compounds had nearly equivalent biodegradability index values of 0.94 and 1.04.

In summary, these fish toxicity data emphasize the importance of *in vivo* detoxication of the DDT analogues in determining both toxicity and tissue accumulation compounds with an alkyl and an alkoxy aryl substituent are considerably more biodegradable than dialkoxy-substituted compounds. It appears that the multi-function oxidase enzymes cannot readily attack aliphatic groups such as $-C(CH_3)_3$ and $-CH(CH_3)NO_2$ and that these compounds are not much better degraded environmentally than the DDT series with $-CCl_3$. It appears then that biodegradability in fish is primarily associated with aryl degradophores of which $-CH_3$ is one of the most effective.

Table 4. Approximate Half-Lives of DDT Analogues in Water

p,p' substituents (GLC)		T ½ weeks
CH_3O	CH_3O	1.0
CH_3	Cl	0.30
CH_3	C_2H_5O	0.45
C_2H_5	C_2H_5O	0.35
C_2H_5O	C_3H_7O	0.85
C_2H_5	Cl	0.40
CH_3	C_2H_5	0.50
CH_3O	3,4-OCH_2O	1.0
C_2H_5O	C_2H_5O	0.85
C_2H_5O	Cl	0.65

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Table 1 Detailed Knockdown and Mortality Studies with DDT Analogues on Plywood to *Culex fatigans* - initial deposit

OMS-		% knockdown			% mortality				
		15'	30'	60'	24h	48h	72h	96h	120h
15	DDT	0	15	35	50	60	77	97	100
44	methoxychlor	0	0	45	50	55	75	85	95
1425	CH_3-Cl	0	10	45	15	20	25	56	100
1476	CH_3-OC_2H_5	0	10	40	90	95	95	100	--
1395	CH_3O-CH_3S	0	20	20	5	10	15	45	90
1445	aniline	0	5	10	0	0	19	69	100

Table 2 - Comparative Toxicity of DDT Analogues to *Anopheles albimanus* Adults in Plywood Huts with Window Exit Traps treated with EC at 2g. per m^2

		days after treatment				
		1	8	16	23	40
OMS-1395	% in traps	28	32	17	18	4
	mortality in traps-%	92	83	75	55	0
	total mortality-%	82	47	32	9	0
OMS-1425	% in traps	30	25	23	29	29
	mortality in traps-%	100	100	100	100	100
	total mortality-%	100	100	100	100	100
OMS-1476	% in traps	32	39	30	48	36
	mortality in traps-%	100	100	100	100	100
	total mortality-%	100	100	99	100	100
OMS-1406	% in traps	47	10	17	40	30
	mortality in traps-%	100	100	100	100	100
	total mortality-%	92	100	98	90	94
methoxychlor	% in traps	33	19	46	34	32
	mortality in traps-%	100	100	100	99	100
	total mortality-%	100	98	100	99	100
DDT	% in traps	43	54	50	41	41
	% mortality in traps	100	100	100	99	100
	total mortality-%	100	100	100	99	100

