Symposium on Pesticide Interaction Phenomena

INTRODUCTION

Synergism of insecticide action by certain substances is a well known phenomenon that has been extensively exploited to enhance insecticidal potencies. In a related manner, certain pairs of organophosphates interact directly and thus exhibit potentiation of their toxic action.

A recent symposium of the ACS Division of Agricultural and Food Chemistry reviewed newer developments in these and related aspects of pesticide interaction events. Several of the symposium papers are presented in this issue of the JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY; however, others are to be published elsewhere. In essence, many interaction types focus on the same fundamental biochemical mechanism, the reactions of microsomal enzymes in liver and the insect fat body. J. R. Gillette and B. B. Brodie keynoted the symposium with their paper on "Variations in Metabolism of Foreign Compounds by Liver Microsomes." A review of that topic by Gillette has appeared in *Advances in Pharmacology*. Undoubtedly, one of the most striking developments in the field of organochlorine insecticides is the discovery of the marked interactions occurring with drugs and many other substances. Such pesticide effects on drug and steroid metabolism were discussed by A. H. Conney, R. Welch, R. Kuntzman, and J. J. Burns; their paper is to be published in *Clinical Pharmacology and Therapeutics*. Other papers covered related aspects of that type of interaction. The symposium was concluded by W. F. Durham with a general review of nonchemical interactions affecting pesticide toxicity; it will be published in *Residue Reviews*.

The following section comprises a group of original contributions from the symposium.

J. C. STREET, Symposium Chairman
Department of Animal Science
Utah State University
Logan, Utah

MICROSOMAL CHANGES

Interactions between DDT Analogs and Microsomal Epoxidase Systems

JAMES W. GILLETT, TIMOTHY M. CHAN, and L. C. TERRIERE

Department of Agricultural Chemistry, Oregon State University, Corvallis, Ore.

Liver microsomes, isolated from white rats fed diets containing DDT, DDD, DDE, or Kelthane, were incubated with a NADPH-generating system and either aldrin or heptachlor. The rates of formation of the corresponding epoxides, determined gas chromatographically, were used as a measure of microsomal epoxidase activity. A litter-mate comparison approach was used to minimize differences. Microsomes from rats fed DDT showed an increase in epoxidase activity related to the concentration of DDT in the diet. DDE and Kelthane promoted a smaller increase in epoxidation than did DDT, and DDD stimulated epoxidation only slightly. Age, sex, and duration of the exposure to dietary DDT analogs also appeared to affect the extent of increases, termed induction, brought about by DDT. Only male rats and female quail were inducible. Induced and control microsomes differed in their sensitivities to various inhibitors and in their temperature stability. Accordingly, DDT-induced epoxidase is believed to be an altered form of the natural activity.

THE CONVERSION OF ALDRIN, isodrin, and heptachlor to their corresponding epoxides has been demonstrated (*20*, *25*, *29*) in microsomal fractions from both vertebrate and invertebrate species. Cyclodiene epoxidation requires NADPH and oxygen and may be considered typical of microsomal mixed function oxidases performing a variety of metabolic reactions—*N*-dealkylation, hydroxylation, ring-cleavage, and side-chain oxidation. In 1963, Hart and Fouts (*14*, *15*) reported that microsomal drug metabolism could be induced by treating rats with chlordan or DDT. Unaware of this discovery and seeking to conserve tissue, Wong used microsomes from rats fed DDT to study the effect of dietary DDT on soluble liver proteins (*29*) in experiments on aldrin epoxidation and found them to be more active than microsomes from untreated rats (*28*). Subsequently, others (*9*, *10*) demonstrated an inductive effect of DDT on microsomal oxidations. The phenomenon of induction, noted by the appearance of increased amounts of an enzymic activity, may be contrasted too with alterations in metabolism brought about by genetic selection, as in the case of developing insecticidal resistance, in that changes occur in only one generation and after only brief exposure to the inducing agent. Induction differs from in vitro activation, such as by cofactors, in that the intact animal or cell is required to

show the effect and in that the inducer is inactive in vitro. This paper considers those factors affecting cyclodiene epoxidation and the interaction of epoxidation with dietary DDT analogs.

Under the "Pesticides in the Environment" program, microsomal epoxidation is being studied in white rats, Japanese quail, and rainbow trout. As a base for this study, rat liver microsomes have been investigated to demonstrate the dependance of cyclodiene epoxidation on age, sex, and nutritional status. Inclusion of DDT or its analogs in the diet effected definite increases in activity related to the compound and level fed, the age and duration of treatment, and the sex and species involved. Comparisons of the epoxidative activity of both normal and DDT-induced microsomes in regard to substrate and inhibitor specificities and temperature stability have been made.

The study of the effect of DDT analogs was undertaken to assess the role of structure and metabolism on induction. Additionally, occurrence of DDT analogs as environmental residues along with DDT suggested a practical aspect to such a study. These experiments tested three analogs which represent different routes of DDT metabolism (dehydrochlorination to DDE, reductive dechlorination to DDD, and hydroxylation to Kelthane) and which might demonstrate that metabolic activation of DDT was required for induction.

