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16. ABSTRACT

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CHLORINATED HYDROCARBONS: INSECTICIDE VERSUS CARCINOGENIC ACTION

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NOTICE

Although the research described in this report has been funded wholly or in part by the United States Environmental Protection agency (EPA) through Grant No. R805008 to Ohio State University, Columbus, Ohio 43210, it has not been subjected to EPA review and therefore does not necessarily reflect the views of EPA and no official endorsement should be inferred.

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 complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory conducts a coordinated environmental health research program in inhalation toxicology, genetic toxicology, neurotoxicology, developmental and experimental biology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, water pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides and other chemical pollutants. The Laboratory participates in and provides data for the development and revision of criteria documents on pollutants for which national ambient air quality and water 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 primarily responsible for providing 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 environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

Identify those structural features of pesticides (halogenated hydrocarbon class) that are responsible for the species specific effects.

F. Gordon Hueter, Ph.D.
Director
Health Effects Research Laboratory

PREFACE

Population growth and an increase in groups which do not directly participate in food production have increased the demand for more efficient agricultural productivity. This demand has been satisfied by both higher crop yields and by eliminating man's natural competitors with pesticides. Pesticides have thus become some of the most widely used environmental chemicals today. Some of these chemicals although not showing an immediate effect *in vivo* at concentrations normally used in agriculture, may pose a significant long term hazard to man. The ideal pesticide should selectively affect a desired species for a specified period of time and then disappear without any trace. There is no such pesticide and the present policy of pesticide use is a compromise between desirable and undesirable effects. It was the purpose of this study to develop an approach to effectively evaluate the relationships that exist between insecticidal agents used in agriculture and the induction of genetic damage in mammalian systems.

This study was directed towards identifying those structural features of the halogenated hydrocarbons class of pesticides that are responsible for the species specific effects observed. We found that it was possible to selectively reduce the deleterious genetic effects of many of these agents in mammalian systems while maintaining a high level of insect toxicity. This research suggests that more effective and environmentally safe pesticides may be attainable through an interdisciplinary approach combining chemical, cellular, molecular and entomological studies.

ABSTRACT

The purpose of this grant was the determination of those structural characteristics responsible for the deleterious vs beneficial effects of chlorinated pesticides. These studies have led to the development of a model system for the rational design, synthesis and evaluation of insecticidal compounds with reduced genetic hazard.

Various halogenated hydrocarbons and their analogs were designed and synthesized for this study. The test systems employed for evaluation of the mammalian and insect effects were: a) normal (CI and 153) and SV-40 transformed (VA-4) human fibroblasts; b) DNA repair by UDS and BUdR photolysis; c) measurement of association of ^{14}C-labeled synthetic analogs with genetic material; d) cytotoxicity; e) metabolic activation studies using liver homogenates; and f) topical and oral toxicities of standards and test compounds in house flies (Musca domestica) and mosquito larvae (Aedes aegypti).

These studies indicated that modification of the 6,7-double bond of aldrin and the 6,7-epoxide of dieldrin could lead to potent insecticidal agents with reduced cytotoxicity, DNA repair and DNA association. These studies included the syathesis and evaluation of three distinct series of pesticide analogs including two series of halogenated cyclodienes with modified π-electron character and a series of aromatic pyrethrin-related agents.

This report was submitted in fulfillment of Grant No. R-805008 by the CBERG Group under the sponsorship of the U.S. Environmental Protection Agency covering the period July 18, 1977 to July 17, 1980.

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LIST OF ABBREVIATIONS

BUdR - Bromodeoxyuridine
CBERG - Chemical Biomedical Environmental Research Group
CS - Calf Serum
DMSO - Dimethylsulfoxide
DNA - Deoxyribonucleic acid
EPA - Environmental Protection Agency
FCS - Fetal calf serum
GLC - Gas liquid chromatography
HU - Hydroxyurea
IR - Infrared spectroscopy
J - Joules
LC_{50} - Lethal concentration, 50%
LD_{50} - Lethal dose, 50%
MFO - Mixed function oxidase
mMEM - Modified minimal essential medium
MS - Mass spectrometry
Mw - Weight average molecular weight
NADP - Nicotine adenine diphosphonucelotide
NMR - Nuclear magnetic resonance spectrometry
PBO - Piperonyl butoxide
PBS - Phosphate buffer saline
SAR - Structure activity relationship
SDS - Sodium dodecyl sulfate
TCA - Trichloroacetic acid
UDS - Unscheduled DNA synthesis
UV - Ultraviolet

SECTION I

INTRODUCTION

OBJECTIVES

The primary objective of this grant was to determine the structural characteristics responsible for the deleterious vs beneficial action of chlorinated pesticides. The overall purpose of this study was to develop a model system for the rational design, and synthesis of non-carcinogenic, yet biologically effective pesticides. Specifically the purpose of this project was to:

1) Synthesize and rationally design a selected series of compounds with systematically juxtaposed functional groups.

2) Evaluate the toxicities of those chemical agents in a variety of insect model systems.

3) Evaluate the in vitro mammalian cellular effects.

4) Determine the pesticide effects on DNA repair and replication in vitro in mammalian systems.

5) Synthesize, as needed, radiolabeled molecules of primary interest to determine the extent and type of macromolecular interaction.

6) Determine the molecular features of pesticides which contribute to: a) mammalian genetic damage; and b) assess the relationship of this damage to insect toxicity.

SUMMARY OF RESULTS

Synthesis and Selection of Halogenated Pesticides

The halogenated pesticides evaluated during the course of this study period included numerous E.P.A. standards and a variety of analogs of the halogenated polycyclic insecticidal agents, aldrin and dieldrin. Individual analogs (Figure 1 and Figure 2) were synthesized (1,2) and evaluated for their insecticidal and mammalian effects. These compounds represented the chemical manipulation of the metabolically reactive portions of the molecule and the studies included several series of halogenated cyclodienes (3). Additionally, a second study was carried out assessing the effects of chemical manipulation on extended aromatic and π-electron rich moieties in other categories of pesticides based upon findings observed in the aldrin related series of

molecules. The synthetic approaches were extended to the pyrethrin classes (Figure 3) of insecticidal agents based on results (4) obtained early in the grant period. The chemical approaches resulted in the synthesis of a variety of novel chemical agents with observed insecticidal activities. Several of the agents synthesized were radiolabeled (5) for further macromolecular interaction studies which led to the eventual synthesis of chemical agents which retained their insecticidal action while demonstrating a markedly reduced deleterious effect on mammalian systems. The structures of the chemical agents selected and/or synthesized for these studies are summarized in Figures 1-4.

Insect Toxicity Studies

All chemical agents and EPA standards were evaluated for their insecticidal action in house fly (Musca domestica) and mosquito (Aedes aegypti) test systems (6,7) utilizing both topical and oral administration routes. Additionally, experiments were carried out to determine the effects of synergistic insecticidal chemicals (8) and the effects of metabolism onthe observed toxicities of pesticides from the several classes involved in these studies.

The studies undertaken indicated little direct relationship between high halogen content, aromatic ring content and insecticidal action. The halogenated cyclodiene analogs (Fig. 2) (1) demonstrated only very weak insecticidal action by either oral or topical routes of administration. The compounds with extended π-electron rich systems (utilizing pyrethrin-related agents) (Figure 3) failed to demonstrate decreased mammalian toxicity while retaining effective pesticidal action. Analogs of aldrin and dieldrin (Fig. 1) provided the most effective insecticidal agents and demonstrated the greatest activities when used in conjunction with synergistic agents such as PBO. The results of these studies clearly demonstrated that structure-action correlations could be established based upon probable routes of metabolism in the insect species.

Our studies further demonstrated a stereochemical selectively of cis isomeric pyrethrine-related analogs as the most effective insecticidal agents. Naturally occurring and other synthetic analogs usually favor the trans isomeric structures (Figure 3). Additionally, the most effective insecticidal analog of aldrin, 6,7-dihydroaldrin (Figure 1,4) was shown to be converted by mammalian liver homogenates to aldrin (Figure 1,1), dieldrin (Figure 1,2) and 6-hydroxydihydroaldrin (Figure 1,14).