Table 3. Toxicity of Degradable DDT Analogues to Green Sunfish

		0.10 ppm in water	
		Duration of fish toxicity-days*	
OMS#		16-35°C	1-15°C
15	$ClC_6H_4CH(CCl_3)C_6H_4Cl$ (DDT)	18	
44	$CH_3OC_6H_4CH(CCl_3)C_6H_4OCH_3$ (methoxychlor	2	41
1395	$CH_3OC_6H_4CH(CCl_3)C_6H_4SCH_3$	0	
1425	$CH_3C_6H_4CH(CCl_3)C_6H_4Cl$	0	
1476	$CH_3C_6H_4CH(CCl_3)C_6H_4OC_2H_5$	0	15
1477	$CH_3C_6H_4CH(CCl_3)C_6H_4C_2H_5$		0
1671	$C_2H_5C_6H_4CH(CCl_3)C_6H_4OC_2H_5$		5
1637	$C_2H_5C_6H_4CH(CCl_3)C_6H_4Cl$		0
1473	$C_2H_5OC_6H_4CH(CCl_3)C_6H_4OC_3H_7$		41
1355	$C_2H_5OC_6H_4CH(CMe_3)C_6H_4OC_2H_5$		49
1356	$C_2H_5OC_6H_4CH(CHMeNO_2)C_6H_4OC_2H_5$		52
1461	$CH_3OC_6H_4NHCH(CCl_3)C_6H_4Cl$		0
1536	$ClC_6H_4OCH(CCl_3)C_6H_4OC_2H_5$		17
1399	$CH_3OC_6H_4CH(CCl_3)C_6H_3OCH_2O$		28
1074	$C_2H_5OC_6H_4C_3H_2Cl_2C_6H_4OC_2H_5$	23	
1027	$ClC_6H_4C_3H_2Cl_2C_6H_4Cl$	5	
467	$ClC_6H_4CH(CHMeNO_2)C_6H_4Cl$	2	

Figure 1. Evaluation of Biodegradable DDT analogues.
Above: plastic house and tanks for fish toxicity tests.
Lower left: plywood huts for evaluation of persistence and excitorepellency of DDT analogues
Lower right: hut showing exit traps for mosquitoes

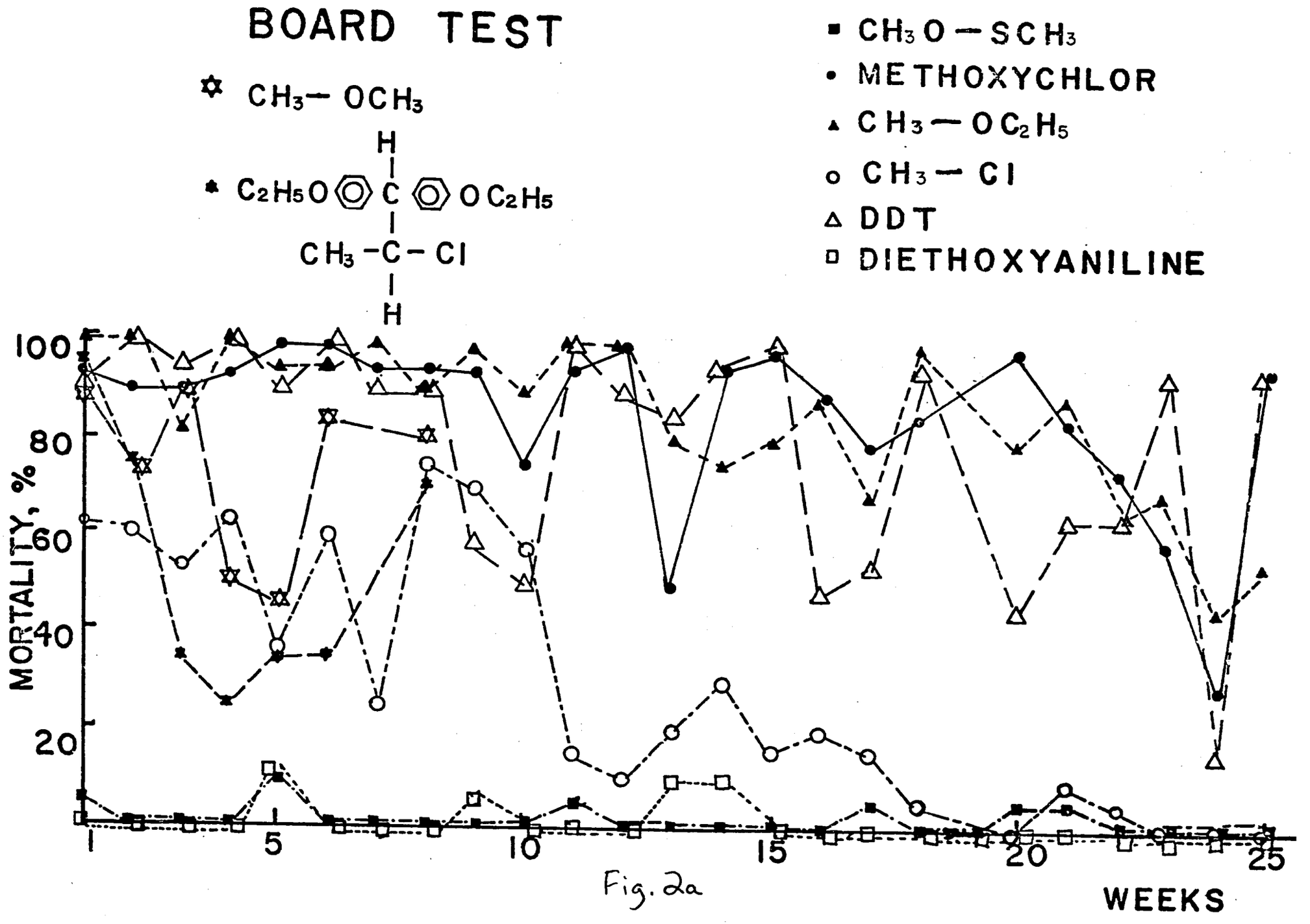
BOARD TEST
CH3— OCH3
C2H5O C OC2H5
H
CH3 -C- Cl
H
CH3O — SCH3
METHOXYCHLOR
CH3 — OC2H5
CH3 — Cl
DDT
DIETHOXYANILINE
MORTALITY, %
100
80
60
40
20
1
5
10
15
20
25
WEEKS