Experimental

Treatment of the Rats. White rats of the Corvallis strain, a highly inbred variety of the Wistar strain, were placed on a synthetic basal ration (*27*) at four weeks of age or later. Between weaning and initiation of the studies, the rats and their mothers were maintained on Rockland Rat Pellets. Where possible, littermate groups of rats were sacrificed on the same day. DDT or its analogs were added to the basal ration by substituting a solution of pure insecticide in corn oil for all or a portion of the corn oil used to make up the diet. In early experiments, the rats were maintained in individual cages with their own supply of food and water, but later litter-mate groups were reared together from weaning on the control (DDT) ration, then separated into pairs while DDT was being fed to one group.

Treatment of the Quail. Japanese quail (*Coturnix coturnix japonica*) received a turkey starter ration (*23*) containing corn oil, for which oil containing DDT could be substituted as in the rat diet. Mature females were provided with oyster shell *ad libitum*. Males and females were segregated at 17 days.

Dietary Residue Determinations. Chlorinated hydrocarbon insecticide residues were monitored in all diets by periodic sampling and analysis according to the following procedure:

The powdered or ground sample (10 grams) was suspended in 25 ml. of benzene and extracted 3 times with 25 ml. of acetonitrile. The combined acetonitrile layer was washed once with 50 ml. of hexane, then 300 ml. of water was added to the acetonitrile and the mixture extracted 3 times with 100-ml. portions of hexane. The combined hexane phase was dried with sodium sulfate, filtered, and evaporated to dryness. The residue was taken up in distilled hexane for analysis by gas chromatography. Identity of components was checked by use of two different detectors—electron capture and microcoulometric—and two different columns.

Preparations of Liver Microsomes. All operations were performed at 0° to 4° C., and preparations were stored at 2° to 4° C. until assay. The basic operations remained unchanged, although some variations between experiments were made in attempts to increase yield and purity.

The rat was anesthetized with diethyl ether, and the liver was removed and washed free of blood and hair in ice cold 1.15% (w./v.) KCl solution. Quail were decapitated and exsanguinated, then the livers were handled in the same manner as those of rats. The livers of groups of rats or quail were pooled prior to homogenization, which was accomplished by blending the blotted and weighed livers in a VirTis "45" homogenizer at 45,000 r.p.m. in 9 ml. of 1.15% KCl solution per gram of tissue. The crude homogenate was centrifuged at 14,000 r.p.m. (25,000 × G) for 30 minutes in the Servall SS-2 head. The pellet was discarded, and the supernatant was recentrifuged in the Spinco Model L ultracentrifuge at 2×10^6 G_{av}-minutes. The resultant pellet was washed by resuspension in about 5 ml. of KCl solution per gram of liver and high-speed centrifugation as before. The final microsomal suspension was made by homogenizing the washed pellet briefly with sufficient buffer or KCl solution to give approximately 1 gram of whole liver equivalents per milliliter.

Epoxidase Assay. The incubation procedure used by Wong and Terriere (*29*) was modified only slightly. Determinations were made at pH 8.0 and 38° C., and nicotinamide was omitted from the reaction medium. Microsomal protein levels, as determined by a modified biuret procedure (*6*), were reduced to 1 to 5 mg. per flask. Flasks containing aldrin were incubated 15 minutes, and flasks containing heptachlor were incubated for 30 minutes. The addition of 10 ml. of 2-propanol–hexane (2:3, v./v.) mixture stopped the reaction.

The organic layer was separated, and the aqueous layer was re-extracted twice with 10-ml. portions of hexane. The combined organic layer was made up with hexane to 50 ml. in a volumetric flask. Anhydrous sodium sulfate then was added to remove traces of moisture. The extracts were analyzed for epoxides on a Wilkins Aerograph Hy-Fi Model 500 (electron capture detector) fitted with a borosilicate glass column packed with 5% QF 1-0065 on Chromosorb W (27-inch inlet end) and 5% Dow 11 on Chromosorb W (21-inch outlet end).

A unit of epoxidase activity was defined as that amount of enzyme forming 1 mμ-mole of epoxide per minute, at an initial substrate concentration of 20 μg.—approximately 55 mμmoles for the various substrates—per flask, after correction for the controls. A flask stopped at 0 minutes gave a correction for loss during extraction, and flasks incubated without microsomes or with boiled microsomes indicated nonenzymatic conversions.

Materials. Cyclodiene insecticides were donated by the Shell Development Co., Modesto, Calif., *p,p'*-DDT and G-6-P DH were obtained from Nutritional Biochemical Corp. *p,p'*-DDE was synthesized from DDT, and *p,p'*-DDD and Kelthane were analytical standards from Rohm and Haas, Philadelphia, Pa. NADPH, NADP, G-6-P and Tris buffer were purchased from Sigma Chemical Co., St. Louis, Mo. All chlorinated hydrocarbons were checked for identity and purity by gas chromatography. All solvents were redistilled until free of interfering contaminants.