In Vitro Mammalian Cellular Effects

The cytotoxic effects of the chlorinated hydrocarbons synthesized were determined in normal fibroblasts (C-153) and transformed human fibroblast (VA-4) cell lines. Aldrin, dihydroxyaldrin (Figure 1,6) and dieldrin at a 100 uM concentration reduced the colony forming ability to 0% in both C-153 and VA-4 fibroblasts. Other structural modifications of the active moieties of the chlorinated hydrocarbons greatly reduced cytotoxicity, but not to the level observed with the above three compounds. Allethrin and pyrethrin

(Figure 5), of the pyrethroid class, exhibited a high level of cytotoxicity resulting in 0% survival at 100 uM concentration in the C-153 and VA-4 cell lines. All compounds tested exhibited typical cytotoxic curves dependent upon dose.

Induction of DNA Repair

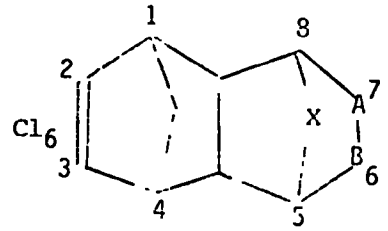
Dieldrin induced UDS in the VA-4 cell line (9), while it showed comparatively less UDS in the C-153 and CI (10,11) (normal human skin fibroblasts) cell lines. Aldrin did not show any UDS in the cell lines tested. Of the structural analogs tested for UDS, those containing a reactive moiety at the 6,7 position induced UDS. The other analogs did not appear to induce significant levels of UDS. Repair was also measured using the BUdR photolysis technique. Aldrin and dieldrin both showed photolyzable sites in the CI cell line, while dihydroaldrin did not (11). Aldrin and dieldrin both inhibited normal DNA replication. Pyrethrin showed repair while allethrin and permethrin did not.

Radiolabeled Agents for Assessment of DNA-Association

In order to assess the relationship of pesticidal association with macromolecular cell components, ^{14}C-aldrin, ^{14}C-dieldrin, ^{14}C-dihydroaldrin and ^{14}C-dihydroxydihydroaldrin (5), were required at different stages of research in this project. The ^{14}C-aldrin and ^{14}C-dieldrin were obtainable through commercial sources at 80 mCi/mM. These agents were used for biological studies and served further as the starting materials for radiosynthetic procedures that resulted in novel synthesis of the ^{14}C-dihydroaldrin and ^{14}C-dihydroxydihydroaldrin (12). Coincident with UDS data, ^{14}C-dieldrin was found to be associated with VA-4 and 153 cells to the greatest extent. ^{14}C-aldrin and ^{14}C-dihydroaldrin did not associate with the DNA of the VA-4 and 153 cells while ^{14}C-dihydroxydihydroaldrin did associate to a limited extent. Activation studies utilizing S-9 rat liver microsomes increased the binding of ^{14}C-aldrin and ^{14}C-dihydroaldrin in both VA-4 and 153 cell lines. It was also demonstrated that ^{14}C-dieldrin associated with single- and double-stranded isolated calf thymus DNA.

FIGURE 1

Structures of Halogenated Cyclodienes for SAR Studies - Aldrin and Dieldrin Analogs



1) X = CH_2; A-B is CH = CH (aldrin)

2) X = CH_2; A-B is CH_2 (dihydroaldrin)

3) X = CH_2; A-B is  (dieldrin)

4) X = CH_2; A-B is CH(OH)-CH(OH) (dieldrindiol)

5) X = O; A-B is CH=CH (oxyaldrin)

6) X = O; A-B is CHOH-CHOH (dihydrodihydroxyaldrin)

7) X = O; A-B is  (oxydieldrin)

8) X = O; A-B is CH(OH)-CH(OH) (oxydieldrindiol)

9) X = CH_2; A-B is $CHCH_2$

10) X = CH_2; A-B is CHFCHF

11) X = CH_2; A-B is (CHOH)  (monoacetoxymonohydroxy)

12) X = CH_2; A-B is  (diacetoxydihydro)

13) X = CH_2; A-B is  (6-oxo)

14) X = CH_2; A-B is $CH_2CH(OH)$ (monohydroxydihydro)

15) X = CH_2; A-B is CH_2CF_2

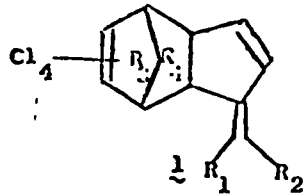
16) X = CH_2; A-B is $CH_2CHOCCH_3$ (monoacetoxydihydroaldrin)

17) X = CH_2; A-B is 

18) X = CH_2; A-B is

FIGURE 2

Chemical Structures of Halogenated Cyclodienes -
Electron Rich Analogs



19) $R_1=R_2=C_6H_5$ $R_3=R_4=Cl$

20) $R_1=R_2=C_6H_5$ $R_3=R_4=OCH_3$

21) $R_1=R_2=C_6H_5$ $R_3=R_4=H$

22) $R_1=R_2=CH_3$ $R_3=R_4=Cl$

23) $R_1=R_2=CH_3$ $R_3=R_4=OCH_3$

24) $R_1=R_2=CH_3$ $R_3=R_4=H$

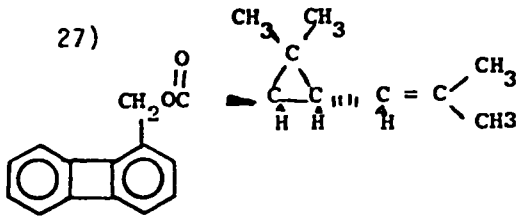
25) $R_1=CH_3$; $R_2=C_6H_5$ $R_3=R_4=Cl$

26) $R_1=CH_3$; $R_2=C_6H_5$ $R_3=R_4=OCH_3$

FIGURE 3

Extended Aromatic Systems -
Pyrethrin-Related Analogs

27)



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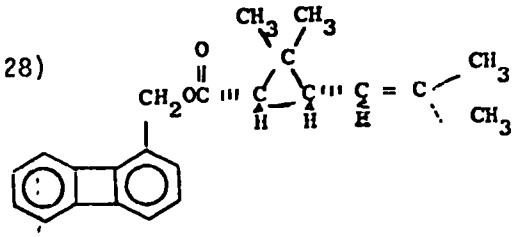

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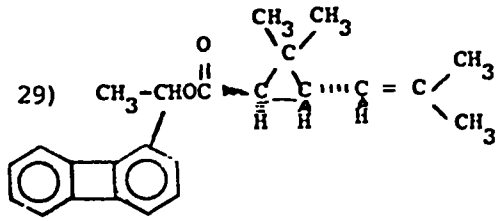

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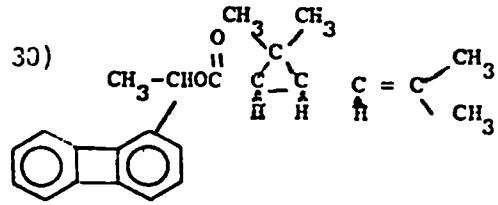

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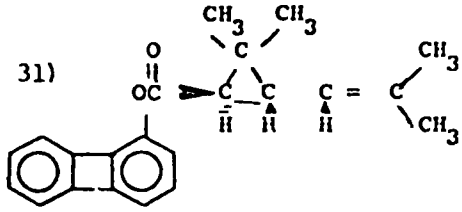

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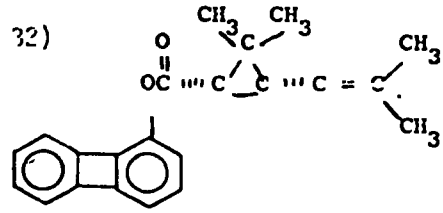

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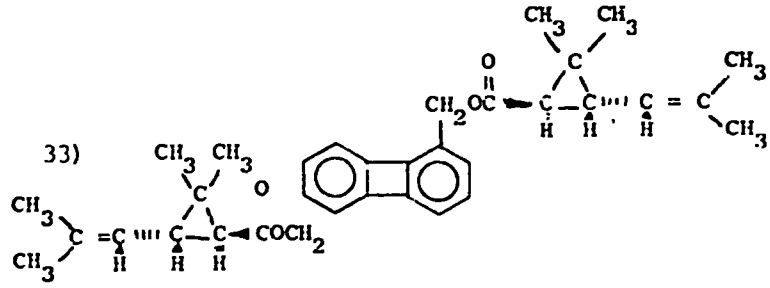


