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Mechanisms of Pesticide Degradation

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MECHANISMS OF PESTICIDE DEGRADATION

by

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is preparing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

This report represents a research effort to enhance our knowledge of the mechanisms by which pesticides are degraded in living organisms. The results of several studies dealing with biochemical defense mechanisms and transformation of pesticides are addressed in this report.

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Acting Director,
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PREFACE

As a group of chemicals pesticides certainly represent one of the most toxic materials that are intentionally broadcast in the environment by man. Many of these chemicals are biologically active, persistent enough, and accumulate in biological systems; and therefore must be regarded as environmental contaminants. Yet, these chemicals are often very useful in maintaining quality of agricultural products, our environment, and protecting man from diseases and famines. Assessment of risk/benefit ratio for a pesticide or any compounds for that matter, is not easy, however. Ideally such judgements must be based upon sound scientific facts and economic or aesthetic values that are produced by the chemical. And yet, few environmental events are definitive, and clear cut. As a result, we are often forced to come to a decision before all facts become available. If the past decade can be viewed as a typical period, environmental issues of pesticidal toxicology are crisis filled. The cases such as DDT, dieldrin, 2,4,5-T (TCDD) attest to this view. Urgent issues may suddenly crop up and disturb daily routines or basic work schedules. My personal feeling is that the U.S. scientific community has really reacted well, and time and time again have proven that many able scientists are willing to spend many hours to face the issue.

In this report we have covered three important issues: they are questions on a) toxaphene, now number 1 chlorinated insecticide, b) chlordimeform, a controversial, behavior modifying pesticide, and c) anaerobic degradation systems of various pesticides, a new area of research design to probe into unanswered degradative force in animals. All these topics have been chosen as a result of deliverate considerations on acute needs for toxicological information. Toxaphene has been on the RPAR (rebuttable presumption against registration) list, and yet at the time this study was initiated nobody even knew what the molecular structure of the toxic ingredient of toxaphene was. Chlordimeform has been suspended for use by the request of the manufacturer because of its health implications. It is now reinstalled with stringent precautionary measures. Our own viewpoint is that scientific facts are the best basis for any regulatory decisions. On the other hand, since the resources and time are limited all scientists make very conscious efforts to concentrate on matters of utmost significance. We are confident that the data generated by this project are of good use to that end and that our collaboration between university communities and the government agencies such as EPA will continue in the future.

ABSTRACT

This research project was initiated with the overall objective of determining (1) the chemical structures of toxic components of toxaphene, (2) to study anaerobic metabolism to degrade toxaphene and other pesticides, and (3) to understand toxic action mechanism of chlordimeform.

As a result of intensive efforts the molecular structures of three of the most toxic principles of toxaphene were identified. Together these components comprise at least 70% of toxaphene's toxicity toward mice. This is the first time that the structure of toxic components of toxaphene became apparent despite the widespread use (over 1 billion pounds, which is comparable to DDT) of toxaphene in the last 3 decades. Toxaphene on the other hand degrades relatively faster than other chlorinated pesticides such as DDT and dieldrin. The reason for it is that toxaphene is susceptible to reductive degradative forces.

Chlordimeform was found to affect amine regulatory mechanisms in animals. Such actions explain some of the subtle effects of this pesticide on animals. Inasmuch as that biogenic amines are known to play many important biological roles such as controlling emotion, behavior and circulatory functions of the body.

This report was submitted in fulfillment of Research grant, R801060 from the U.S. Environmental Protection Agency. This report covers the period of May 1, 1975 through April 30, 1978, the date that the project was completed.

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CHAPTER I

CHEMICAL STUDIES ON TOXAPHENE

ABSTRACT

Toxaphene is the most widely used chlorinated insecticide in the U.S.A. today with an annual production of about 50 million pounds, and a total usage of one billion pounds in the last 25 years. Because of its extreme complexity, it would be both difficult and impractical to thoroughly chemically characterize all the components of toxaphene, thus it is very important to limit study only to the major toxic components. At the time this project was initiated nobody had knowledge on molecular structure of toxaphene. Our isolation and identification efforts led us to elucidation of three major toxic components of toxaphene. Together they constitute at least 1/2 to 2/3 of the toxicity of toxaphene to mice.

INTRODUCTION

Toxaphene is a widely used insecticide. Two-thirds of its production is used for cotton insect control while other uses include vegetables, small grains, soybeans, and control of external insects on livestock. It has also been employed extensively in fish eradication programs. Its annual production is about 50 million pounds (1971 estimate, Environmental Protection Agency, 1972) with a total usage of one billion pounds in the past 25 years. Despite this wide usage, little has been known about the chemistry, toxicity, metabolism, or environmental fate of its components.

Only recently a major effort toward answering these questions has been made by Casida *et al.* (1974), who were successful in isolating and identifying a toxic component of toxaphene, 2,5-*endo*,6-*exo*-8,9,10-heptachlorobornane. According to them, at least 175 polychlorinated 10-carbon compounds were recognized by their methods (Holmstead *et al.*, 1974). The components were described as polychlorobornanes, polychlorbornenes, and polychlorotricyclenes with 6 to 10 chlorine atoms per component. Also isolated was a $C_{10}H_{10}Cl_8$ component which was more toxic to mice and houseflies than the above component; however, no structure was proposed for the latter component.

Isolation and identification of all the components of toxaphene would be a monumental task. Therefore, at this stage of our understanding of toxaphene, it was necessary to limit study to the chemical elucidation of major toxic components. Also from an environmental standpoint, it is important to assess the hazards in terms of its toxicity to nontarget organisms, particularly aquatic species. This is of obvious importance since results from several studies on the toxicity of toxaphene to nontarget organisms (Pimentel, 1971) indicate toxaphene to be quite toxic to fish, many aquatic invertebrates, and most insects.

CONCLUSIONS

Toxaphene is a very unusual chlorinated insecticide in that its residues are not generally found in the human tissues, foods and drinking water despite its extensive use which is comparable to DDT. The reason for this lack of residue data can be multifold: e.g. (a) the difficulty in establishing analytical procedures because of its chemical complexity, (b) the lack of knowledge as to which component to study, and (c) general lability of the major components via metabolic and photochemical processes etc. To answer the question whether toxaphene is an environmentally damaging chemical or not, one must first clearly identify and establish its most toxic fraction or fractions so that their environmental fates and effects can be closely studied. Also the basic requirement for any toxicological study is the knowledge on the structure of the toxicant involved. The data shown in this work provide the basic means to that end.

At least now we know the chemical structures and some properties of toxic components of toxaphene. With this knowledge scientists can start searching their residues in the environment because they will know what to look for. Also metabolic studies which were impossible before can be initiated to increase our knowledge on toxicological behavior of toxaphene.

RECOMMENDATIONS

1. With the knowledge on these toxic components of toxaphene, residue monitoring programs specifically aimed at finding them should be initiated.

2. Metabolic studies particularly aimed at finding possible toxic metabolic products of toxaphene should be encouraged in animals and in the environment.

3. Determine parameters for environmental toxicological information such as bioaccumulation, half-life in soil, evaporation, ecotoxicity etc. of these toxic components of toxaphene.

MATERIALS AND METHODS

Components and fractions of toxaphene have been separated by a combination of methods including column chromatography, thin-layer chromatography, reverse phase thin-layer chromatography, and preparative gas chromatography.

For analysis of purified components, mass spectra were taken on a Finnigan 1015 quadropole mass spectrometer. Infrared (IR) spectra were obtained on a Beckman IR33 spectrophotometer. Fourier Transform NMR spectra were obtained on a 90 MHz Bruker FTNMR spectrometer.

A number of test organisms were used for bioassay of toxaphene fraction toxicity. They were: 1) mosquito larvae, *Aedes aegypti*; 2) freshwater blue-green algae, *Anacystis nidulans* (TX20); 3) brine shrimp, *Artemia salina*; and 4) fathead minnow, *Pimephales promelas* Bioassay procedures involved LC_{50} determinations for brine shrimp and mosquito larvae (Nelson, 1974), while toxicity to algae was measured as a decreased k value, a growth rate constant (Batterton *et al.*, 1971). Chandurkar *et al.*, (1978) provided toxicity testing on minnows.