Results and Discussion

Enzyme Assays. Figure 1 shows a typical plot of activity *vs.* time for aldrin epoxidation. The rate of epoxide formation was linear for the first 30 minutes if the amount of microsomal protein was between 0.1 and 4 mg. per flask.

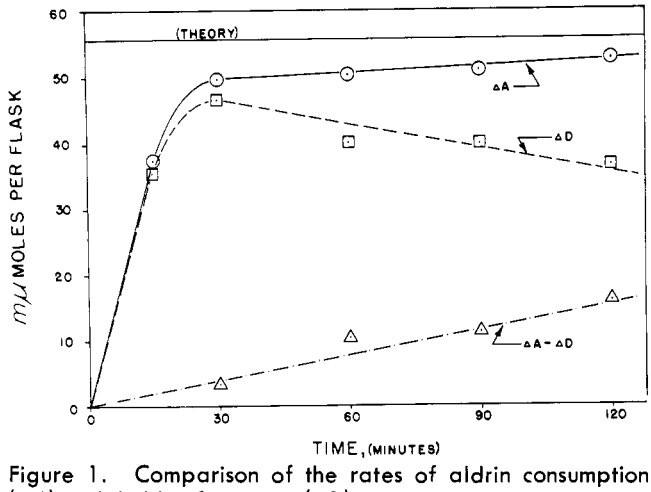

Figure 1. Comparison of the rates of aldrin consumption (ΔA) and dieldrin formation (ΔD)

5.6 mg. of microsomal protein, 38° C.

For longer incubations and higher protein levels, the rate of aldrin disappearance usually exceeded the rate of appearance of the epoxide and showed a greater disparity between activity and increasing level of microsomes than did dieldrin formation. Although Figure 1 suggests progressive loss (ΔA-ΔD) of aldrin and/or dieldrin, no further metabolism of dieldrin could be measured. Incubation of dieldrin with microsomes, with and without added NADPH and/or reduced glutathione, at various protein levels, and for up to two hours, gave recoveries of 95 ± 5% at levels of 4 and 53 mμmoles per flask. Routes of aldrin loss other than metabolism have been minimized by improvement of the extraction and analytical procedures, but some losses might come from preferential absorption of aldrin on the walls of the incubation flask or in the protein. During the incubation period used in the assay of liver microsomes, aldrin appears to be converted only to dieldrin, but alternatives to and extensions of this route have not been eliminated.

Little success was achieved in increasing the purity (specific activity) of aldrin epoxidation in liver microsomes. No difference between CO_2 and diethyl ether anesthesia or between perfused and unperfused livers could be detected. However, use of the VirTis "45" homogenizer increased the yield of microsomal protein and epoxidase activity about 50% over that obtained by the Waring Blendor or glass-Teflon homogenizer. Resuspension of the final pellet in either 0.05*M* Tris buffer (pH 8.0) or 1.15%*M* KCl solution made little difference in the activity.

Epoxidase Activity. Table I summarizes the data on epoxidation of aldrin and heptachlor in male rats of varying age on a control diet. The lower activity from 8 to 16 weeks is associated with sexual maturation (*5*) and may reflect hormonal influences and interactions. No difference in activity could be seen between control animals raised on the laboratory chows and those on the synthetic diet; hence, these changes were not introduced by the semisynthetic ration. However, considerable variation in the level of epoxidase activity of microsomes from control rats of any given age has been observed in these experiments. Whereas replicate rats of the same litter sacrificed on the same day yielded microsomes having identical (±5%) epoxidase activity, litter differences have been as much as 20- to 30-fold (compare controls in Table V with Table I). Therefore, the authors have used a litter-mate comparison approach in evaluating the various effects reported herein. Even then, no statistical significance may be attached to these results.

Effect of DDT and Its Analogs. DDT has no effect on the epoxidation of aldrin in vitro (Table II), but marked increases in the level of epoxidase in the microsomes can be obtained by administering DDT or its analogs in vivo. The magnitude of this increase is determined by the test substrate, the inducer compound used, the level of inducer in the diet, the length of time the rat is on the diet, the age at which treatment started, and the sex and species of the animal tested. Table III compares the substrate specificity of normal and induced microsomes of male rats on a 25-p.p.m. DDT diet for two weeks. Naphthalene hydroxylation was determined by Schonbrod (*25*).