Figure 3 (cont.'d)

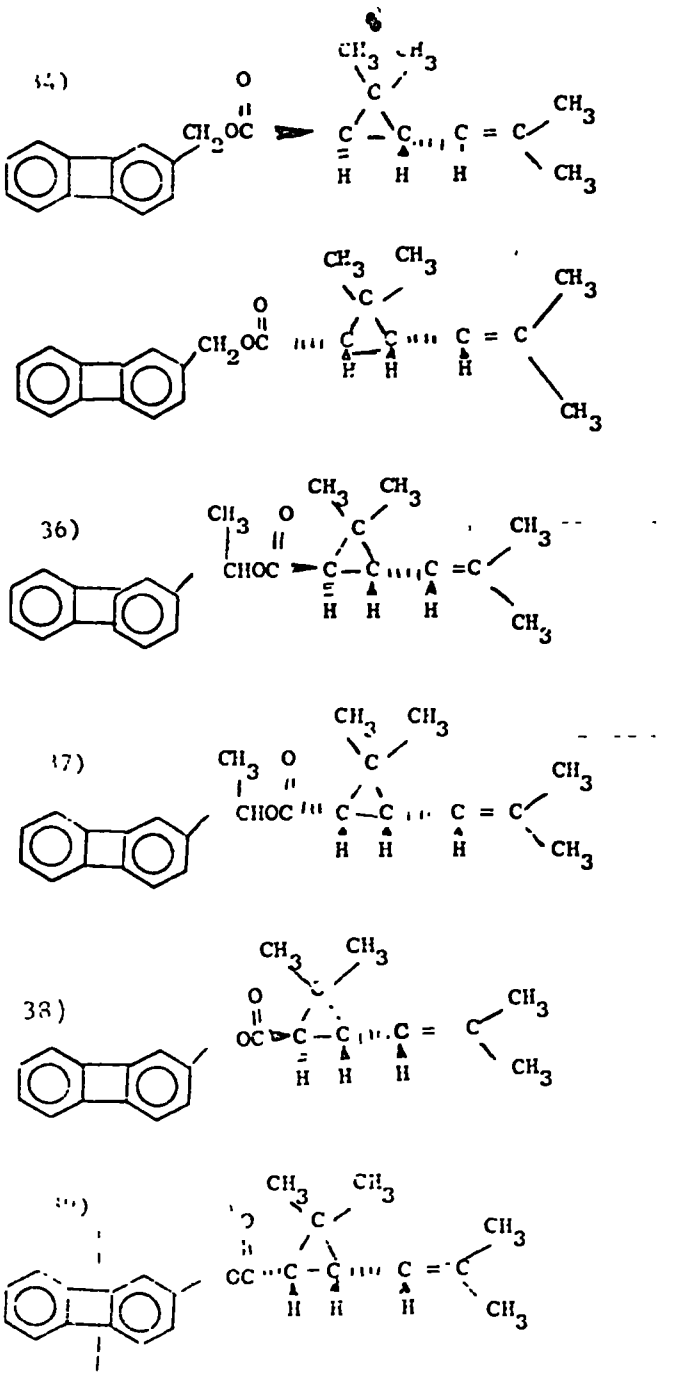
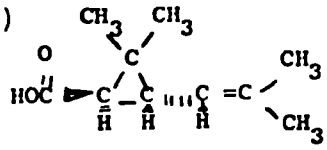


FIGURE 4

Synthetic and Precursor Components for Extended π-System Analogs

40)



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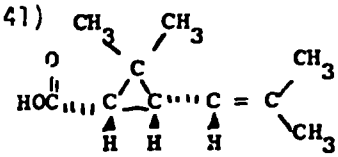

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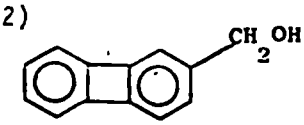

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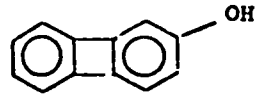

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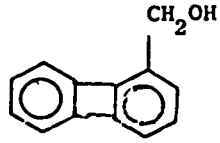

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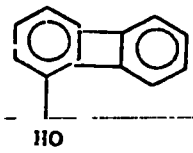

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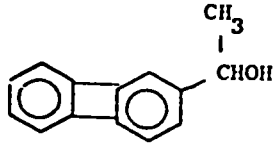

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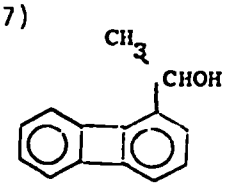

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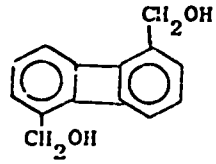
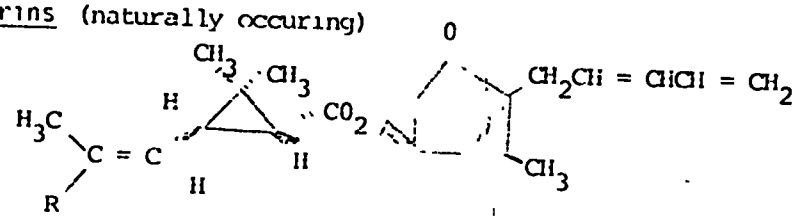


FIGURE 5

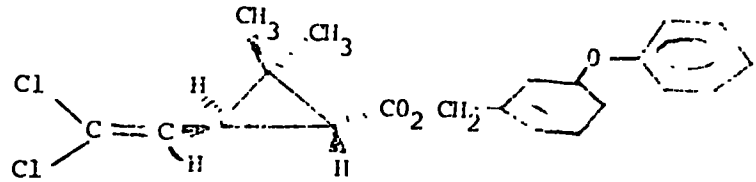
Structures of Commercially Important Pyrethrins Studied

Pyrethrins (naturally occuring)



R = CH_3, CO_2CH_3

Permethrin



Allethrin (synthetic)

see pyrethrins (above) R = CH_3

SECTION II

CONCLUSIONS

1. Aldrin and dieldrin as the parent compounds of a series of halogenated pesticides are cytotoxic to mammalian cells *in vitro*.

2. The metabolic activation of aldrin produces a product with greater DNA damaging potential than the parent compound.

3. Modification of the 6,7 double bond of aldrin to the epoxide (dieldrin) results in extensive damage to mammalian cells *in vitro* relative to the parent compound.

4. Reduction of the 6,7-double bond greatly reduces the mammalian cytotoxicity and DNA damaging capabilities of the parent compound. Incorporation of a 6-fluoro group into the reduced molecule produced an agent with minimal DNA damaging capabilities.

5. Chemical manipulation of the bridge carbon to produce oxyaldrin (Figure 1, 5) and oxydieldrin (Figure 1, ') produced agents with DNA-damaging capabilities.

6. Insecticidal studies indicated that the most potent insecticidal analogs of the halogenated cyclodienes were metabolically convertable to aldrin, dieldrin or the 6-monohydroxy-6,7-dihydroaldrin (Figure 1, 14).

7. The use of synergistic agents such as PBO proved effective in enhancing the insecticidal potency of those compounds demonstrating markedly reduced mammalian toxicities.

8. The use of π-electron rich moieties in structures of known pesticidal activity caused either a loss of pesticidal activity or a sterochemical reversal of previously observed activity in related insecticidal agents.

9. Studies utilizing 6,7-dihydroaldrin indicated that reduced mammalian cytotoxicities and DNA damage could be obtained while retaining a high level of insect toxicity.

The results of this study support the feasibility of the rational design of pesticides that will not induce genetic damage resulting in mutagenesis, carcinogenesis and cytotoxicity. The selection of a limited number of model compounds with modified chemical functional groups served as an effective basis of identifying the probable sites of reactivity responsible for insecticidal and mammalian cellular effects in the aldrin/dieldrin class of halogenated pesticides.

SECTION III

NARRATIVE

MATERIAL AND METHODS

Cell Culture

The VA-4 cell line, a SV-40 transformed human skin fibroblast was obtained from Dr. James Blakeslee in the Department of Veterinary Pathobiology, at The Ohio State University. This cell line was originally established by Dr. A.J. Giradi (13). The CI and C-153 cell lines were established in our laboratory from neonatal foreskins. The cell lines were maintained in mMEM supplemented with 5% FCS and 100 ug/ml streptomycin, 100 ug/ml penicillin, and 100 ug/ml fungazone. Cells were incubated at 37°C in a humidified 5% CO_2: 95% air atmosphere.