Fig. 2a

III. Results of Investigations

A. Refinement of knowledge regarding biological properties of biodegradable analogues of DDT.

1. Biodegradability and Ecological Magnification

The number of biologically active analogues of DDT is very large and it is estimated that at least 1000 active compounds of this nature have been made. Comprehensive evaluations of their basic insecticidal action to flies and mosquitoes have been published (Metcalf and Fukuto 1968, Metcalf et al 1971). Adding the added requirement for biodegradability has substantially increased the complexities of the search for the most useful compounds. A detailed investigation of degradation pathways and model ecosystem evaluation for ecological magnification (E.M.) and biodegradability index (B.I.) has been made for eight DDT-like compounds (Kapoor et al 1973).

	E.M.		B.I.	
	fish	snail	fish	snail
DDT	84,500	34,500	0.015	0.045
methoxychlor	1,545	120,000	0.94	0.13
ethoxychlor	1,536	97,645	2.69	0.39
methylchlor	140	120,270	7.14	0.08
methiochlor	5.5	300	47	0.77
OMS-1395 (CH_3O-SCH_3)	310	3,400	2.75	105
OMS-1476 (CH_3-OC_2H_5	400	42,000	1.20	0.25
OMS-1425 (CH_3-Cl)	1,400	21,000	3.43	2.0
OMS-$CH_3OC_6H_4CHC(CH_3)_3$ $C_6H_4OCH_3$	1,636	23,300	1.04	0.23