THE INDUCER. Of the compounds tested, *p,p'*-DDT produced the greatest increase in the ratio of specific activities of treated to control preparations. Table IV compares the effect on epoxidation of *p,p'*-DDT, *p,p'*-DDE, Kelthane, and *p,p'*-DDD fed at 100 p.p.m. for two weeks to groups of rats sacrificed at seven weeks of age. Kelthane and DDE appear to be about equally effective, although neither promoted a change as great as did DDT. DDD is a considerably less potent inducer. Hart and Fouts (*12*) reported DDE and Kelthane to have effects as great or greater than DDT, and all much more effective than DDD, in induction of several drug metabolizing activities when these compounds were injected into or fed to the rat. Apparently, metabolism of DDT to either DDE or DDD (*17*), or to Kelthane by the hydroxylative pathway found in some insects (*1*), is not only unnecessary for induction but may even lessen the effectiveness of a given dose of DDT. Morello (*19*) has reported induction of DDT-metabolizing enzymes by DDT fed to the rat, but the pathway of degradation is not known.

THE LEVEL OF INDUCER. Table V shows the effect of 2.5, 10, 25, and 100 p.p.m. of both DDT and DDD

Table I. Microsomal Epoxidation by Male Rats at Various Ages[a]

Age, Weeks	Specific Activity (μμMoles/Minute/Mg. Protein) Aldrin	Heptachlor
5	108	20
6	66	10
7	36	24
8	15	10
10	26	1
12	18	9
16	16	6
20	70	46

[a] Rats were weaned onto the basal ration at 4 weeks of age. Activities toward aldrin and heptachlor were determined on the same preparations.

Table II. Effect of DDT on Epoxide Formation in Vitro[a]

DDT (MμMoles/Ml.)	MμMoles Epoxide from Aldrin	Heptachlor
0	24.9	17.7
0.4	22.4	16.5
1.4	23.4	16.8

[a] Each reaction contained 20 mg. of liver microsomes from a six-month-old male rat. Incubation time was 30 minutes, all other assay conditions as described in test.

Table III. Epoxidase Activity with Various Substrates

Substrate	DDT in Diet, P.P.M.	Specific Activity (μμMoles/Minute/Mg.) 7 weeks[a]	10 weeks[b]
Aldrin	0	84	62
	25	450	150
Heptachlor	0	25	16
	25	300	170
Isodrin	0	...	31
	25	...	110
Naphthalene	0	69	51
	25	540	410

[a] Microsomes from three male rats 7 weeks old.
[b] Microsomes from one male rat 10 weeks old.

Table IV. Comparison of DDT Analogs as Inducers of Epoxidation of Aldrin and Heptachlor

Expt.	Analog Added to Diet, 100 P.P.M.	Specific Activity (μμMoles/Minute/Mg. Protein) Aldrin	Heptachlor
6a	None	1.9	2.4
	DDT	33	54
	DDE	16	26
	Kelthane	19	25
7b	None	1.9	1.1
	DDD	9.2	6.4

Table V. Effect of Concentration of DDT and DDD on Epoxidase Induction

Expt.	Analog Added to Diet, P.P.M.	Specific Activity (μμMoles/Minute/Mg. Protein) Aldrin		Heptachlor	
5	None	28		29	
	DDT, 2.5	67		24	
	DDT, 10	227		131	
	DDT, 25	480 (420[a])		400 (380)	
	DDT, 100	920[b] (650)		273 (470)	
6d	None	1.5		0.5	
	DDD, 2.5	1.9		0.7	
	DDD, 25	2.8		1.8	
		b	c	b	c
6b,c	None	1.9	36.2	1.1	23.5
	DDD, 10	2.8	17.1	1.1	19.5
	DDD, 100	9.2	162	6.4	141

[a] Figures in parentheses indicate single mate reared alone; all other figures for pooled sample of pairs reared and sacrificed together.
[b] $P < 0.10$.

on the specific activity. This increase is not directly proportional to the dosage because of a general increase in microsomal synthesis promoted by DDT (*21*). The increase in specific activity of epoxidase indicates a specific response of the cell to DDT, whereas the net effect of DDT can be seen in a comparison of the activity per gram of liver at the various levels of DDT. Figure 2, which shows a double reciprocal plot of the activity per gram of liver against the concentration of DDT in the diet, resembles the familiar Lineweaver-Burk plot (*18*) demonstrating the dependence of enzyme activity on substrate concentration. If one takes the synthesis of epoxidase as the reaction, DDT then appears to participate in a rate-limiting step, such that formation of new or additional activity depends on the concentration of ingested DDT. Several workers have shown drug- and pesticide-induced changes in the microsomes to be sensitive to inhibitors of protein synthesis (*13*). Should DDT interact with DNA to depress or accelerate expression of the system for microsomal epoxidation, expression might be expected to be dependent on the concentration of DDT. This view would be complicated, however, by the dosage-dependent variation in rates of absorption, metabolism, excretion, and storage.

An alternative manner of relating this data can be seen in Figure 3; the ratio of epoxidase activities of control rats to DDT-fed rats is plotted against the reciprocal of the concentrations of DDT and DDD in the diet. Extrapolation of the line to a C/T ratio of 1 indicates the probable level of no effect. The over-all analytical procedure (enzyme isolation, incubation, and product measurement) has an internal accuracy of about 20% (Figure 3, shaded area) on replicate rats of the same litter sacrificed on the same day and assayed under identical circumstances. A level of 0.9-p.p.m. dietary DDT probably would not exert a detectable inductive effect. Similarly, even a level of 10-p.p.m. dietary DDD is approaching the no-effect dosage.