Cells were seeded onto 11 x 22 mm coverslips contained in a 100 mm diameter glass petri dish at a density of 1 x 10^4 cells per cm^2. Twelve hours later, after cells had attached, the media was removed and fresh medium containing 5% CS and 2 mM HU was added to inhibit scheduled DNA synthesis. At the time of chemical addition, the media was removed the fresh-medium-containing 5% CS, 2 uCi/ml [^{3}H]tnymidine (specific activity 5 Ci/mM) and 2 mM HU was added to the plates along with the compound to be tested. For cells to be irradiated with UV radiation, the cells were washed twice with PBS and irradiated with 254-nm radiation at a fluence of 1 J/m^2/sec. Immediately following irradiation, medium containing CS, [^{3}H]tnymidine and HU were added as described above with the chemical. Cover slips were pulled at the time points indicated in the figures, legends and tables, and washed in Hank's buffered salt solution, fixed in 95% ethanol: 5% acetic acid (9). Slides were washed and rehydrated by dipping in 95% ethanol, 70% ethanol and distilled water. After air drying, the coverslips were mounted onto slides and dipped in 2-fold diluted Ilford 44 emulsion (Eastman Kodak) and kept in the dark four days at 4°C. Slides were then developed in D-19 Kodak Developer, fixed and stained with Harris hematoxylin and eosin. Approximately 100 cells were randomly selected and the number of grains over nucleus were counted and corrected for background grains. All grain counting was done using an Artex model 880 counter with an Hitachi video screen connected to a Leitz Aus Jena Docuval microscope. Pesticides wre dissolved in DMSO. The highest concentration of DMSO used was 1.0%. Results obtained with test agents were compared to controls containing DMSO only and no DMSO.

Cytotoxicity

The cytotoxicity of the chlorinated hydrocarbons was determined by assaying for the number of surviving cells following treatment with the compounds. The assay as described previously (14) is based upon the capacity of a cell to replicate and form a visible colony after staining. Approximately 600 cells were seeded in the 100 mm diameter tissue culture dishes (Corning). Following attachment various concentrations of chlorinated hydrocarbons were added to the cultures and incubated for 12 hours at 37°C. The media was removed and the plates were washed twice with 10 ml of PBS and fresh mMEM containing 5% FCS. After 7 days of incubation at 37°C the plates were washed twice with Hank's basic solution and stained by incubating for 10 minutes with a saturated Giemsa solution. After rinsing, the number of colonies per plate were counted using an Artex model 880 counter with a Hitachi video screen. Three counts were made on each plate and at least 10 plates were used in determination of each point on the survival curves shown in the figures. Two controls were used in each experiment, one with media and the other with DMSO as a solvent.

BUdR Photolysis

The BUdR photolysis method was described previously (15,16). Cells were labeled with either [^{3}H] or [^{14}C] thymidine. After labeling, the cells were incubated with pesticide as reported above. The ^{3}H-labeled cells were incubated with 0.1 uM BUdR, while the ^{14}C-labeled cells were incubated with 0.1 mM thymidine for 15 hours. The ^{3}H- and ^{14}C-labeled cells were mixed together and irradiated for 0, 6, and 9 minutes with 313-nm radiation. The DNA was sedimented on alkaline sucrose gradients and the number of single-strand breaks (Photolyzable sites) calculated (16).

DNA Association Determination

Cells were incubated with ^{14}C-labeled pesticide. At the time indicated, cells were scraped from the plates and the DNA harvested using SDS-isoamyl alcohol (17). The purified DNA was precipitated with absolute alcohol and counted for radioactivity. Calf Thymus DNA which was reacted with radioactive pesticide was precipitated with TCA, washed with alcohol and acetone and collected on Whatman #3 paper discs (18). The discs after drying were counted for radioactivity. Single-stranded DNA was prepared by heating at 100°C for 15 minutes followed by rapid cooling at -10°C. The amount of pesticide associated with DNA was calculated from the specific activity after correcting for dilution of isotope and quenching.

Insect Toxicity Assays

Housefly - Topical Application

Contact toxicity was determined as LD_{50} by topical application of chemical dilutions to mixed-sex houseflies, Musca domestica (6,7) using reagent grade acetone as solvent. One-microliter droplets of each solution were applied with an ISCO microapplicator to the thoracic region of 20 adult

flies, 3 ± 1 days of age. Dead and moribud flies were recorded at 24 and 48 hours. Reconstituted powdered milk was offered as food during the observation period. Acetone only was administered to the control flies.

Housefly - Oral Exposure

Oral toxicity was determined by feeding experiments on mixed-sex house flies. An appropriate volume of acetone dilutions of each chemical was uniformly mixed with granulated sugar, and the solvent was evaporated to provide a w/w% concentration in the food. The 48 hour LC_{50} was determined for each exposure group containing 20 flies in a ventilated container. Dead and moribund flies were counted at 24 and 48 hours.

Mosquito Larvae - Immersion Toxicity

Immersion toxicity was determined by exposing 20 fourth instar mosquito larvae (Aedes aegypti) in water containing the chemicals to be assayed. Chemicals were dissolved in acetone and 0.5 ml of hot solution was added to 500 ml of water. Dead and moribund larvae were recorded after 24 and 48 hours. Control groups were exposed to acetone and water.

Determination of Toxicity Values, All Insects

LD_{50} and LC_{50} values were interpolated from regression lines of probit mortality vs. $\log_{10}$ dose when graded responses occurred. Otherwise, the maximum response at the highest dose was recorded. Insecticides of known potency (aldrin, dieldrin, allethrin) were included in each experiment as standards of toxicity.

In Vitro Metabolism of Chemicals by S-10 Fraction of Rat Liver

Incubation and Extraction

Chemicals were incubated with S-10 liver fraction and the necessary co-factors (NADP, isocitrate, isocitrate dehydrogenase, Mg^{+2}, Mn^{+2}) for various periods of time. The mixture was extracted three times with petroleum ether, dried with Na_2SO_4, reduced in volume and analyzed. In some cases the extract was purified by column chromatography using silicic acid and florosil.

GLC Analysis of Metabolites

Petroleum ether extracts were analyzed by gas-liquid chromatography utilizing electron capture detection. Qualitative analysis was accomplished by comparing retention times of unknowns and standards. Quantitative analysis was obtained by peak height measurements.

Chemical Synthesis of Standards and Analogs

The syntheses of the novel halogenated cyclodienes have been reported by us (1,2). The synthesis of the radiolabeled derivative ^{14}C-6,7-dihydroaldrin has also been published (3). The synthetic methods utilized for the pyrethrin

related family of agents have been described (4) and literature preparations were utilized for the synthesis of oxoaldrin (3), oxodieldrin and the derivatives (Figure 1) of 6,7-dihydroaldrin and 6,7-dihydroxydihydroaldrin. All chemical/samples were analyzed by GLC chromatography for purity, and were purified as required by preparative thin layer chromatography. Samples were evaluated for chemical composition by standard spectral analysis (IR, NMR, mass spec) and microanalysis for carbon hydrogen and chlorine where applicable. Mass spectroscopy was employed as needed for metabolism studies and conclusive identification of reaction mixture components.

RESULTS

Cytotoxicity

The effects of chlorinated hydrocarbons and pyretheid pesticides on the colony forming ability (survival) of VA-4 (Figure 6) and C-153 (Figure 7) human fibroblast is shown in Table 1. The cytotoxic effect of chlorinated hydrocarbons was similar in both the transformed (VA-4) and normal (C-153) cell lines. Aldrin, dieldrin, allethrin and pyrethrin exhibited the greatest cytotoxic effect at the 100 uM dose. 6-Oxodihydroaldrin (Figure 1,13) and dihydroxyaldrin exhibited a comparatively less cytotoxic effect at all concentrations tested.