RESULTS & DISCUSSION

Identification of toxic components

Fractions separated by the above methods show varying toxicities to four aquatic organisms, a blue-green alga, brine shrimp, fathead minnow and mosquito larvae. Employing a combination of preparative TLC and GC methods, a toxic fraction of toxaphene 1.87, 1.75 and 1.35 times more toxic than toxaphene to mosquito larvae, brine shrimp, and algae, respectively, has been isolated.

The toxic A fraction, though it behaves as a single component in various chromatographic systems, was found to consist of two components on the basis of nuclear magnetic resonance (NMR) spectroscopy. They were further characterized by infrared and mass spectrometry as octachlorobornanes. We have also noticed that there is a persistent contaminant in the preparations of toxic fraction A. In view of the implication of recent reports (Turner *et al.*, 1971) we have examined this contaminant (hereinafter referred to as toxicant Ac), and now report the identification of such a component of toxaphene.

The toxicity of this toxicant Ac was determined using fish, fathead minnow and mosquito larvae as test organisms. The data are presented in Table 1. The comparison of LC_{50} values of this toxicant Ac with that of standard toxaphene shows that it is approximately four times more toxic to fish than standard toxaphene, and that it is just as toxic to mosquito larvae as standard toxaphene.

The toxicity data against mosquito larvae show that toxicant Ac is not as toxic as toxic fraction A[12], indicating that the former cannot account for the toxicity of the latter. On the other hand, the toxicity of toxicant Ac to fish raises the possibility that there are components in toxaphene with unfavorable selective toxicity to nontarget organisms as compared to insects.

TABLE 1

Toxicities of Toxaphene and Toxicant Ac to Fathead Minnow and Mosquito Larvae

Compound(s)	Fathead Minnow (48-hr LC_{50}, ppb)	Mosquito Larvae (24-hr LC_{50}, ppm)
Toxaphene	77.55	0.3755
Toxicant Ac	18.77	0.3788

Our finding here gives strong support to the idea that toxic fraction A (= toxicant A) is the most toxic component of toxaphene. In view of the uncertainties about the real toxicities of the other two major components, toxicant B and toxicant C, the identification of the structures and the toxicities of toxicant Ac and A at this stage certainly helps clarify the question as to which component of toxaphene to study for its environmental behavior in order to assess the potential ecotoxicity of toxaphene.

Degradation of toxic components in lake sediments by microorganisms

Degradation of toxicant A (= toxic fraction A) and toxicant B was found to proceed at a faster rate than that of crude toxaphene in lake sediments. Under the experimental condition the half-life of A and B was about 30 days. In all tests, degradation of toxicant C, on the other hand, was found to be even faster than A or B. The rate of degradation did not vary appreciably when the incubation condition was changed from anaerobic to aerobic. This is contrary to the generally held belief that toxaphene degrades faster under anaerobic conditions.

To study microbial degradation *Pseudomonas putida* was selected because of its well known ability to degrade camphor-type molecules. Degradation of toxaphene by the microorganism proceeds under aerobic conditions. Again the degradability of toxicant C was much higher than A or B by microorganisms. It was not possible to isolate and determine any of the metabolic products.

However, because of the stimulatory action of oxidative cofactors (e.g. NADPH) it was concluded that the metabolism is mainly carried out in an oxidative enzyme system. The rates of degradation of A and B were slightly faster than that of the total crude toxaphene (faster by 10 to 30%).

Degradation in animals (rats)

Toxaphene was found to be metabolized in the rat via dechlorination and oxidation reactions. The enzyme systems responsible for such degradation were studied in detail. In short the animal systems degrade toxaphene in two step reactions. First chlorine molecules are stripped off from toxaphene via dechlorination system which is stimulated by a cofactor, NADPH. Oxidative attacks occur either directly on the less chlorinated members of the original toxaphene complex, or on the dechlorination reaction products. One dechlorination product of toxicant C was isolated and identified. Also several hydroxylation (= oxidation) products of toxicant C exist as judged by the result of a specific derivatization reaction. Apparently several nonchlorinated sites on the bornane ring are susceptible for oxidative attacks by the animal enzymes.

CHAPTER II

REDUCTIVE DEGRADATION OF PESTICIDAL CHEMICALS

ABSTRACT

A low molecular weight flavoprotein from rat gut walls is active in reductive reactions, namely, dechlorination and N-demethylation. Reductive dechlorination is stimulated by FAD addition. It provides a logical explanation as to how mammalian systems could initiate DDT degradation process which involves the initial reductive dechlorination step as a rate limiting reaction.

INTRODUCTION

Biotransformation of foreign compounds (Xenobiotics) is of utmost importance to the survival of living organisms against environmental pollutants. The three major primary enzymatic processes involved are oxidative, reductive and hydrolytic systems. The mechanisms of oxidation and hydrolysis are reasonably well understood. On the other hand, the reductive reactions are not fully established, and even among the known reactions their mechanisms are not fully understood (Gillette, 1971). Exception to this is the reduction of nitro and azo compounds where microsomes are capable of forming corresponding anilines under anaerobic conditions (Rose and Young, 1973; Symms and Jachau, 1974). Recently several papers have appeared describing reductive reactions on pesticidal chemicals, indicating the trend for a renewed interest to the role of reductive reactions in respect to detoxication of foreign compounds. Under anaerobic conditions, DDT is converted to TDE by avian and mammalian livers, microsomes, intestinal microflora, and several bacterial and soil microorganisms.

Our interest in this subject stems out of the observation of reductive metabolism of DDT to TDE, the first indispensable step for DDT degradation. It has been debated for some time whether the reaction is due to the microbial action in the alimentary system. To be sure there have been some evidence that avian and mammalian livers are capable of metabolizing DDT to TDE under anaerobic conditions *in vitro*. However, the functional meaning of such systems *in vivo* as well as the enzymatic basis of such activities have not been fully clarified.

CONCLUSIONS

The discovery that flavoproteins-flavin cofactor combinations, whether they are microbially derived or mammalian in origin, are capable of degrading various xenobiotic substrates in the presence of FAD or other flavin cofactors under anaerobic conditions helps clarify many phenomena hitherto considered and unexplainable. For instance, it has been shown by French and Hoopingarner (1971) that cell membrane fractions from *Escherichia coli* actively convert DDT to TDE, and that the reaction is strongly stimulated by FAD. Similarly Wedemeyer (1966) observed earlier that the reduction activity on DDT in the cell-free extract from *Aerobacter aerogenes* was stimulated by the addition of flavine mononucleotide (FMN) with light illumination. Such reductive reaction was strongest under an anaerobic condition at an acidic pH. As for mammalian systems, it is interesting to note that basically similar biochemical characteristics are found in the DDT reducing enzyme in the liver. According to Hassall's (1971) description, the system is heat-stable, is optimal at an acidic pH, and is stimulated by exogenously added riboflavin. Thus, it is likely that the underlying basic mechanism common throughout these phemonena is anaerobic reduction by involving flavoprotein-flavin cofactor systems.

RECOMMENDATIONS

The meaning and significance of such a flavoprotein involved system *in vivo* in elimination of toxic foreign compounds must be further examined, particularly in relation to other documented reductive systems such as porphyrin -Fe^{++} involved systems (Castro, 1964; Khalifa *et al.*, 1976; Miskus *et al.*, 1965) and specific NADPH requiring nitro reductase systems (Gillette, 1971; Hitchcock and Murphy, 1967; Rose and Young, 1973; Symms and Jachau, 1974). Nevertheless, this system can be easily distinguished from others by the stimulated effect of FAD (and other flavins), characteristic heat stability, and acidic pH requirements, and therefore, we feel certain that its contribution will be properly assessed in the near future.

MATERIALS AND METHODS

To study the reductive system in mammals, we have chosen the 20,000 g supernatant fraction from the homogenate of the intestinal wall (small intestine) of rats as the source. Anaerobic degradation activities on DDT and mexacarbate were investigated. At the end of the reaction, the products were extracted with diethyl ether, and the degradation activity was mainly monitored by assaying the radioactivity in the aqueous phase.