Duration of Exposure. Apparently, the reaction of the rat liver to dietary DDT is much less marked than that of the DDT by interperitoneal injection. Hart and Fouts (*12*) have found the rat to show enhanced drug metabolism within one day after a single injection of DDT or its analogs. However, about two weeks on a 2.5- or 25-p.p.m. DDT diet is needed for the ratio of treated to control animals to reach a maximum, although the change has been noted at one week. In two studies of this phenomenon, changes were taking place in the control animals also, but there appeared to be a gradual decrease in the ratio of treated to control animals kept on the diet after this first two-week period. The failure of DDT to continue to induce epoxidase activity may be related to the effect of age on the level of epoxidase or to the lack of effectiveness of DDT as an inducer in older animals. Alternatively, induction of DDT metabolism may establish a new status quo that elicits no response from the animal (*12*).

Age at Time of Exposure. In Table VI, rats treated for two-week periods with 25-p.p.m. DDT are compared with litter mates raised on the control diet over a 12-week period. Weanling rats show relatively high activity and strong inducibility but there is a drop in both as the rats reach sexual maturity at eight to 10 weeks. A recoupment in inducibility occurs thereafter, but the activity of control rats continues to rise (Table I) even after inducibility has dropped to lower levels in older mature rats.

Sex and Species. Epoxidase levels in female rats are less than half that of their male litter mates and sometimes are immeasurably low (*29*). In the present experiments, only insignificant differences were found between normal and DDT-treated females. As a check of the possibility that female microsomes contained an endogenous inhibitor, male and female microsomes were incubated together, but the rates of product formation were simply additive (*3*). Other authors have found similar sex differences in drug metabolism (*8*), and Kato, Chiesara, and Vassanelli (*16*) showed the male hormones to be associated with activity and inducibility.

In preliminary experiments on the effect of DDT on Japanese quail, however, the opposite was observed. Female quail could be induced by 100-p.p.m. DDT, but male quail were unaffected or even slightly depressed (Table VII). Variations in inducibility have been noted for various species (*12*) and strains (*4*).

Differences between Normal and Induced Microsomal Epoxidation. Two explanations for the inductive effect of DDT and other compounds have been

Table VI. Relationship between Age at Exposure to DDT and Inducibility of Aldrin Epoxidation

Age, Weeks	Epoxidase Activity per Gram of Liver[a]		Ratio
	Normal	DDT-induced	Induced/Normal
5[b]	2.1	7.6	3.6
6	0.8	6.4	8
8	0.3	2.3	7.7
10	0.9	6.0	6.7
12	0.9	13.4	15
16	0.5	3.2	6.4

[a] Determined as mμmoles dieldrin produced per minute per gram of liver.
[b] Rats sacrificed after only one week on the diet; all others were treated for two weeks at 25-p.p.m. DDT.

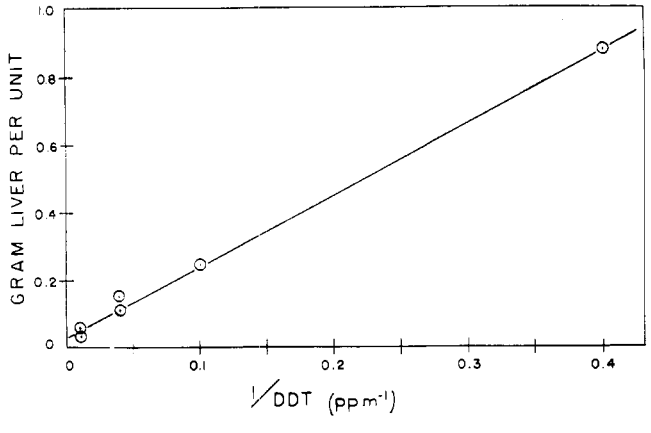

Figure 2. Relationship between reciprocals of epoxidase activity and concentration of dietary DDT

Activity unit = mμmoles of dieldrin per minute; DDT as p.p.m. in diet

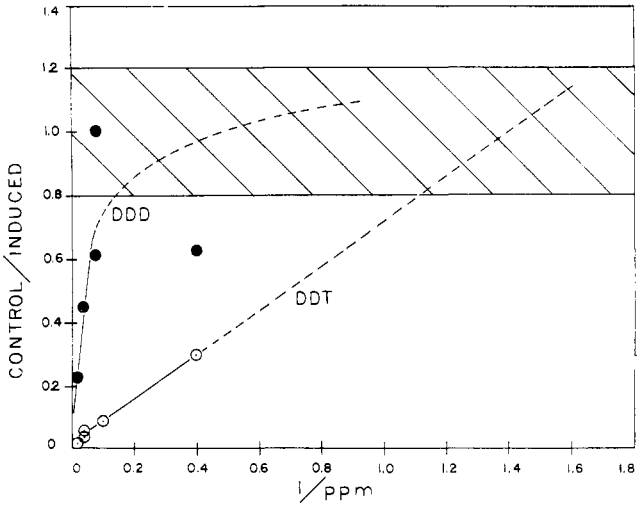

Figure 3. Epoxidase induction by DDT and DDD in the diet of rats

Plotted as ratios of control epoxidase (no insecticide) to induced epoxidase vs. reciprocal of insecticide concentration