DNA Damage and Repair

The induction of DNA damage by chlorinated hydrocarbons and the repair of such damage in VA-4 cells was determined using UDS. A series of chlorinated hydrocarbon analogs plus UV as an internal control were tested for their ability to induce UDS in VA-4 cells. As shown in Table 2, VA-4 cells exhibited an expected level of UDS characteristic of normal human skin fibroblasts following UV irradiation. Dieldrin induced a lower level of repair then UV, while aldrin exhibited little if any UDS.

These results could be expected if the epoxide at the 6,7 position of dieldrin played a role in reacting with DNA. Aldrin on the other hand would have to undergo metabolic activation for conversion to dieldrin in order to exhibit the same effect. The monoacetoxy (Figure 1,16) and diacetoxy (Figure 1,12) analogs, which contain reactive groups on either the 6 and/or 7 position of aldrin also exhibited a high level of UDS equivalent to that observed with dieldrin. Oxyaldrin and oxydieldrin, which are similar to the parent compound except that the bridge carbon is replaced by a bridge oxygen, also appeared to induce UDS in these cells. On the other hand diehydroaldrin and dihydroxydihyroaldrin retard the formation of the 6,7 epoxide and exhibited little if any UDS. These data point to the involvement of the 6,7 position of chlorinated hydrocarbons as a likely site of reactivity with cellular DNA.

A series of pyrethroid analogs were tested for induction of UDS in VA-4 cells. Pyrethrin, allethrin, cis-chrysanthemumic (Figure 4,41) acid, and trans-chrysanthemumic (Figure 4,41) acid induce comparitively few (0.3 to 2.24) grains per nuclei. The 1-hydroxyaethyl-biphenylene (Figure 3,33) induced a higher (5.9) number of grains indicating that DNA damage induced by this compound was repaired to some extent.

Studies using the BUdR photolysis method for measuring repair, indicated (Table 3) that aldrin, dieldrin, dihydroaldrin and pyrethrin induce repair.

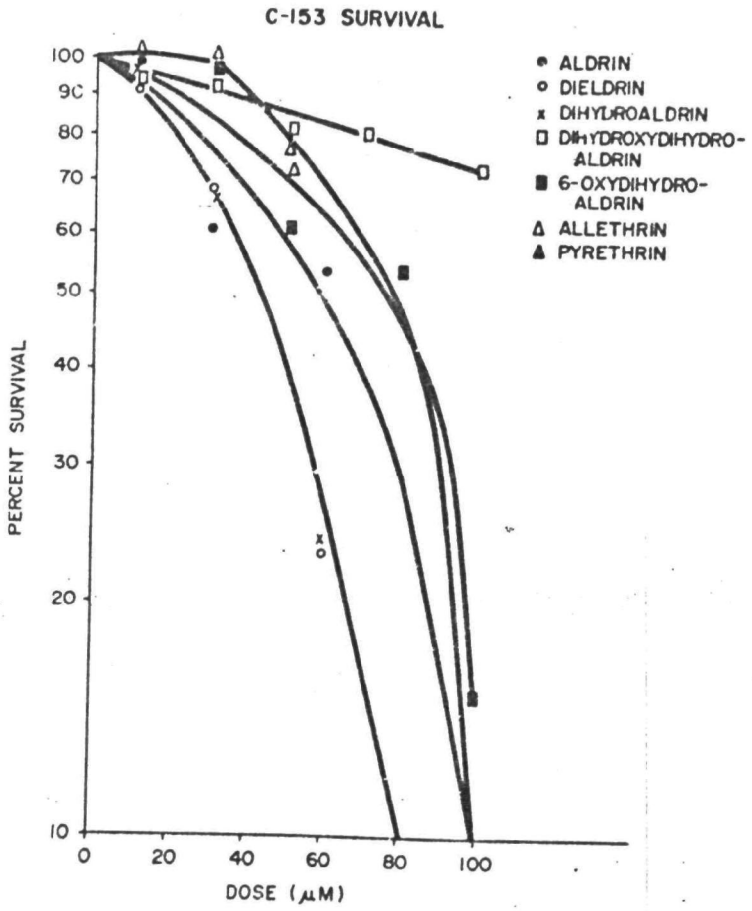


Figure 6. Pesticide induced cytotoxicity in VA-4 cells

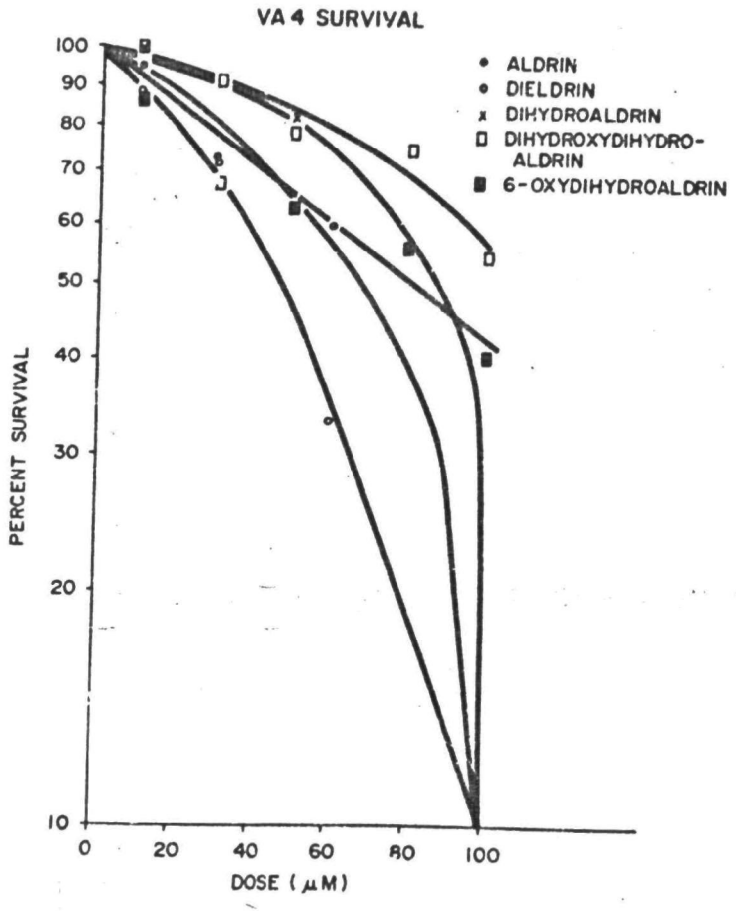


Figure 7. Pesticide induced cytotoxicity in C-153 cells

TABLE 1

Cell Survival of Human Fibroblasts to Pesticide.

Compound	Concentration μM	% Survival VA-4	C-153
Aldrin	30	73.8 ± 6.6	61.4 ± 4.3
	60	60.0 ± 9.8	53.7 ± 3.2
	100	0	0
Dieldrin	30	73.3 ± 5.1	68.3 ± 7.5
	60	33.5 ± 2.0	23.9 ± 2.4
	100	0	0
Dihydroaldrin	30	72.3 ± 6.5	67.7 ± 6.1
	60	33.1 ± 3.3	24.5 ± 2.7
	100	0	0
Dihydro-dihydroaldrin	30	91.1 ± 5.5	91.8 ± 9.2
	60	79.4 ± 6.4	81.2 ± 5.7
	100	76.7 ± 6.1	80.9 ± 7.3
6-Oxydihydro-aldrin	30	68.0 ± 5.4	± 3.1
	80	56.1 ± 5.6	53.7 ± 2.1
	100	40.2 ± 3.2	15.2 ± 0.8
Allethrin	-	-	-
	50	-	77.0 ± 9.2
	100	-	0
Pyrethrin	-	-	-
	50	-	71.5 ± 7.9
	100	-	0

TABLE 2

Unscheduled DNA Synthesis[a] in VA-4 Cells after Treatment with a Series of Chlorinated Hydrocarbons

Treatment	Dose	Exp. #1	Exp. #2	Average
Control (1% DMSO)	-	0.8 ± 0.4[b]	1.5 ± 0.4	1.1
UV- -	10J/M^2	15.4 ± 1.0	20.4 ± 0.7	17.9
Dieldrin	100 µM	11.0 ± 0.8	13.6 ± 1.6	12.3
Aldrin	100 µM	0.7 ± 0.4	1.7 ± 0.5	1.2
Dihydroaldrin	100 µM	0.4 ± 0.6	0.2 ± 0.4	0.3
Dihydroxydihydroaldrin	100 µM	0.9 ± 0.4	1.4 ± 0.3	1.2
Monoacetoxyaldrin	100 µM	11.2 ± 0.8	10.7 ± 1.1	10.9
Diacetoxyaldrin	100 µM	10.7 ± 0.8	12.4 ± 1.0	11.6
Oxyaldrin	100 µM	9.3 ± 0.9	6.3 ± 1.0	7.8
Oxydieldrin	100 µM	7.0 ± 0.8	6.3 ± 0.9	6.7

[a]Figures represent the mean number of grains/nucleus of 50 cells from which background was substracted. Treatment time = 5h.