Anaerobic degradation *in vitro* of ^{14}C-mexacarbate by the 20,000 g supernatant

The incubation mixture consisted of 1 ml of the 20,000 g supernatant containing 36 mg fresh weight tissues equivalent in phosphate buffer and ^{14}C-mexacarbate in 10 µl ethanol. The cofactors FAD or NADPH were added by using 0.2 ml buffer as the vehicle. The volume of incubation mixture in each tube was adjusted to 1.2 ml by using phosphate buffer. Incubation was carried out in Thunberg tubes. After addition of all constituents, the tube was evacuated and nitrogen was flushed; this process was repeated two additional times and finally the nitrogen was evacuated and the incubation carried out under reduced pressure. The system was maintained in a metabolic shaker at 37°C for two hours. At the end of incubation, the products were extracted with diethyl ether, and analysis was carried out by using thin-layer chromatography (TLC), along with autoradiography, using benzene-methanol (95:5 v/v) as the mobile phase.

Sephadex gel-filtration

In an attempt to partially purify the protein responsible for such degradation activities the 20,000 g supernatant was first prepared by homogenizing the rat intestinal wall in two parts of buffer, and centrifuging it successively at 8,000 g for 10 minutes and 20,000 g for one hour. Hydrolysis was carried out by incubation of the 20,000 g supernatant with protease at the ratio of 2 mg per ml supernatant. The resulting hydrolysate was subjected to a gel-filtration column using sephadex G-75. Absorption at 260 or 280 nm was used to monitor the column elution pattern, and the degradation activity was measured individually for each fraction, using water-soluble ^{14}C-metabolites of mexacarbate as the parameter. Two major peaks (I and II) were observed as shown in Fig. 1. Hereafter, peak II will be referred to as flavoprotein preparation.

Anaerobic degradation *in vitro* of DDT and ^{14}C-mexacarbate by the flavoprotein preparation

Incubation mixtures consisted of 5 ml of the flavoprotein preparation in case of DDT and 2.5 ml in case of ^{14}C-mexacarbate; FAD or NADPH; and DDT or ^{14}C-mexacarbate in 10 µl ethanol. Incubation conditions, extraction, and expression of data are as mentioned above. With respect to DDT, analysis was carried out by gas chromatography using an electron capture detector and two columns, QF-1 and OV-101 at 185°C. The first column was employed for qualitative identification of the metabolites, while the latter was used for both qualitative and quantitative estimations. As for mexacarbate degradation, ether extracts were analyzed by TLC (mobile phases were: chloroform-methanol 99:1, benzene-

methanol 95:5, ether-hexane 4:1, chloroform-acetonitrile 4:1) along with autoradiography on X-ray films against authentic reference compounds. The spot corresponding to desmethyl mexacarbate was isolated and characterized by proton magnetic resonance spectroscopy.

RESULTS AND DISCUSSION

By using the 20,000 g supernatant from rat intestine, we could first establish that there was indeed a mexacarbate degradation system which was strongly stimulated by FAD under an anaerobic incubation condition (Table 2). In this system mexacarbate was found to be degraded to relatively polar compounds as judged by the increase in radioactivities in the aqueous phase. In addition, the major ether-soluble product, N-desmethyl mexacarbate as judged by TLC analyses and autoradiography also increased in the presence of FAD.

To study the characteristics of this reductive system, several tests were conducted using the 20,000 g supernatant. The continuous treatment of the system with 1 ppm of streptomycin from the time of dissection to the final incubation (added in the buffer) had no effect on the activity. Neither did the addition of cations Fe^{++}, Co^{++}, Mg^{++} cause the change in the degradation activity. The treatment which caused the activity changes were 10^{-3}M $HgCl_2$ (80% inhibition), CO bubbling (28% inhibition), 10^{-4}M DFP (50% inhibition), 10^{-3}M N-ethyl maliemide (60% stimulation), and mersalyl acid (130% stimulation). The pH optimum was found to be 6.

Degradation activity on mexacarbate was associated with the first 3-5 fractions in each peak. However, further examination of peak I by rechromatography on sephadex G-25 revealed three peaks, all with very low specific degradation activity on mexacarbate. Hence, it was decided that peak II is a better source for the degradation systems on mexacarbates.

Upon examination of these two peaks, it was found that fluorescence spectroscopic characteristics (i.e., fluorescence peak at 520 nm and excitation peak at 450 nm) of peak II and FAD were practically identical. Moreover, both FAD and peak II showed an identical UV-absorption peak at 260 to 270 nm (Fig. 2). Other characteristics such as the resistance to protease, heat (up to 90°C for 20 min), etc., agree well with our diagnosis that the system isolated here is a flavoprotein. The molecular weight of the flavoprotein is likely to be in the order of 6,000 to 10,000 as judged by its relative elution position against standard components (i.e., cytochrome c:13,000) on the same sephadex column.

DDT and mexacarbate were incubated with this flavoprotein preparation (peak II) in the presence and absence of either NADPH or FAD (Table 3). It was found that the addition of FAD, but not NADPH, greatly stimulated the metabolism of both substrates. The most conspicuous increase was observed in the production of TDE in the case of DDT, and desmethylmexacarbate and water soluble metabolites in the case of mexacarbate. The reaction of TDE formation is reductive dechlorination. Moreover, the formation of desmethylmexacarbate must involve reductive desmethylation which has never been reported in any biological systems to our knowledge.

To study the effects of light and oxygen the tubes were incubated in the dark under the same conditions as described before. Two flavin cofactors were used, namely, FAD and riboflavin. The overall degradation activities on mexacarbate, in the presence of FAD, amounted to 36% as of initial substrate added (Table 3). When the reaction was carried out in the dark, the overall degradation activities were decreased to 1/3 and was further decreased in the presence of oxygen to 1/10. When riboflavin was used as the cofactor, degradation activities amounted to 80% of mexacarbate degraded in the presence of light. When the reaction was carried out in dark, the same degradation activity on mexacarbate was experienced. Only in the presence of oxygen in dark did the degradation activities decrease to 1/10 of the standard value.

The first question one must ask is whether the flavoprotein system discovered here represents a genuine enzymatic system or not. Gillette (1971) has previously noted that azo and nitro compounds may be nonenzymatically reduced to amines by reduced cofactors such as NADPH, NADH and reduced flavins such as FADH. Kamm and Gillette (1963) also showed that FAD can be reduced by a purified cytochrome c - NADPH system anaerobically, and that this reduced FAD was capable of nonenzymatically converting p-nitro-benzoate to p-aminobenzoate.

The system described herein is not stimulated by NADPH or NADH, and therefore, is different from the above cases. The degradation activity of mexacarbate was, on the other hand, reduced in the absence of light indicating that at least some part of FAD reduction in the standard reaction scheme is carried out nonenzymatically by some electron donors. The rest of the FAD reducing reaction which takes place in the dark could be carried out by enzymatic systems or by flavoprotein itself. It has been known that flavoproteins are capable of reducing flavin cofactors. Thus, in view of the essential role of flavoproteins, it is most logical to assume that the sole function of flavoprotein here is to convert FAD to FADH which actually reacts with DDT or mexacarbate to produce respective degradation products. It must be stressed here that FAD, as low as 10 µg per reaction tube, resulted in maximum stimulation of reductive

activities, and furthermore, this FAD concentration is roughly in the same magnitude of its level in various animal tissues. Thus, even though the actual reductive reaction itself could be carried out nonenzymatic, the phenomenon itself should not be regarded as irrelevant phenomenon *in vivo*. Also, flavoproteins are known to be omnipresent in various biological systems. In animals, they are present in the liver as well as in the alimentary canal. Thus, along with the evidence that favorable anaerobic conditions exist in the alimentary canal *in vivo*, the chance is that such a flavoprotein-flavin cofactor catalyzed reaction do play significant roles for degradation of certain xenobiotics.

TABLE 2

Degradation of ^{14}C-mexacarbate by the 20,000 g supernatant from rat intestines*

Incubation conditions	Mexacarbate Remaining (%)	Degradation Products (%)		
		Desmethyl Mexacarbate	Other Ether Soluble	Water Soluble
20,000 g Supernatant	88	1	1	0.5
20,000 g Supernatant + FAD	74	7.5	4	5
20,000 g Supernatant + NADPH	85	2	3	0.5

* Averages of 2-3 independent experiments, each experiment consisting of duplicate sets.