Table VII. Effect of Dietary DDT on Epoxidation of Aldrin and Heptachlor by Quail Liver Microsomes

Sex	DDT in Diet, P.P.M.	Specific Activity[a] (μμMoles/Minute/Mg. Protein) Aldrin	Specific Activity[a] (μμMoles/Minute/Mg. Protein) Heptachlor	Liver Activity (MμMoles/Minute/G. Liver) Aldrin	Liver Activity (MμMoles/Minute/G. Liver) Heptachlor
Male	0	108	26	2.1	0.50
Male	100	61	16	1.1	0.28
Female	0	9.4	5.1	0.17	0.08
Female	100	32	12.5	0.75	0.22

[a] Determined in microsomes from five-week old birds that had been on the diet for two weeks.

offered: greater production of the normal enzyme or production of an enzyme with a different (and increased) activity. Rubin, Tephly, and Mannering (*24*) examined the phenobarbitol-induced *N*-dealkylating system in rats. They found no difference in the K_Ms or K_is between treated and control animals but an increased V_{max} of treated rat microsomes, suggesting a greater production of normal enzyme. During the authors' investigations, various inhibitors of epoxidation were tested in both treated and control animals. The results of one such experiment are shown in Table VIII. The treated animals received 25-p.p.m. dietary DDT from the fifth to the seventh week when they were sacrificed with their litter-mate controls. Most striking is the failure of the methylenedioxyphenyl compounds, safrole and sesamex, to inhibit significantly the treated preparation. The control microsomes responded to these two inhibitors in the same manner as did housefly microsomes assayed for inhibition of naphthalene hydroxylase by methylenedioxyphenol compounds (*22*). The degree of response to cyanide and azide was surprising also, since Gillette, Brodie, and La Du (*11*) reported that aminopyrine *N*-dealkylation was not inhibited by NaCN. These phenomena are presently under further investigation.

Another difference between the two preparations is illustrated in Figure 4, where temperature stabilities of control and DDT-treated preparations are shown. Epoxidase activity in microsomes, incubated for 10 minutes at the various temperatures before being added to substrate and cofactors, is expressed as per cent of that obtained after preincubation of 0° C. using both aldrin and heptachlor as substrates. Aldrin epoxidation shows nearly 2.5-fold enhancement on warming the enzyme for 10 minutes at 38° C. prior to adding the enzyme to the reaction mixture but only in the control preparation. Heptachlor epoxidation was enhanced only slightly in the same preparation, and epoxidation of neither substrate was stimulated greatly in the treated preparation. A time study showed that enhancement of aldrin epoxidation reached a maximum after 30 minutes at 38° C. Incubation of the treated enzyme at 50° C. left about 15% of the activity towards either substrate, whereas the control preparation lost almost all activity. In terms of specific activity, the two preparations differ by only a factor of 2 at 38° C. but by a factor of 25 at 50° C. A similar phenomenon was observed in frozen microsomal preparations. After one day at −20° C., the control preparation was more active, whereas the treated preparation had lost activity. After three days, the rate of loss of activity in the two preparations was equal.

Conclusion

Not only does DDT or its analogs effect an increased level of microsomal epoxidation in the rat liver, but the studies of temperature stability and inhibitor sensitivity of DDT-induced and normal epoxidase lead the authors to believe that the former has altered characteristics. These changes could affect sensitive animals in two major ways. First, enhanced epoxidation without a concommitant increase in metabolism of the resultant epoxide should increase toxicity of cyclodiene insecticide to that animal. Male rats are more sensitive to aldrin poisoning than females (*7*), and this may be attributed to the higher level of epoxidase in the male. The generalization might be made that those insecticides and drugs which require oxidative activation for toxicity, such as aldrin, parathion, and Thimet, would show enhanced toxicity to animals exposed and susceptible to inducers. Alternatively, those insecticides which undergo oxidative detoxication, such as carbaryl and lindane, would show decreased toxicity under the same circumstances.