[b]Standard deviation

TABLE 3

Relationship of $^{1}/_{Mw}$ of VA4 cells treated with 10 μM pesticide for 12 hours.

Pesticide	Photolysis time min	$^{1}/_{Mw}$
Dihydroaldrin[2]	0	0
	6	0
	9	.44
Aldrin	0	0
	6	.018
	9	.104
Dieldrin	0	0
	6	.025
	9	.135
Allethrin	0	0
	6	0
	9	0
Pyrethrin	0	0
	6	.015
	9	.028
Permethrin	0	0
	6	0
	9	0

Allethrin and permethrin did not.

Repair was measured in the CI cell line by UDS and BUdR photolysis. Aldrin and dieldrin both induced repair (Table 4) as measured by UDS and BUdR photolysis. The number of repair sites induced in the CI cell lines by aldrin and dieldrin was greater than in the VA-4 cell line. Other studies using later passage of CI cells, passage 17, showed that aldrin and dieldrin induced a lower level of repair in the presence or absence of S-9 liver microsomes. Diacetoxydihydroaldrin, 6-acetoxy-dihydroaldrin, oxyaldrin and oxydieldrin all induced significant levels of UDS. The dihydroaldrin and 6-hydroxyaldrin compounds induced UDS only in the presence of the S-9 liver microsomal fraction. The monofluoro (Figure 1,9) and 6-oxo analog of aldrin did not induce UDS while they retain pesticidal activity.

Effect Upon DNA Replication

Aldrin and dieldrin were tested for their ability to inhibit scheduled DNA synthesis and their ability to replicate daughter DNA. For this study we used the Syrian hamster cell system described previously (19) for polyaromatic hydrocarbons. As shown in Table 5, aldrin and dieldrin inhibited scheduled DNA synthesis in a dose related manner. Also, both aldrin and dieldrin inhibited the ability of cells to synthesize daughter DNA (Table 5). These data indicate that aldrin and dieldrin act as blocks for normal DNA replication.

Association to Cellular DNA

The extent of ^{14}C-pesticide association with the DNA of VA-4 and 153 cells was determined 12 hours after addition of the compound. Even though the cells and extracted DNA were extensively washed, we prefer not to use the term "binding" in the interpretation of these results. Association to the DNA is used here as a broader term to include interaction with DNA as well intercalation with DNA and covalent binding to DNA. As shown in Table 6 radioactive dieldrin associated with the DNA of both VA-4 and C-153 cells. On the other hand little of the radioactive aldrin, wihtout metabolic activation, associated with DNA. These data correspond with the above UDS studies showing that dieldrin induced UDS while aldrin did not. Two analogs, dihydroaldrin and dihydroxydihydroaldrin, were ^{14}C-synthesized and tested for their association with cellular DNA. Little association of these compounds to DNA was observed.

When cells were incubated with compounds in the presence of the S-9 liver microsomal fraction, the level of aldrin and dihydroaldrin associated with the DNA increased. The small amount of dihydroxydihydroaldrin associating with DNA without activation, decreased to background with the S-9 fractions.

It is interesting to note that the compounds tested for association to cellular DNA: a) dieldrin, aldrin, dihydroaldrin are cytotoxic to the cells: and b) only dieldrin associated to a great extent with DNA. These results suggest that although UDS (an indirect measurement of DNA damage) parallels the radioactive association of the chlorinated hydrocarbon, the cytotoxicity

TABLE 4

Repair of Pesticide induced DNA damage in the C-1 human cell line.

Agent[1]	Conc	UDS[2]	Photolysis[3]
Dieldrin	100 µM	5.8	0.15
Aldrin	100 µM	7.1	0.06
UV	10J/m^2	12.2	0.2.

[1]Chemical dissolved in DMSO.

[2]Average number of grains per nuclei.
Cells were incubated with pesticide 6 hr.

[3]1 Sv per 10^8 daltons. Cells were incubated for 14 hr.
with pesticide and BUdR.

TABLE 5

Effect of dieldrin and aldrin in scheduled DNA synthesis and daughter DNA.

Compound[1]	Conc ..M	% Inhibition of scheduled DNA synthesis	Ratio of neutral daughter DNA co-sedimenting with control DNA
Aldrin	10	37.5	0.91
	100	70.0	0.77
Dieldrin	10	34.0	0.84
	100	50.0	0.77

[1]Compounds were dissolved in DMSO.

does not appear to be directly related to either UDS or to binding.

Association to Cell-free DNA

To determine whether the association observed with cellular DNA was due to covalent binding or to some other factor, we performed a series of experiments on purified calf thymus DNA. In these experiments single-stranded and double-stranded DNA were incubated with radiolabeled dieldrin and aldrin. The amount of radiolabeled compound was assayed following a 12 hour period or allowed to dialyze against buffer for an extended period of time. As shown in Table 7, dieldrin, aldrin, dihydroaldrin and dihydroxydihydroaldrin all associated with DNA. However, following dialysis we observed that only dieldrin remained associated, while approximately 3, 73 and 63% of the aldrin, dihydroaldrin and the dihydroxydihydroaldrin, respectively, were lost from single-stranded DNA. These data would suggest that the association observed with aldrin, dihydroaldrin and dihydroxydihydroaldrin is probably due to hydrophobic and/or hydrogen bond interactions and not covalent binding to single-stranded DNA. Radioactive dieldrin appears to associate with double-stranded DNA approximately 2.5 times greater than with single-stranded DNA. However, the greater percentage of this dieldrin is lost from the DNA upon dialysis. Similar results were obtained with the other three radioactive compounds when tested for their association with double-stranded DNA. This loss of radiolabeled compound upon dialysis probably indicates that a large amount is strongly associated with, but not covalently bound to the double-stranded DNA.

Toxicity of Synthetic Pyrethroids

The toxicity of biphenylene esters of cis and trans chrysanthemic acid was measured by topical application and immersion (Tables 13, 14 and 15). It is apparent that these pyrethroids are metabolized oxidatively and that housefly MFO is more sensitive than mosquito MFO to PBO, based on the higher synergistic ratios with houseflies. None of the synthetic pyrethroids were within an order of magnitude of the toxicity of the natural pyrethrins (Table 13). However, contrary to results with most synthetic pyrethroids, the most toxic isomers of certain compounds were the cis isomers (compounds 28, 35, Table 14).

Insecticidal Evaluation

Topical application is an effective, standard assay for evaluating toxicity and studying structure activity relations. However, chemicals that penetrate the cuticles slowly may exhibit low potency in the laboratory tests even though they have high intrinsic insecticidal activity. As a result, oral measures of toxicity are often used in conjunction with topical application. However, a limitation of oral toxicity measurement can be the instability of many compounds in the gut. If this is the case, then little or no oral toxicity will be measured with most test agents. The oral exposure to many of the pyrethroid and organochlorines proved to be of limited value as a screening technique.

TABLE 6

^{14}C-Chlorinated hydrocarbon association with human cell DNA.

Compound[1]	VA-4[2]		C-153[2]	
	+S9[3]	-S9	+S9[3]	-S9
Dieldrin	-	96.2	-	32.5
Aldrin	23.0	3.1	27.1	8.5
Dihydroaldrin	34.0	1.5	24.0	1.3
Dihydroxydihydroaldrin[4]	3.9	12.6	1.2	8.3

[1]Compound were dissolved in DMSO, final concentration was 10 μM.

[2]Cells were incubated for 12 hours with compound.

[3]S-9 rat liver microsomes.

[4]Compound was approximately 90% pure.