TABLE 3

Anaerobic degradation of DDT and ^{14}C-mexacarbate by the flavoprotein preparation from rat intestine.[1]

Substrate and Metabolites	Flavo-protein	Flavoprotein + FAD	Flavoprotein + NADPH	Control[2]
		DDT as Substrate		
DDT	88	71	88	96
TDE	6	21	7	2
DDE	6	8	5	2
		^{14}C-Mexacarbate as Substrate		
Mexacarbate	87	54	90	91
Desmethyl mexacarbate	9	22	6	7
Other ether solubles	2	10	2	1
Water-solubles	2	14	2	1

1) Figures in this Table are percent of initial substrate added, average of 2 to 3 separate experiments; each carried out in duplicates.

2) Control samples refer to flavoprotein incubated without substrate; then after incubation substrate was added and extracted immediately with ether.

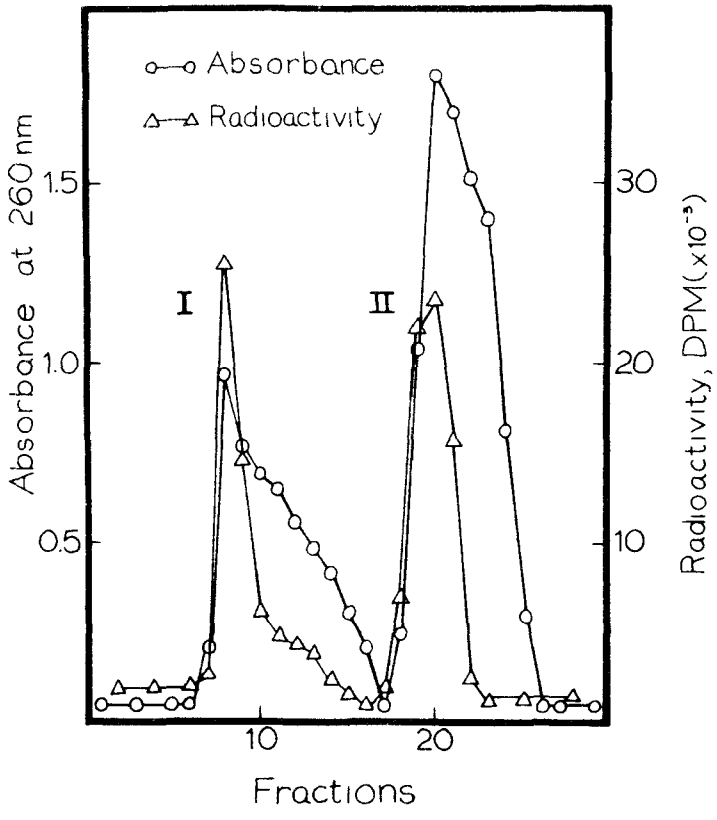


Figure 1. UV absorption and degradation activity (DPM) for the column fractions.

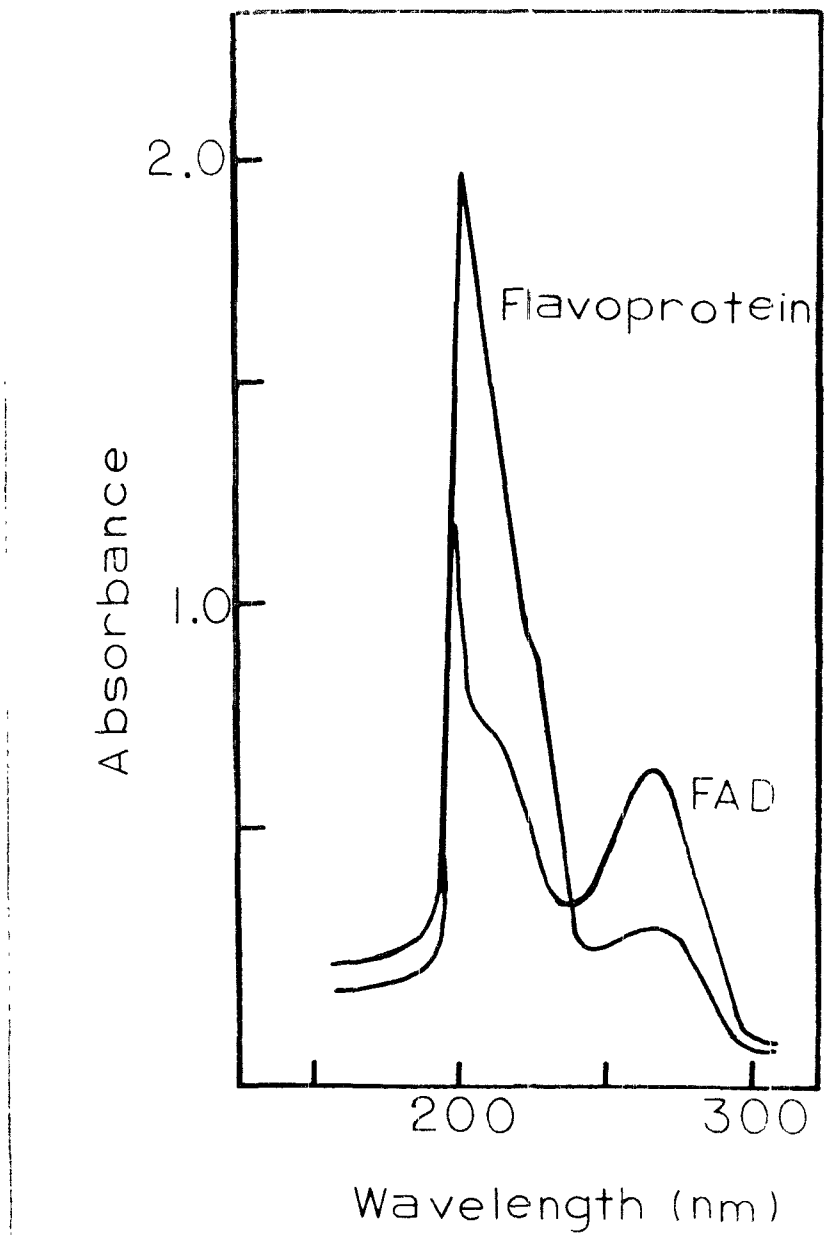
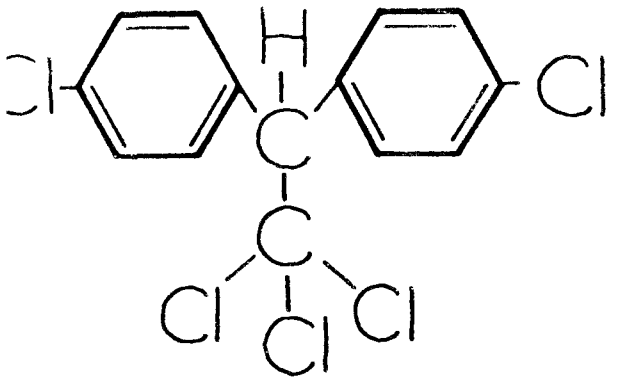


Figure 2. UV spectra of flavoprotein preparation and FAD in phosphate buffer.



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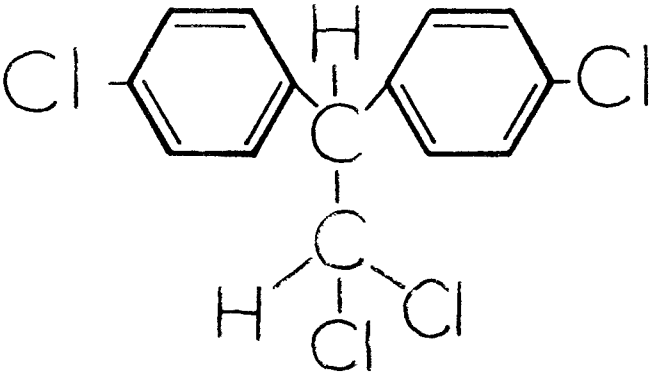