Second, in insect control, the induction of an enzyme insensitive to methylenedioxyphenyl compounds could alter drastically the toxicity of mixtures of insecticides and synergists. In resistant insects, which depend on oxidative metabolism, blockage of oxidation by a synergist such as sesamex leads to enhanced toxicity of an otherwise detoxified compound (*26*). DDT-induced insects, as opposed to DDT-selected insects, would not only be protected more highly by having a greater metabolic capacity, but would not have this mechanism blocked by the synergist. Agosin and coworkers (*2*) have demonstrated that DDT induces NAD-kinase in vivo in nymphs of *Triatoma infestans* and increases other NADP-requiring activities and protein synthesis. Further investigation

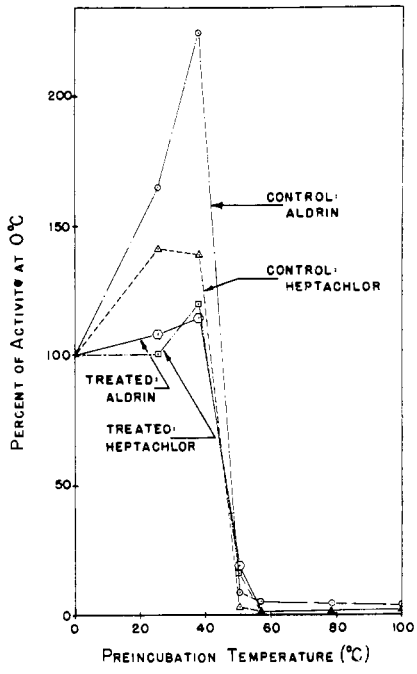

Figure 4. Stability of DDT-induced and control microsomes to preincubation temperatures

Table VIII. Effect of Various Inhibitors on Aldrin Epoxidation in Vitro by Rat Liver Microsomes

Inhibitor	Concn., Molar	Epoxidase Activity[a] Normal Microsomes Units	Normal Microsomes % of control[c]	Induced Microsomes[b] Units	Induced Microsomes[b] % of control
None	...	95	100	570	100
Safrole	5×10^{-4}	19	20	630	112
	5×10^{-5}	22	23	490	86
	5×10^{-6}	38	41	700	124
Sesamex	5×10^{-4}	36	38	590	104
	5×10^{-5}	39	41	450	80
	5×10^{-6}	56	58	460	81
NaCN	10^{-3}	0	0	170	30
	10^{-4}	0.6	0.6	390	69
NaN_3	10^{-3}	7	7	270	48
	10^{-4}	73	78	570	100
$HgCl_2$	10^{-4}	0	0	0	0
	10^{-5}	0	0	(+)	(+)

[a] μμMoles/minute/mg. protein.
[b] Litter mates of normal rats that had been given 25-p.p.m. DDT for two weeks.
[c] Per cent of control (no addition).

should reveal if actual changes in microsomal detoxifying enzymes are brought about in *T. infestans* by DDT, as they are in the rat.

Acknowledgment

We particularly acknowledge the contributions of Kathleen Morack, Robert Schonbrod, Ian J. Tinsley, and G. H. Arscott.

Nomenclature

NADP, NADPH = oxidized and reduced forms of nicotinamide–adenine dinucleotide phosphate

DDT = 1,1,1 - trichloro-2,2 - bis(*p*-chlorophehyl)ethane

DDD = 1,1 - dichloro-2,2 - bis(*p*-chlorophenyl)ethane

DDE = 1,1 - dichloro-2,2 - bis(*p*-chlorophenyl)ethylene

Kelthane = 4,4′ - dichloro - α - (trichloromethyl)benzhydrol

Aldrin = 1,2,3,4,10,10 - hexachloro-1,4,4a,5,8,8a - hexahydro - 1,4 : 5,8 - endo-exo - dimethanonaphthalene

Isodrin = endo-endo isomer of aldrin

Dieldrin = 1,2,3,4,10,10 -hexachloro-6,7 - epoxy - 1,4,4a,5,-6,7,8,8a - octahydro-1,4 : 5,8 - endo - exo - dimethanonaphthalene

Endrin = endo-endo isomer of dieldrin

Heptachlor = 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a - tetrahydro-4,7-methanoindene

Heptachlor epoxide = 1,4,5,6,7,8,8-heptachloro-2,3 - epoxy - 2,3,3a,4,-7,7a - hexahydro - 4,7-methanoindene