TABLE 7

Effect of Dialysis on ^{14}C-Pesticide Association to Calf Thymus DNA

Treatment	Single Strand			Double Strand		
	no dialysis	dialysis	% lost[a]	no dialysis	dialysis	% lost[a]
^{14}C-Dieldrin	19.01[b]	16.72	12	50.01	26.00	48
^{14}C-Aldrin	10.68	7.15	33	9.46	3.32	65
^{14}C-Dihydroaldrin	12.65	3.41	73	11.08	1.88	83
^{14}C-Dihydroxydihydroaldrin	10.79	3.45	68	6.41	1.34	79

[a] % lost = (no dialysis - dialysis)/no dialysis

[b] moles bound per mole nucleotide

Toxicity of Standards and Precursors

The use of aldrin, dieldrin and certain other insecticidally active compounds confirmed the utility of the study and established them as sufficiently sensitive assays of toxicity (6,7). None of the precursors were very toxic to houseflies as external doses. Hexachlorocyclopentadiene, 1,2,3,4,7,7-nexachlorobicyclo (2,2,1) 2,5 heptadiene and exo-2,3-epoxy norbornane were highly toxic orally (houseflies) and by immersion (mosquito), calling attention to the need for purification of synthetic compounds (Table 8) to eliminate any starting materials.

Insecticidal Activity of Diels-Alder Adducts of Fulvenes and Halogenated Dienes

These novel cyclodiene adducts demonstrated weak topical insecticidal action but were approximately three orders of magnitude weaker as topical pesticides than were heptachlor, chlordane, or aldrin. In the oral toxicity studies, a further reduced activity was observed as compared to various standard controls (Table 9).

Compounds 19-26 represent systematic studies of substitutions at two sites of the molecule: a) symmetrical attachment of chlorine (19, 22, 25) methoxy (20, 23, 26), or hydrogen (21 and 24) on the bridge carbon (C_8) of the norbornene ring and b) dimethyl (22, 23, 24) diphenyl (19, 20, 21), or methyl and phenyl (25 and 26) additions to the exocyclic vinyl group.

Chlorination of the bridge carbon (C_8) is essential in the highly chlorinated insecticides aldrin and chlordane but in compounds 19-26 the bridge chlorines are replaced with alkoxyl groups or hydrogen. LD_{50} values were unobtainable at the highest dose of most of these compounds, thereby precluding complete structure activity analysis, but the dimethoxyl drivative 24 was more toxic than the dichloro analogue 25 in contrast to aldrin and chlordane.

The loss of toxicity, compared to aldrin and chlordane, may be due to changes in molecular size and shape, electronegativity, penetration, or metabolism.

Toxicity of Chlorinated Cyclodiene Analogs

These data (Tables 10 and 11) demonstrate the equivalence of relative toxicity of aldrin, dieldrin, oxyaldrin and oxydieldrin among two assays (topical or immersion), with two different species, with and without a MFO inhibitor. The intrinsic toxicity of 6,7 dihydroaldrin is equal to aldrin and dieldrin if MFO metabolism is prevented. The major metabolite, 6,7 dihydroxyaldrin is much less toxic than the parent compound.

In Vitro Metabolism of Dihydroaldrin and Aldrin

Using S-10 fraction of rat livers and appropriate co-factors, the conversion of aldrin to dieldrin was demonstrated as was the conversion of

6,7-dihydroaldrin to 6-hydroxydihydroaldrin (Table 12; Figure 8). Or intrigaing phenomenon, the appearance of aldrin and dieldrin in incubations dihydroaldrin with rat liver microsomes, was confirmed in two separat experiments.

TABLE 8

Toxicity of Standards and Precursors to Adult Mixed-Sex *Musca domestica* (house fly) and *Aedes aegypti* (mosquito larvae).

Compound Tested	Topical Application[a] LD_{50} µg/fly	Oral LC_{50}[b] ppm	Immersion Toxicity[c] LC_{50}, ppb
chlordane	0.05 µg/fly	0.5 ppm	0.016
dieldrin	0.05	0.5 ppm	0.016
DDT	----	0.8 ppm	0.013
bicyclo (2.2.1) 2 heptene (norbornene)	50 µg/fly	-------	-----
5-norbornene-2-ol	>40 µg/fly	-------	-----
1, 6-dideoxyheptenditene	40 µg/fly	>10 ppm	>10 ppm
(d, l) 1,2,3,4-tetrachlorobutene	>40 µg/fly	10 ppm	>10 ppm
bicyclo (2.2.1) 2, 5-heptadiene	>40 µg/fly	10 ppm	>10 ppm
hexachlorocyclopentadiene	12 µg/fly	0.5 ppm	>0.5 ppm
1,2,3,5,7 7-hexachloro bicyclo (2.2.1) 2,5 heptadiene	>40 µg/fly	>3 ppm	>3 ppm
$C_{10}H_{12}O_2$	20 µg/fly	>10 ppm	>10 ppm
furan	>10 µg/fly	>5 ppm	>5 ppm
endrin	---------	0.4 ppm	---------
exo-2, 3-epoxynorbornene	>40 µg/fly	0.4 ppm	0.4 ppm
1, 2-epoxy-3, 3, 3-trichloropropene	>40 µg/fly	10 ppm	>10 ppm
heptachlor	0.05	-------	---------
heptachlor epoxide	0.01	-------	---------

a. Data represent responses of treated flies after 48 hours.

b. Data represent 48-hour responses of groups of 30 flies fed test compounds in sucrose.

c. Data represent 24-hour responses of fourth instar mosquito larvae.

TABLE 9

Insecticidal Activity of Cyclodiene Adducts in Houseflies (Musca domestica).

Compound	Topical Application: LD_{50}, µg/fly	Oral: LC_{50}, ppm
19	>20[a]	>12 000[b]
20	>20[a]	>12 000[b]
21	>20[a]	>12 000[b]
22	16	10 000
23	>20[a]	10 000
24	>20[a]	10 000
25	>20[a]	10 000
26	12	12 000
β-chlordane[c]	0.05	3.0
aldrin[c]	0.02	0.5
heptachlor[c]	0.04	

a. 0% mortality at 20 µg/fly. b. 0% mortality at 12 000 ppm.
c. Source: USLPA standards.

TABLE 10

Toxicity of Organochlorines to House Flies.

Chemical	LD_{50}, µg/fly Topical		Ratio*	Oral, LC_{50}, ppm
	alone	+5 µg PBO		
Aldrin	0.02	0.02	1	0.5
Dieldrin	0.0?	--	-	0.5
Oxyaldrin	0.02	0.02	-	0.4
Oxydieldrin	0.015	0.015	1	
6,7 Dihydroaldrin	0.2	0.02	10	20
6,7 Dihydrooxyaldrin	20 µg = 10% mortality			
Heptachlor	0.04			
Heptachlorepoxide	0.01			

* Ratio = $\frac{LD_{50} \text{ alone}}{LD_{50} + PBO}$

TABLE 11

Immersion toxicity of organochlorines to Aedes aegypti larvae.

Chemical	LC_{50}, mg/L		Notes
	alone	+5 mg/L PBO	
Aldrin	0.016	0.015	No synergism
Dieldrin	0.035	---	--------
Oxyaldrin	0.048	0.048	No synergism
Dihydroxydihydroaldrin	2.0mg/L = 0% mortality		--------
Oxydieldrin	0.18	0.18	No synergism

TABLE 12

Quantitative Analysis: Incubation of Dihydroaldrin
With S-10 Fraction of Rat Livers.

Incubation Time	% of Initial Dihydroaldrin[a,b]				
Hours	Dihydroaldrin	6-hydroxy dihydroaldrin	Aldrin	Dieldrin	Total % Recovery[c]
0.25	40.8	5.0	0.2	0.04	46
0.5	14.3	7.7	0.1	0.1	22
1.0	26.4	16.3	0.1	0.1	37
2.0	9.2	7.1	0.1	0.2	17

[a]Corrected for recovery efficiencies after florisil column clean up. Recovery efficiencies were dihydroaldrin (94%), aldrin (51%), dieldrin (44%) and 6-hydroxydihydroaldrin (24%).

[b]Uncorrected for extraction efficiencies from incubation mixture.

[c]Qualitative analysis based on GLC retention times.

TABLE 13

Immersion Toxicity of Pyrethroids with 4th Instar Mosquito Larvae, Aedes aegypti.