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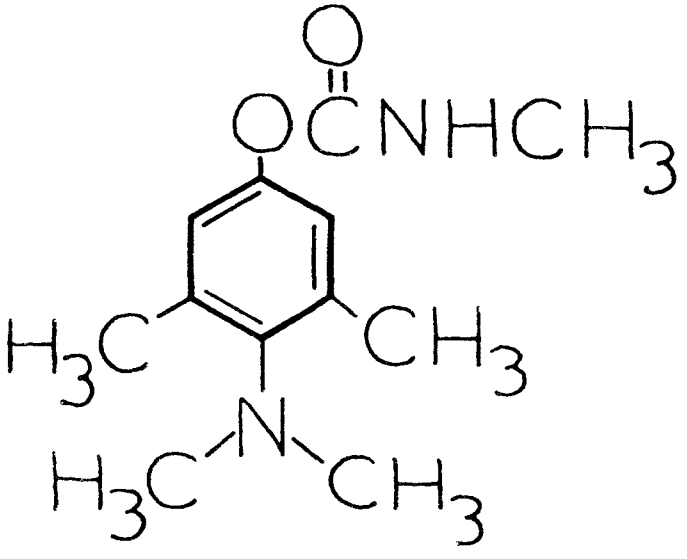
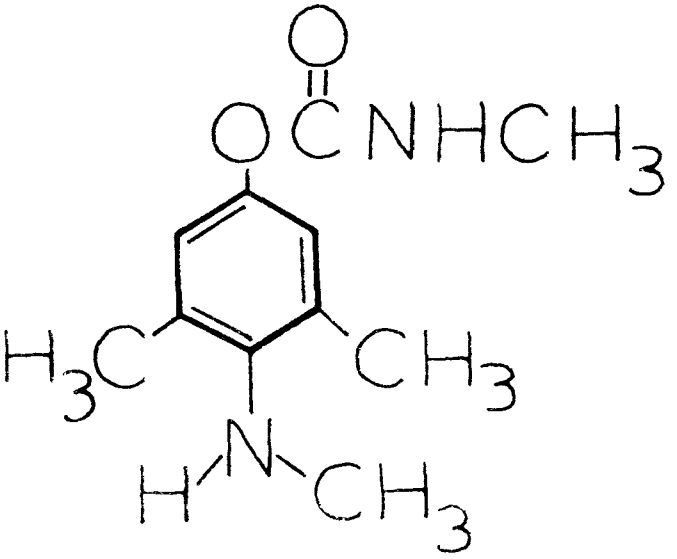


Mexacarbate



Desmethyl
Mexacarbate

CHAPTER III

HEALTH EFFECTS OF CHLORDIMEFORM

ABSTRACT

Chlordimeform, a novel type of insecticide-acaricide was found to cause changes in the level of biogenic amines in rats and cockroaches. It also causes a marked decrease in blood pressure in the carotid arteries of rabbits: an observation consistent with the changes in amine levels.

INTRODUCTION

Chlordimeform, also known as Galecon© or Fundal®, is an important insecticide and miticide. Despite its importance and usefulness, exactly how it poisons has been a mystery until quite recently. Abo-Khatwa and Hollingworth (1972) reported that chlordimeform was a respiratory poison in cockroaches. Although such a property of chlordimeform might explain its effects upon respiratory function (oxygen utilization) in insects, it does not explain the effects on the nervous system. Beeman and Matsumura (1973) found that chlordimeform is a potent inhibitor of monoamine oxidase (MAO) in the rat. Furthermore, they could demonstrate the actual accumulation of amines in the rat brain, the expected result of MAO inhibition. The importance of these findings in the mechanism of toxic action of chlordimeform is the subject of this research.

CONCLUSIONS

In conclusion, we have shown that chlordimeform interferes with amine function in the nervous system in a variety of ways. Specifically, chlordimeform causes a build-up of the amines 5-hydroxytryptamine and to a lesser extent norepinephine in the rat brain, prevents the behavioral effects of reserpine in the rat (reserpine depletes amine stores in the brain), inhibits monoamine oxidase from rat liver, and causes low blood pressure in rabbits.

In the American cockroach it directly stimulates the heart, enhances the toxicity of the amine, tryptamine, inhibits amine N-acetyltransferase from cockroach head, causes accumulation of indoleamines in living cockroaches, and blocks the action of octopamine in the cockroach nervous system. It also inhibits tryptamine metabolism in mites.

RECOMMENDATIONS

On the basis of the findings summarized here, we propose that a major biological effect of chlordimeform is its action on amine-related systems. In the future, chlordimeform and its metabolites should be tested in more detail for their effects on amine function, specifically re-uptake, leak-out from presynaptic storage, metabolism (not just MAO but many other enzyme systems) and the range of its action on amine receptors either as agonist or antagonist.

MATERIALS AND METHODS

General Procedures

The effects of chlordimeform on a variety of amine functions in insects, mites and mammals were assessed using established procedures.

Brain Amine Levels and Amine Oxidase (MAO)

To study the change in amine levels male rats were first treated with 200 mg/kg of chlordimeform, were killed after 1 hr, and their brains quickly removed. The serotonin and norepinephrine levels in the whole brain were then measured by fluorometer (Maickel *et al.*, 1968). To study the relationship between this increase in amine levels, and a decrease in amine breakdown by MAO, we have examined the effect of chlordimeform on the MAO of the rat liver.

Fluorescence Histochemistry

Histochemical investigation in the roach brain: To ascertain that the basic amine regulatory mechanisms in the central nervous system of the American cockroach are similar to those found in mammalian brain, the histochemical experiment of Frontali (1968) was repeated. In this experiment biogenic amines were made visible by treating the freeze-dried roach brain with formaldehyde vapor. The brains were then embedded and sectioned, and the sections viewed through a fluorescence microscope.

Measurement of Effects of Chlordimeform on Amine Receptors in the Cockroach CNS: To study the effects of chlordimeform on the CNS receptors for biogenic amines to the cockroach we adopted the method of Nathanson and Greengard (1973). This method measures stimulation of adenylate cyclase in the cockroach central nervous system as a result of the addition of exogenous biogenic amines.

RESULTS AND DISCUSSION

Studies in Higher Animals

Acute toxicity and poisoning symptoms: The acute toxicity of chlordimeform to mammals is relatively low. By our estimation the acute, intraperitoneal LD_{50} values for rats is 200 mg/kg. Acute oral and intraperitoneal LD_{50} values for mice and rabbits are also estimated to be of the same order of magnitude (CIBA information sheet).

Initially, chlordimeform causes nervous excitation in rats and mice. They exhibit tremors and become extremely hypersensitive. In rats, gradual dilation of pupils took place over a 1-hr period. Throughout the entire duration of this early excitation period the animals have not been observed to show cholinomimemtic symptoms as slowing of the heart beat, salivation, urination or muscle spasms.

Following these initial periods of hyperexcitation the animals gradually fall into a state of sedation. The transition can be clearly recognized, since they no longer make attempts to run around. Instead, they stay motionless in a characteristic low posture unless they are disturbed. The state of sedation induced by chlordimeform differs from the one induced by general sedatives such as phenobarbital, in that in the former case the animal remains alert to disturbances such as clapping of hands, showing quick jumping and running responses. The recovery occurs gradually and in most cases, the animals behave seemingly normal within 24 hours.

Physiological and biochemical effects *in vivo*: An intraperitoneal injection of 200 mg/kg chlordimeform into rabbits caused a marked decrease in blood pressure of almost 50% within 30 min of injection. The brain itself also gives evidence of amine dysfunction. Whole brain level of amines were measured after chlordimeform treatment.

The result, shown in Table 4, indicates that the amine levels, particularly that of serotonin, were noticeably high in the brains of chlordimeform-treated rats.

TABLE 4

Serotonin and norepinephrine levels in whole rat brain

	Chlordimeform	Control
Serotonin, μg/g wet weight	0.75 ± 0.07	0.44 ± 0.06
Norepinephrine, μg/g wet weight	0.22 ± 0.01	0.18 ± 0.03

Biochemical effects *in vitro*: The results of MAO assay in rat liver homogenates indicate that chlordimeform is an inhibitor of monoamine oxidase (MAO). Also, the degrees of inhibitory potency of the chlordimeform analogs correlate roughly with those of the toxicity of these compound to mites.

Acetylcholine (ACh) receptor: It was found that 10^{-3}M chlordimeform had no effect on a muscle which was sensitive to 7×10^{-7}M acetylcholine. Thus, chlordimeform poisoning is not mediated by acetylcholine-related systems as far as its excitatory aspects are concerned.