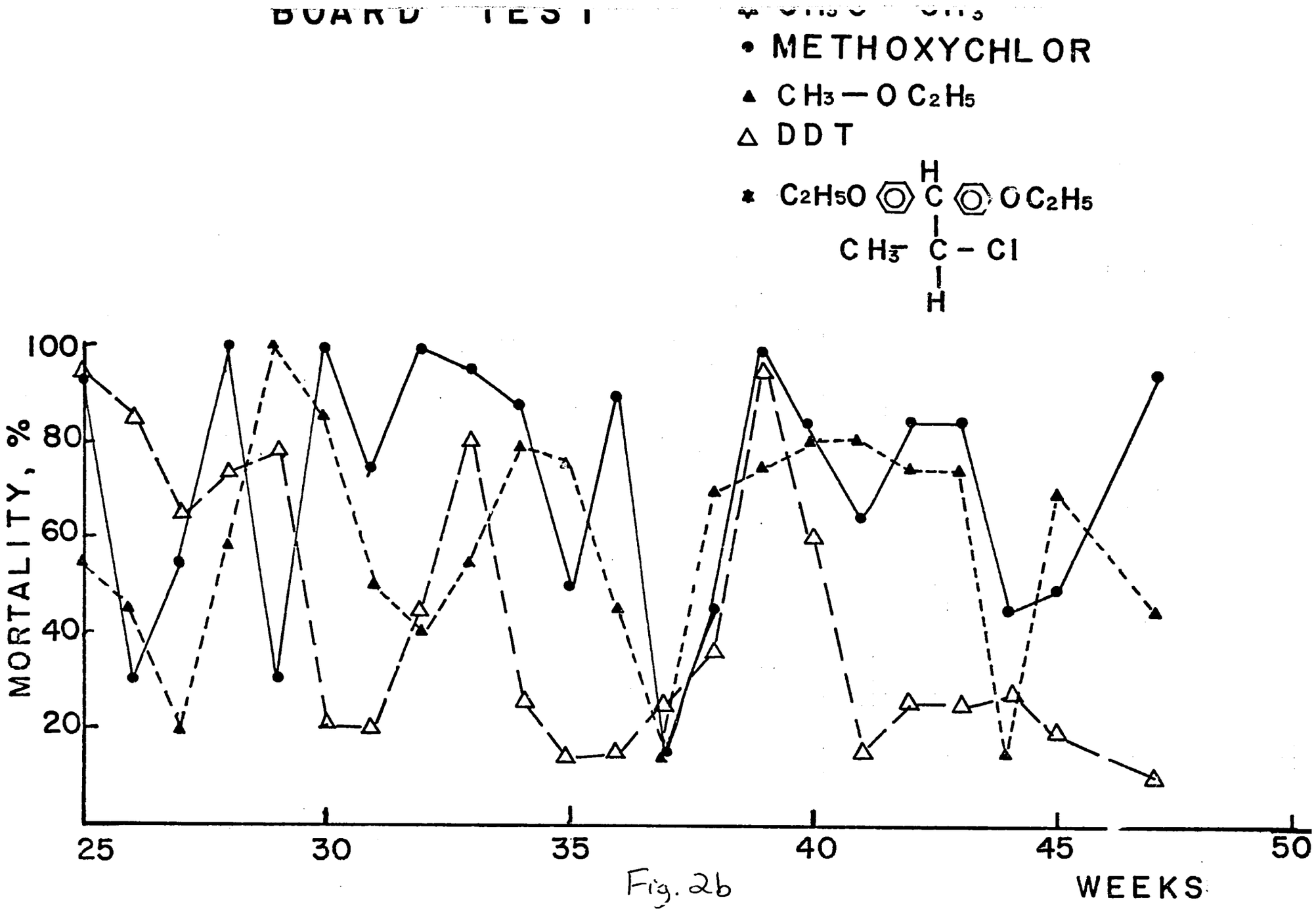
BOARD TEST
METHOXYCHLOR
CH3—OC2H5
DDT
C2H5O
H
C
OC2H5
CH3
C
Cl
H
MORTALITY, %
100
80
60
40
20
25
30
35
40
45
50
WEEKS