G-6-P = Glucose 6-phosphate

G-6-P DH = Glucose 6-phosphate dehydrogenase

Literature Cited

(1) Agosin, M., Michaeli, D., Miskus, R., Hoskins, W. M., *J. Econ. Entomol.* **54,** 340 (1961).
(2) Agosin, M., Scaramelli, N., Dinamarca, M. L., Aravena, L., *Comp. Biochem. Physiol.* **8,** 311 (1963).
(3) Chan, T. M., Gillett, J. W., Terriere L. C., *Ibid.*, to be published.
(4) Cram, R. L., Juchau, M. R., Fouts, J. R., *Proc. Soc. Exptl. Biol. Med.* **118,** 872 (1965).
(5) Farris, E. J., in "The Rat in Laboratory Investigation," E. J. Farris and J. Q. Griffiths, Jr., Eds., 2nd ed., p. 3, Lippincott, Philadelphia, 1949.
(6) Fincham, J. R. S., *J. Gen. Microbiol.* **11,** 236 (1954).
(7) Gaines, T. B., *Toxicol. Appl. Pharmacol.* **2,** 88 (1940).
(8) Gelboin, H. V., Blackburn, N. R., *Cancer Res.* **24,** 355 (1964).
(9) Gerboth, G., Schwabe, U., *Arch. Exptl. Pathol. Pharmakol.* **246,** 469 (1964).
(10) Ghazal, A., Koransky, W., Portig, J., Vohland, H. W., Klempau, I., *Ibid.*, **249,** 1 (1964).
(11) Gillette, J. R., Brodie, B. B., La Du, B. N., *J. Pharmacol. Exptl. Therap.* **119,** 532 (1957).
(12) Hart, L. G., Fouts, J. R., *Arch. Exptl. Pathol. Pharmakol.* **249,** 486 (1965).
(13) Hart, L. G., Fouts, J. R., *Biochem. Pharmacol.* **14,** 263 (1965).
(14) Hart, L. G., Fouts, J. R., *Proc. Soc. Exptl. Biol. Med.* **114,** 388 (1963).
(15) Hart, L. G., Schultice, R. W., Fouts, J. R., *Toxicol. Appl. Pharmacol.* **5,** 371 (1963).
(16) Kato, R., Chiesara, E., Vassanelli, P., *Japan. J. Pharmacol.* **12,** 26 (1962).
(17) Klein, A. K., Laug, E. P., Datta, P. R., Watts, J. V., Chen, J. T., *J. Assoc. Offic. Agr. Chemists* **47,** 1129 (1964).
(18) Lineweaver, H., Burk, D., *J. Am. Chem. Soc.* **56,** 658 (1934).
(19) Morello, A., *Can. J. Biochem.* **43,** 1289 (1965).
(20) Nakatsugawa, T., Ishida, M., Dahm, P. A., *Biochem. Pharmacol.* **14,** 1853 (1965).
(21) Ortega, P., *Federation Proc.* **21,** 306 (1962).
(22) Philleo, W. W., Schonbrod, R. D., Terriere, L. C., J. AGR. FOOD CHEM. **13,** 113 (1965).
(23) Rachapaetayakom, P., Brown, W. G., Arscott, G. H., *Feedstuffs* **36,** 22 (1964).
(24) Rubin, A., Tephly, T. R., Mannering, G. J., *Biochem. Pharmacol.* **13,** 1007 (1964).
(25) Schonbrod, R. D., Gillett, J. W., Terriere, L. C., *Bull. Entomol. Soc. Am.* **11,** 157 (1965).
(26) Sun, Y. P., Johnson, E. R., J. AGR. FOOD CHEM. **8,** 261 (1960).
(27) Tinsley, I. J., *Nature* **202,** 1113 (1964).
(28) Wong, D. T., Master's thesis, Oregon State University, 1964.
(29) Wong, D. T., Terriere, L. C., *Biochem. Pharmacol.* **14,** 375 (1965).

Received for review April 26, 1966. Accepted July 18, 1966. Symposium on Pesticide Interaction Phenomena, Division of Agricultural & Food Chemistry, Winter Meeting, ACS Phoenix, Ariz., January 1966. Technical Paper Number 2129, Oregon Agricultural Experiment Station. Work supported by Grant ES-00040-01 from the United States Public Health Service.

INSECTICIDE INTERACTIONS

Insecticide Interactions Affecting Residue Storage in Animal Tissues

J. C. STREET, R. W. CHADWICK, M. WANG, and R. L. PHILLIPS

Animal Science Department, Utah State University, Logan, Utah

SURVEYS during the past few years have provided mounting evidence that food and other sectors of the environment have been contaminated with traces of several organochlorine insecticides. Correspondingly, tissues of humans residing in many parts of the world also were found to contain mixtures of such insecticides in trace amounts.

Some years ago entomologists considered the probability of notable interaction (synergism or antagonism) occurring with combinations of organochlorine insecticides. Certain combinations had greater toxicity to insects than the more toxic component alone (*6*, *13*). Whether these events should really have been described as "synergism," however, is somewhat debatable as it is not clear whether the compounds interacted directly in some biochemical system. Interaction between organochlorine and organophosphate insecticides has been reported to occur in rodents. For example, Ball found the toxicity of parathion to the rat was reduced when it was administered after either aldrin, chlordan, or lindane treatment (*1*).

The authors recently became intrigued with the possibility that the metabolic fate of one organochlorine compound in an animal conceivably might be altered by simultaneous exposure to other such insecticides. A new principle was established when it was demonstrated that DDT and dieldrin interact markedly in the rat (*9*, *10*). The major response was a reduction of dieldrin storage in the fatty tissues when DDT was administered simultaneously.

This paper presents further observations of the effect of DDT on dieldrin