In vivo antagonism by reserpine: We have observed that chlordimeform acts as a reserpine antagonist in the rat. Reserpine at a dose of 10 mg/kg, causes immobility, tremor, and muscle rigidity in the animal. These symptoms appear within 45 min of injection, and last at least 30 hr. Rats which were pretreated with chlordimeform (50 mg/kg, IP) 10 min prior to reserpine administration did not develop tremor, and the muscle rigidity was greatly reduced. These symptoms did not develop in the chlordimeform-pretreated rats, even after 10 hr of reserpinization. To demonstrate the antagonistic action of chlordimeform (50 mg/kg) we injected chlordimeform into rats 2 hr or 30 hr after reserpinization (10 mg/kg). In both cases, chlordimeform treatment was followed within 10 min by the complete disappearance of tremor and rigidity of the muscles. Such an antagonistic action of chlordimeform could be explained by a possible action of chlordimeform on brain amines since tremor and muscle rigidity in reserpine-treated rats have been associated with low levels of dopamine in the brain.

We have decided that cockroaches are the best initial study material, despite their general insensitivity to chlordimeform. Cockroaches, particularly American cockroaches, have been extensively used by physiologists and biochemists. They are most suited for electrophysiological studies. Not only that, but the American cockroach is one of two species that has been shown to have a biogenic amine (serotonin) in the central nervous system. A basic similarity in insect and mammalian amine regulatory mechanisms can be inferred from the effects of reserpine or a MAO inhibitor on the levels of biogenic amine in the cockroach brain.

General observations in the American cockroach: Injection of chlordimeform is followed within 5 min by typical symptoms of intoxication. Symptoms include uncoordination, hyperactivity, arching, and wing flapping. Prostration becomes irreversible over a period of several hours, and paralysis begins in 10-20 hr. Chlordimeform did not cause tremor or twitching, and seldom induced convulsions, even at a high dose (670 ug/g). A sublethal dose of chlordimeform (420 µg/g) was followed by symptoms which lasted at least 6 hr before the insects recovered.

Preliminary experiments have established that chlordimeform does not inhibit housefly head cholinesterase even at 10^{-3}M, nor did it affect the (Na-K) ATPase of the roach head at this concentration.

From our symptomatological observations we suspected central nervous system (CNS) involvement in chlordimeform poisoning in American cockroaches.

Electrophysiological studies in the American cockroach: The effects of chlordimeform on the electrical activity of the exposed cockroach ventral nerve cord were studied. Within 10-20 min after flooding the exposed nerve with 10^{-3}M chlordimeform hydrochloride solution, the first electrophysiological evidence of damage to the CNS becomes apparent. This always consists of short volleys of nerve discharges.

Prolonged exposure (up to 2 hr) to this dose of chlordimeform results in severe hypersensitivity of the CNS, as evidenced by long trains of repetitive nerve discharge, both spontaneous and in response to mechanical stimulation of sensory appendages. Nerve blockade did not occur, even after 2 hr of exposure to 10^{-3}M chlordimeform (Beeman and Matsumura, 1974).

Inhibition of tryptamine, DOPA, and serotonin metabolism: Chlordimeform inhibited metabolism of ^{14}C tryptamine in the cockroach head. To show that such an inhibition of tryptamine metabolism by chlordimeform has some physiological consequences *in vivo* the joint actions of chlordimeform and tryptamine were investigated (Table 5). The results clearly indicate that these two chemicals act synergistically.

TABLE 5

Potentiation of chlordimeform toxicity by tryptamine[a/]

	Mortality, %		
	Chlordimeform + water	Acetone + tryptamine	Chlordimeform + tryptamine
Trial 1	10	0	70
Trial 2	20	0	80

a/ Data are expressed as mortality 30 hr after injection of tryptamine (500 µg) or H_2O. Ten roaches were used for each combination (total, 60 roaches). Chlordimeform (100 µg/roach) was given topically with 5 µl of acetone.

In addition, the effect of chlordimeform on the *in vivo* metabolism of externally applied ^{3}H-L-DOPA (a catecholamine precursor) was studied in male cockroaches. The results (Table 6) show that norepinephrine accumulates in poisoned insects to a greater extent than in unpoisoned ones, in agreement with the observation of Rutschmann *et al.* (1965) with established MAO inhibitors in the rat brain.

Visualization of brain amine in cockroaches

By the fluorescence histochemical approach it was possible to show that reserpine had the expected effects of depleting the amine storage in the roach brain. On the other hand, any changes in amine levels brought about by either chlordimeform or tranylcypromine (a typical MAO inhibitor) were subtle and were not detectable by such a crude qualitative assay method.

TABLE 6

In vivo metabolism of ^{3}H-L-DOPA and accumulation of amines as affected by chlordimeform in the American cockroach[a/] [b/]

	Amounts, % of recovered radioactivity[c/]	
	Control	Total
L-DOPA	5.85	11.36
Norepinephrine	11.05	18.36
Dopamine	9.60	8.65
Other metabolites	74.17	61.75

a/ After 24 hr metabolism by male roaches. Roaches were extracted with 10 volumes of acidified n-butanol and the debris removed by brief centrifugation. The supernatant solution was extracted with 1 ml of 0.1N HCl, with 15 ml of n-hexane added to aid separation. Solvent and aqueous phases were concentrated an spotted on cellulose MN 300 TLC plates along with nonradioactive reference compounds, and the plates were developed in methanol-benzene-n-butanol-water (4:4:4:1).

b/ Chlordimeform given topically at a dose of 100 µg/roach 3 hr prior to the oral administration of ^{3}H-L-DOPA. The total poisoning time for chlordimeform was 27 hr.

c/ Results expressed in percentages of applied radioactivity (0.4 nmole of ^{3}H-L-DOPA, specific activity 15 CI/nmole) recovered in each fraction. Average of two determinations.

Measurement of the effects of chlordimeform on amine receptors in the cockroach CNS: It immediately became apparent that the *in vitro* method was not sensitive enough to measure the increase in the biogenic amine level *in vivo* as a result of either MAOI or chlordimeform treatment. However, when their effects were tested *in situ* (by using isolated half ganglia) two important phenomena became known (Table 7). First chlordimeform itself does not stimulate the adenyl cyclase activity (i.e., it does not act as a false transmitter in this preparation) and second (at 10^{-3}M) it instead prevented exogenously added octopamine from achieving the maximum stimulation of adenylate cyclase activity. On the other hand, chlordimeform at 10^{-5}M was found to increase the rate of cockroach heart beat.

TABLE 7

Effect of chlordimeform on adenylate cyclase and octopamine-induced stimulation of adenylate cyclase in roach thoracic ganglia *in situ*[a/]

	^{3}H-c-AMP bound ± S.D., %[b/]
Control	12.9 ± 0.3
Chlordimeform, 1 x 10^{-3}M	13.3 ± 0.7
Control	15.0 ± 2.6
Octopamine, 2.5 x 10^{-4}M	6.4 ± 1.3
Octopamine, 2.5 x 10^{-4}M + chlordimeform 1 x 10^{-3}M	11.6 ± 0.7

a/ Intact hemiganglia (containing adenylate cyclase) were incubated with no treatment, chlordimeform alone, octopamine alone, or octopamine and chlordimeform together, and accumulated c-AMP was measured by competive binding to a c-AMP binding protein. Procedural details are given in Nathanson and Greengard.

b/ Data are expressed as % ^{3}H-c-AMP bound ± standard deviation. All values are means of three to six determinations. Decreased binding indicates increased cyclase activity.

So far the direct pieces of evidence supporting the "amine-theory" in cockroaches are: (a) chlordimeform inhibits metabolism of ^{14}C-tryptamine in the roach head homogenate *in vitro* at I_{50} of 4.4 x 10^{-4}M; (b) the killing action of chlordimeform is potentiated by tryptamine, which by itself is nontoxic to the roaches; (c) increase in indolamines levels are observed in the whole body after

application of chlordimeform *in vivo*, and changes occur in metabolic patterns of serotonin *in vivo*; (d) chlordimeform at 10^{-3}M (we have not tested lower concentrations) blocks the stimulatory action of octopamine on adenyl cyclase in the roach thoracic ganglion; (e) chlordimeform increases the heartbeat rate in isolated roach heart preparations, the phenomenon being compatible with the report that the cockroach heart is innervated by monoamine-containing axons (Miller, 1975).