Fig. 2b

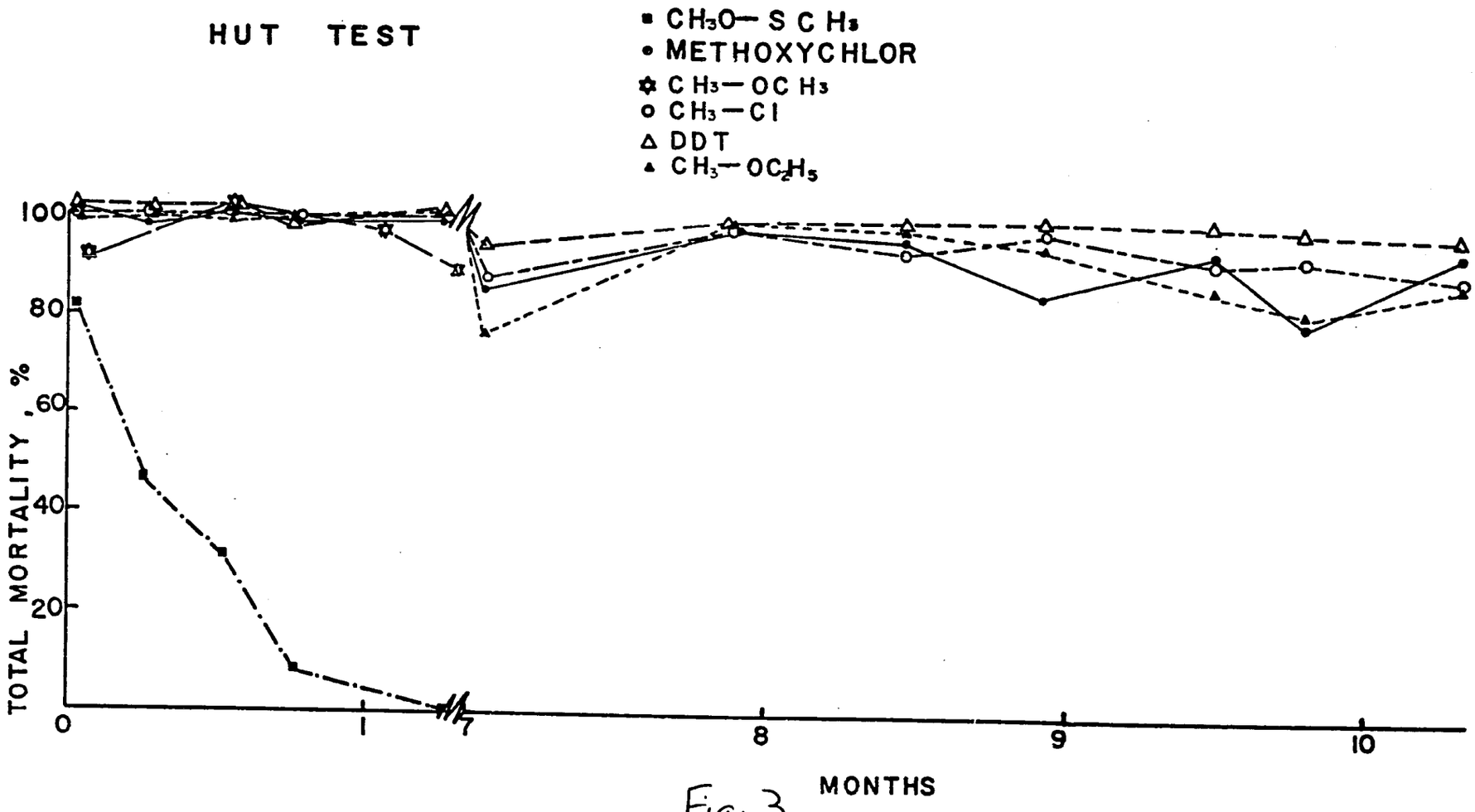
HUT TEST
■ CH3O— S C H3
• METHOXYCHLOR
✡ C H3— OC H3
○ CH3—Cl
△ DDT
▲ CH3—OC2H5
TOTAL MORTALITY, %
100
80
60
40
20
0
1
7
8
9
10
MONTHS