Indirect evidences are that tranylcypromine, a typical MAO inhibitor, produces very similar symptoms in the American cockroach. Also, several MAO inhibitors are good acaricides against the cheese mite, *Tryophagus putrescens*.

On the other hand, there are several unanswered problems. For example; we have so far been unable to detect an increase in amine levels *in vivo* in the central nervous system of the cockroach. Also, it has recently been shown that tryptamine is not metabolized by oxidative deamination in the roach brain, but rather by N-acetylation (Nishimura *et al.*, 1975). In view of the antagonistic action of mixed-function oxidase inhibitors (such as sesamex and piperonyl butoxide), there is a possibility that one of the metabolic products, rather than chlordimeform itself, is an active agent, at least in certain species.

These questions, however, do not directly challenge the working hypothesis that the toxicity of chlordimeform *in vivo* is related to the changes in biogenic amine levels in quantity and/or in quality.

In conclusion, we have shown that chlordimeform can indeed affect amine regulatory mechanisms and in some instances, can react with certain amine receptors. On that basis we have proposed a working hypothesis that chlordimeform acts upon amine-related systems. Certainly much more information is needed to confirm or deny such a hypothesis. In the future, chlordimeform and its metabolic products should be tested for their effects on amine re-uptake, leak-out from the presynaptic storage, metabolism (not just MAO but many other enzyme systems) and the range of its action on amine receptors either as agonist or antagonist.

The key to the safe use of any new pesticide is to provide the basic toxicological data based upon logical explanation of its action mechanism and its side effects. Chlordimeform and its analogs and metabolites possess very peculiar and unfamiliar properties, particularly as pesticides. It appears very important to make efforts at this stage to understand the basic mechanisms of their actions. Hopefully our initial efforts are providing the means to meet the challenge.

REFERENCES

Abo-Khatwa, N. and R. M. Hollingworth. 1972. Chlordimeform: the relation of mitochondrial uncoupling to toxicity in the German cockroach. Life Sci. 11: 1181.

Batterton, J. L., G. M. Boush and F. Matsumura. 1971. Growth response of blue-green algae to aldrin, dieldrin, endrin and their metabolites. Bull. Environ. Contam. Toxicol. 6: 589-594.

Beeman, R. W. and F. Matsumura. 1973. Chlordimeform: a pesticide acting upon amine regulatory mechanisms. Nature 242: 273.

Beeman, R. W. and F. Matsumura. 1974. Studies on the action of chlordimeform in cockroaches. Pestic. Biochem. Physiol. 4: 325.

Casida, J. E., R. L. Holmstead, S. Khalifa, J. R. Knox and T. Ohsawa. 1974. Toxaphene insecticide: a complex biodegradable mixture. Science 183: 520-521.

Castro, C. E. 1964. The rapid oxidation of iron (II) porphyrins by alkyl halide. A possible mode of intoxication of organisms by alkyl halides. J. Amer. Chem. Soc. 86: 2310.

Chandurkar, P. S. and F. Matsumura. 1978. Identification and toxicity of toxicant Ac, a toxic component of toxaphene. Chemosphere 2: 123-130.

French, A. L. and R. A. Hoopingarner. 1970. Dechlorination of DDT by membranes isolated from *Escherichra coli*. J. Econ. Entomol. 63: 756.

Frontali, N. 1968. Histochemical localization of catecholamines in the brain of normal and drug-treated cockroaches. J. Insect Physiol. 14: 881.

Gillette, J. R. 1971. Reductive enzymes. In Handbook of Experimental Pharmacology, B. B. Brodie and J. R. Gillette, eds. Springer-Verlag, New York. pp. 349-361.

Hassall, K. A. 1971. Reductive dechlorination of DDT: the effect of some physical and chemical agents on DDD production by Pigeon River preparation. Pest. Biochem. Physiol. 1: 259-266.

Hitchcock, M. and S. D. Murphy. 1967. Enzymatic reduction of O,O-diethyl O-(4-nitrophenyl) phosphorothioate, O,O-diethyl O-(4-nitrophenyl) phosphate, and O-Ethyl O-(4-nitrophenyl) benzene thiophosphonate by tissues from mammals, birds and fis-es. Biochem. Pharmacol. 16: 1801-1811.

Holmstead, R. L., S. Khalifa and J. E. Casida. 1974. Toxaphene composition analyzed by combined gas chromatography - chemical ionization mass spectrometry. J. Agric. Food Chem. 22: 939-944.

Kamm, J. J. and J. R. Gillette. 1963. Mechanism of stimulation of mammalian nitro-reductase by flavins. Life Sci.

Khalifa, S., R. L. Holmstead and J. E. Casida. 1976. Toxaphene degradation by iron (II) protoporphyrin systems. J. Agric. Food Chem. 26: 277-282.

Marsdel, R. P., R. H. Cox, Jr., J. Saillaut and F. P. Miller. 1968. A method for the determination of seroferrin and norepinephrine in discrete areas of rat brain. Int. J. Neurophrinical. 7: 275.

Matsumura, F. and R. W. Beeman. Biochemical and physiological effects of chlordimeform. Environ. Health Perspect. 14: 71.

Miller, T. A. 1975. Neurosecretion and the control of visceral organs in insects. Ann. Rev. Entomol. 20: 133.

Miskus, R. P., D. P. Blair and J. E. Casida. 1965. Conversion of DDT to DDD by bovine rumen fluid, lake water, and reduced porphyrins. J. Agric. Food Chem. 13: 481-483.

Nathanson, J. A. and P. Greengard. 1973. Octopamines sensitive adenyate cyclase: evidence for a biological role of octopamine in nervous tissue. Science 180: 308.

Nelson, J. O. 1974. Toxaphene: separation and chemistry of toxic components. Ph.D. Thesis, University of Wisconsin, Madison.

Nishimura, K., T. Fukita and M. Nalsajima. 1975. Catabolism of tryptamine by cockroach head enzyme preparation. Pestic. Biochem. Physiol. 5: 557.

Pimentel, D. 1971. Ecological Effects of Pesticides on Nontarget Species. Government Printing Office, Washington, D.C.

Rose, H. A. and R. G. Young. 1973. Nitro-reductases in Madagascar cockroach, *Gromphadorhina portentosa*. Pest. Biochem. Physiol. 3: 243-252.

Rutschuraum, J. et al. 1965. A method for the study of drug-influenced catecholamine metabolism using L-DOPA-(2,5,6-H^3). In: Isotopes in Experimental Pharmacology. W. Roth, ed., Univ. of Chicago Press, Chicago.

Symms, K. G. and M. R. Juchau. 1974. The aniline hydroxylase and nitro-reductase activities of partia-ly purified cytochromes P-450 and P-420 and cytochrome b_5 solubilized from rabbit hepatic microsomes. Drug Mat. Disp. 2: 194-201.

Turner, W. Y. 1977. Paper presented to the Division of Pesticide Chemistry, 173rd American Chemical Society National Meeting, New Orleans, Louisiana, March 21-25.

Wedemeyer, G. 1966. Dechlorination of DDT by *Aerobacter aerogenes*. Science 152: 647.

APPENDIX

List of technical publications resulting from this research project (1973-1978)

Matsumura, F. and H. J. Benezet. 1973. Studies on the bioaccumulation and microbial degradation of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Environ. Health Perspectives 5: 253.

Benezet, H. J., and F. Matsumura. 1973. Isomerization of γ-BHC to α-BHC in the environment. Nature 243: 480.

Beeman, R. W. and F. Matsumura. 1973. Chlordimeform: a pesticide acting upon amine regulatory mechanisms. Nature 242: 273.

Matsumura, F. 1973. Degradation of pesticide residues in the environment. In "Environmental Pollution by Pesticides". C. A. Edwards, ed. 484-513, Plenum Press, N.Y.

Doherty, J. D. and F. Matsumura. 1974. A highly ion-sensitive ATP-phosphorylation system in lobster nerve. Biochem. Biophys. Res. Commun. 57: 987-992.

Doherty, J. D. and F. Matsumura. 1974. DDT effect on ^{32}p incorporation from -labelled ATP into proteins from lobster nerve. J. Neurochem. 22: 765-772.