Fig. 3

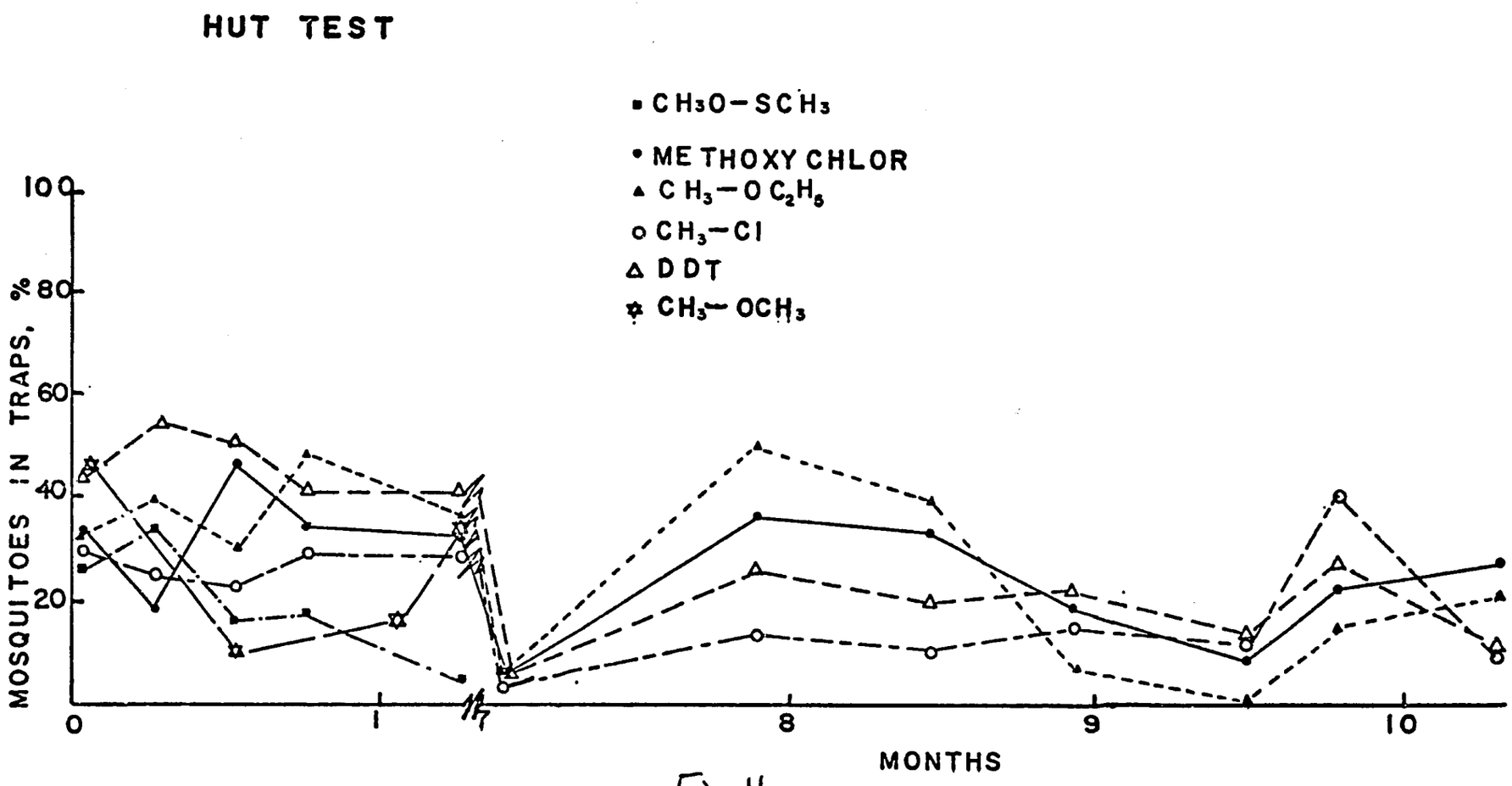
HUT TEST
CH3O—SCH3
METHOXY CHLOR
CH3—OC2H5
CH3—Cl
DDT
CH3—OCH3
MOSQUITOES IN TRAPS, %
100
80
60
40
20
0
1
7
8
9
10
MONTHS