Benezet, H. J. and F. Matsumura. 1974. Factors influencing the metabolism of mexacarbate by microorganisms. J. Ag. Food Chem. 22: 427.

Beeman, R. W. and F. Matsumura. 1974. Studies on the action of chlordimeform in cockroaches. Pesticide Biochem. Physiol. 4: 325-336.

Conaway, C. C. and F. Matsumura. 1975. Inhibition of cellular utilization of thymidine by TCDD. Bull. Environ. Contam. Toxicol. 13: 152.

Nelson, J. O. and F. Matsumura. 1975. A simplified approach to studies of toxic toxaphene components. Bull. Environ. Contam. Toxicol. 13: 464-470.

Nelson, J. O. and F. Matsumura. 1975. Separation and comparative toxicity of toxaphene components. J. Agric. Food Chem. 23: 984-990.

Doherty, J. D. and F. Matsumura. 1975. DDT effects on certain ATP related system in the peripheral nervous system of the lobster, Homarus americanus. Pestic. Biochem. Physiol. 5: 242-252.

Matsumura, F., R. W. Howard and J. O. Nelson. 1975. Structure of the toxic fraction A of toxaphene. Chemosphere 5: 271-276.

Furukawa, K. and F. Matsumura. 1975. Microbial metabolism of PCB's: studies on the relative degradability of PCB components by Alkaligenes sp. J. Agric. Food Chem. 24: 251-256.

Matsumura, F. 1975. Role of microorganisms in altering insecticidal residues in environment. Proc. North Central Br. Entomol. Soc. Am. 30: 43-48.

Matsumura, F., H. J. Benezet and K. C. Patel. 1976. Factors affecting microbial metabolism of γ-BHC. J. Pestic. Sci. 1: 3-8.

Matsumura, F. and R. W. Beeman. 1976. Biochemical and physiological effects of chlordimeform. Environ. Health Perspectives 14: 71-82.

Matsumura, F. 1975. Mechanisms of action of insecticidal and acaricidal chemical. Proc. Int. Controlled Release Pestic. Symp. pp. 200-208. Wright State University, Dayton, Ohio.

Matsumura, F. 1974. Microbial degradation of pesticides. In Survival in Toxic Environments. Kahn, M.A.Q. and J.P. Bederka, Jr., Eds., Academic Press, N.Y. pp. 129-154.

Conaway, C. C., B. V. Madhukar and F. Matsumura. 1977. P,p'-DDT studies on induction mechanisms of microsomal enzymes in rat liver systems. J. Environ. Res. 14: 305-321.

Chandurkar, P. S., F. Matsumura and T. Ikeda. 1978. Identification and toxicity of toxicant Ac, a toxic component of toxaphene. Chemosphere 2: 123-130.

Beeman, R. W. and F. Matsumura. 1978. Formamidine pesticides - action in insects. In Pesticide and Venum Neurotoxicity. Shankland, D.L., R.M. Hollingworth and T. Smyth, Jr., eds. Plenum Press, N.Y. pp. 179-188.

Esaac, E. G. and F. Matsumura. 1978. A novel reductive system involving flavoprotein in the rat intestine. Bull. Environ. Contam. Toxicol. 19: 15-22.

Manuscripts accepted for publication

Chandurkar, R. S. and F. Matsumura. 1978. Metabolism of toxicant B and toxicant C of toxaphene in rats. Bull. Environ. Contam. Toxicol. In Press.

Ward, C. T. and F. Matsumura. 1978. Fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in a model aquatic environment. Arch. Environ. Contam. Toxicol.

Esaac, E. G. and F. Matsumura. 1978. Roles of flavoproteins in degradation of mexacarbate in rats. Pestic. Biochem. Physiol. In Press.

GLOSSARY

adrenomimetic: mimicking the actions of norepinephrine or dopamine.

algae: primitive plants, one or many celled, usually aquatic and capable of growth in mineral materials via energy from the sun and the green coloring material, chlorophyll.

anaerobic: refer to reaction systems carried out in the absence of oxygen.

antagonism: reduction of negation of the effects of one drug by another drug.

bioassay: assay method utilizing biological organisms to assess potential environmental toxins.

biogenic amines: amino acid-derived chemicals, including norepinephrine, dopamine, octopamine, and serotonin, which function as intercellular messengers (transmitters) within the nervous system or between nerve and muscle cells, or nerve and gland cells.

biotransformation: conversion of a foreign chemical, such as an insecticide, to new products (metabolites) in living organisms. These products are usually with lower or higher biological activity.

cholinergic: a type of nerve cell, whose outgoing chemical messages are mediated by the transmitter, acetylcholine.

cyclic AMP: an intracellular "second messenger" present in message-receiving cells, whose production is stimulated by the primary messengers (e.g. biogenic amines) and which is directly responsible for the physiological actions of the primary messengers.

dechlorination: elimination of a chlorine atom.

dehydrochlorination: elimination of a HCl moiety from a chemical.

endocrine: involving long-range chemical messengers, e.g. blood-borne hormones.

flavin: an enzyme cofactor required in the catalytic conversion of substances to metabolites by certain enzymes.

flavoprotein: a protein that contains in its structure a flavin group, such as FAD, FMN.

ganglia: nerve centrs, consisting of masses of nerve cells, where nervous information is analyzed and interpreted.

heme: a biochemical that contains iron such as cytochromes and hemoglobin.

homogenate: a suspension of breakdown-cells in buffer solution. The cells are breakdown by means of certain equipment, such as blender.

hydrolytic: breaking a molecule to more than one part, e.g. ester is hydrolyzed to acid and base.

larvae: immature forms of insects which undergo a complete type of development.

LC_{50}: lethal concentration of some toxic substance which will kill 50% of the assayed population.

metabolism: alteration of chemicals by a biological organism usually in response to intake of nutrients.

microsomes: small particles inside the living cells, obtained by preparing cell homogenate and centrifugation at high speed in vacuum.

monoamine oxidase (MAO): an enzyme which destroys biogenic amines and which is important in regulating biogenic amine levels.

NADPH: an enzyme cofactor required for many oxidative as well as reductive conversion of insecticides in living organisms. Its chemical name is: nicotinamide adenine dinucleotide phosphate.

N-demethylation: elimination of a methyl group.

non-target organisms: organisms which are susceptible to pesticide poisoning(s) even though they are not the pest species themselves.

octa: containing or having eight parts or units.

oxidative: a process by which a given chemical is oxidized, e.g. incorporation of an oxygen atom into the chemical.

receptor: a specialized area of a message-receiving cell, which binds the chemical messenger, and transmits the message to the rest of the cell, e.g. by dispatching the internal messenger, cyclic AMP.

reductive: a process by which a given chemical is reduced, e.g. elimination of an oxygen atom or incorporation of a hydrogen atom into the chemical.

reserpine: a sedative which produces sedation and several other behavioral effects by depleting biogenic amines from the central nervous system.

Sephadex G-75: a carbohydrate polymer used in separation and isolation of biochemical, e.g. proteins, according to their molecular weight.

supernatant: the clear solution that results from centrifugation of the cell homogenate.

synergism: facilitation or enhancement of the effects of one drug by another drug.

Thunberg tubes: glass tubes with a side arm and ground-glass stopper that are used to carry out reactions under anaerobic conditions.

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16. ABSTRACT

This research project was initiated with the overall objective of determining (1) the chemical structures of toxic components of toxaphene, (2) to study anaerobic metabolism to degrade toxaphene and other pesticides, and (3) to understand toxic action mechanism of chlordimeform.

As a result of intensive efforts the molecular structures of three of the most toxic principles of toxaphene were identified. Together these comprise at least 70% of toxaphene's toxicity toward mice. This is the first time that the structure of toxic components of toxaphene became apparent despite the widespread use (over 1 billion pounds, which is comparable to DDT) of toxaphene in the last 3 decades. Toxaphene on the other hand degrades relatively faster than other chlorinated pesticides such as DDT and dieldrin. The reason for it is that toxaphene is susceptible to reductive degradative forces.

Chlordimeform was found to affect amine regulatory mechanisms in animals. Such actions explain some of the subtle effects of this pesticide on animals. Inasmuch as that biogenic amines are known to play many important biological roles such as controlling emotion, behavior and circulatory functions of the body.

17. KEY WORDS AND DOCUMENT ANALYSIS

a DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
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