Fig. 4

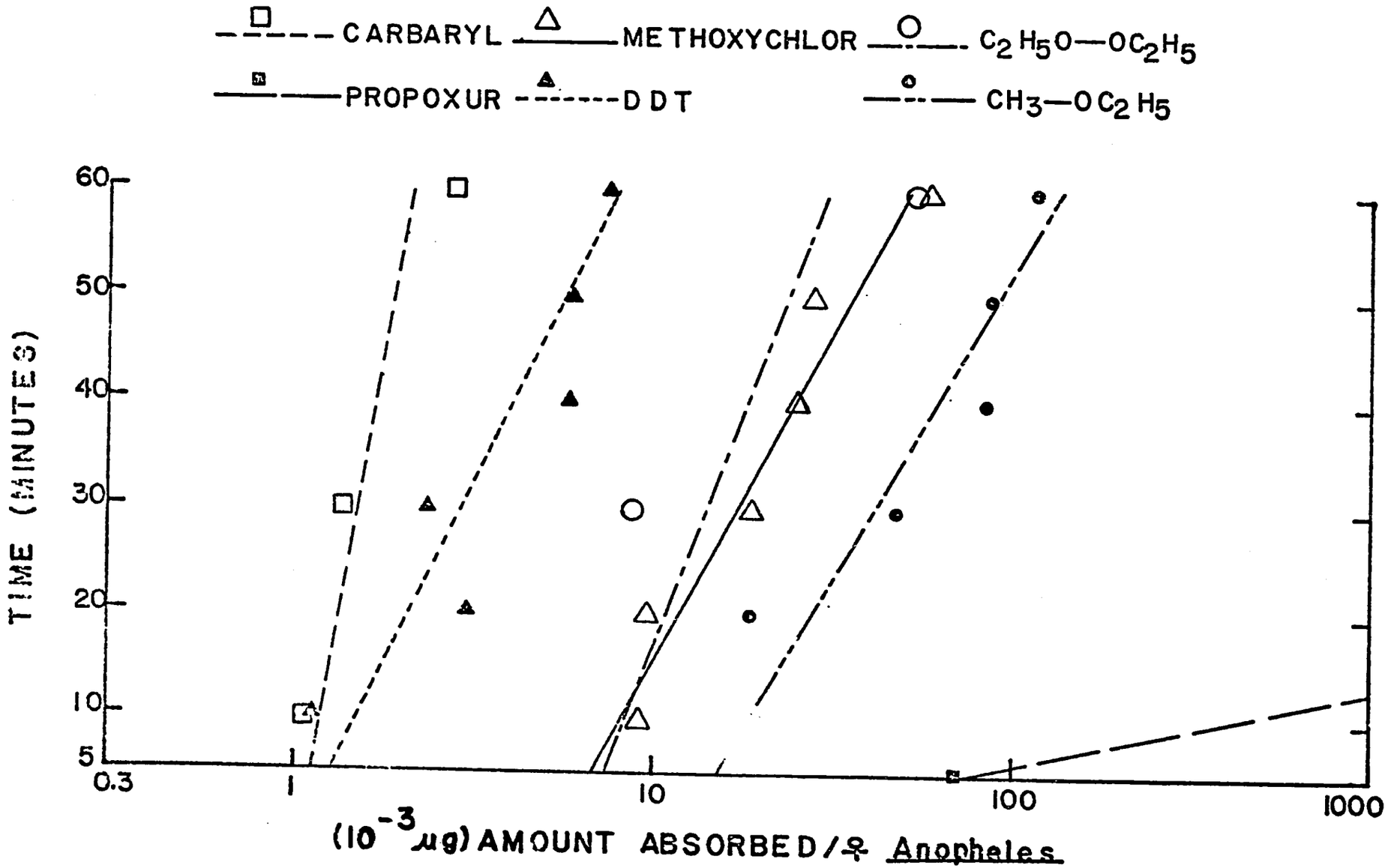
CARBARYL
METHOXYCHLOR
$C_2H_5O—OC_2H_5$
PROPOXUR
DDT
$CH_3—OC_2H_5$
TIME (MINUTES)
60
50
40
30
20
10
5
0.3
1
10
100
1000
(10^{-3} µg) AMOUNT ABSORBED/♀ Anopheles

Fig. 5a