Chemical	Mortality at Concentration, mg/L	
	Alone	+ PBO (5 mg/L)
	10mg/L = 0	10mg/L = 0
36	10mg/L = 0	10mg/L = 0%
34	10mg/L = 0	10mg/L = 70%
33	10mg/L = 0	10mg/L = 0%
27	10mg/L = 0	10mg/L = 0%

	LC_{50} Values in mg/L		Ratio*
Pyrethrins	0.02	0.002	10
Allethrin	0.15	0.025	6
Permethrin	0.0003	0.0004	0.75

* Ratio = $\frac{LC_{50} \text{ alone}}{LC_{50} + PBO}$

TABLE 14

Topical Application of Synthesized Pyrethroids with House Flies: Mortalities at Discrete Doses.

Chemicals	% Mortality at Given Dose	
	Alone, 40 µg	+ PBO (5 µg)
40 (trans)	80%	40 µg = 100% 10 µg = 40%
42	0%	40 µg = 70 10 µg = 0
41 (cis)	20%	40 µg = 90% 10 µg = 10
36 (trans)	0%	40 µg = 70% 10 µg = 30%
34 (trans)	30%	40 µg = 100% 10 µg = 40
33 (trans)	0%	40 µg = 40 10 µg = 0
27 (trans)	10%	LD_{50} = 1.5 µg (Ratio >25)[a]
32 (cis)	20 µg = 0 M	2 µg = 35% M
31 (trans)	20 µg = 0 M	2 µg = 40% M
39 (cis)	20 µg = 0 M	2 µg = 30% M
30 (cis)	20 µg = 25 M	2 µg = 90% M
29 (trans)	10 µg = 0 M	2 µg = 50% M

a. Ratio = $\frac{LD_{50}\ \text{alone}}{LD_{50} + PBO}$

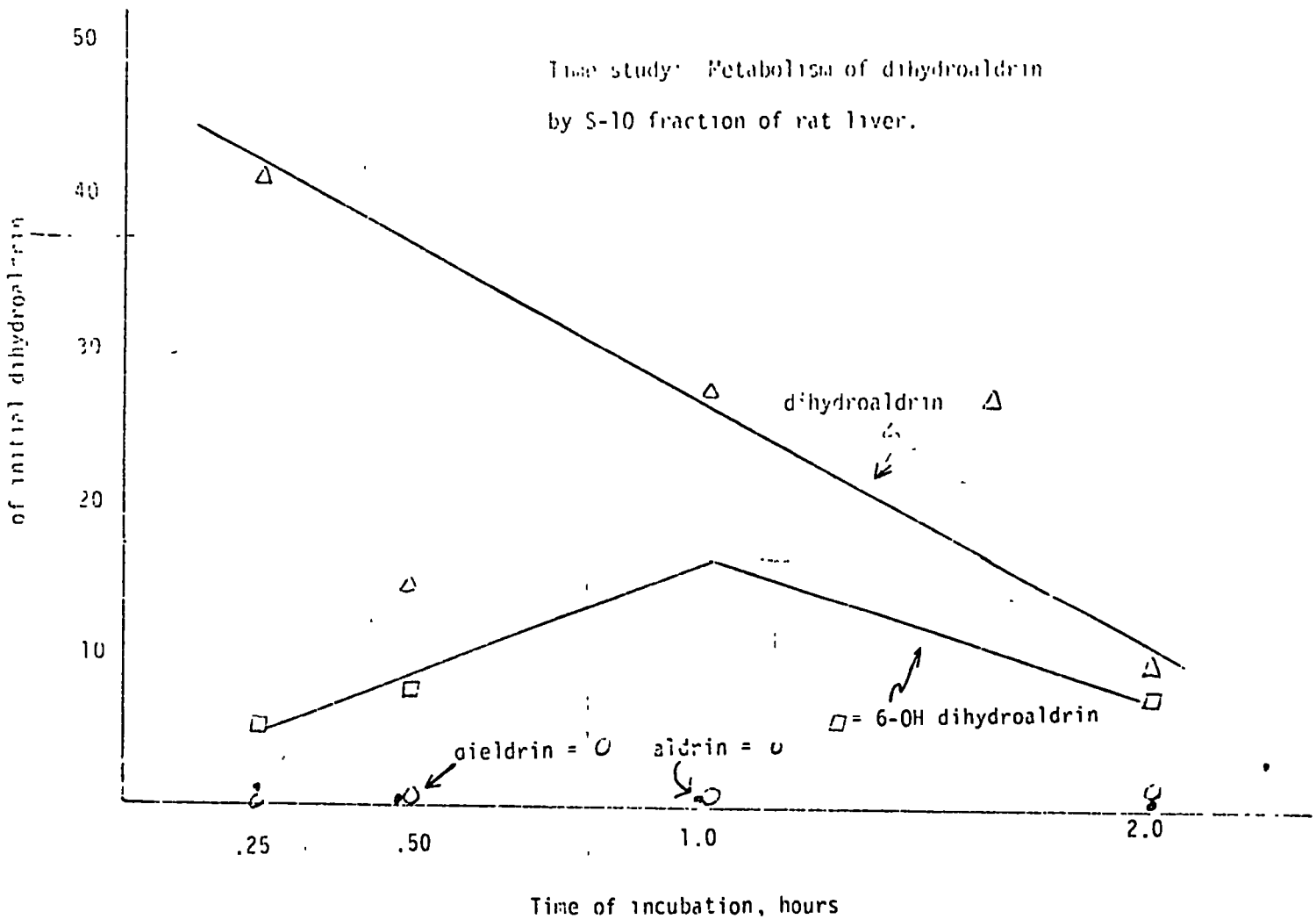
TABLE 15

Topical Application of Biphenylene Pyrethroids to House Flies: Active Compounds and Standards.

Chemical	LD_{50}, µg/fly Alone	LD_{50}, µg/fly + PBO (5 µg)	Ratio*
28	20	0.2	100
35	20 µg = 20 M	0.8	>25
27	40 µg = 40 M	1.5	>25
34	40 µg = 30 M	10 µg = 40	
Pyrethrins (Standard)	0.22	0.006	37

* Synergistic Ratio = $\frac{LD_{50} \text{ alone}}{LD_{50} + PBO}$

FIGURE 8


Time study: Metabolism of dihydroaldrin
by S-10 fraction of rat liver.
50
40
30
20
10
of initial dihydroaldrin
dihydroaldrin
= 6-OH dihydroaldrin
dieldrin = O
aldrin = O
.25
.50
1.0
2.0
Time of incubation, hours

SECTION IV

OVERVIEW

The results obtained from this study support the hypothesis that a limited number of rationally designed and selected analogs can be of value in determining specific chemical toxicities based on functional groups. The choices of aldrin and dieldrin provided us with a chemical model which was readily manipulated for appropriate cellular biological and insecticidal studies. It is important to emphasize that the project was funded at approximately 50% of year 01 requests and that the budget remained constant despite large increases (over 300% in some cases) for supplies, chemicals, medium and personnel. There still remains additional work for: a) detailed cellular studies of the model compounds of greatest interest and b) model ecosystem evaluations for this particular class of agents. It is clear however that this interdisciplinary approach is readily adaptable to other chemical classes of environmental agents.

REFERENCES

1. N.J. Lewis, D.R. Knight and W.J. Collins, "Diels-Alder adducts of fulvenes and halogenated dienes: Synthesis and insecticidal activity" J. Med Chem, 22, 1505-1509 (1979).

2. N.J. Lewis and D.R. Knight, "Diels-Alder adducts of fulvenes and halogenated cyclopentadienes: Application of proton and ^{13}C-NMR to structure determination" Abstracts of the 14th Midwest meeting of the Amer. Chem. Society, Fayetteville, Arkansas (1978).

3. M. Kleiman, U.S. Patent no. 2655513 (1953).

4. N.J. Lewis, N. Ekwuribe, R.W. Hart and W.J. Collins, "Synthesis biological activity of novel pyrethroid-type analogs" Abstracts of the Amer. Chem. Society Congress, Honolulu, Hawaii (1979).

5. N.J. Lewis and F.D. Cazer, "Facile micromolar reduction of (1,2,3,4,10-) [^{14}C] Aldrin to 6,7-dihydro (1,2,3,4,10-) [^{14}C] Aldrin" J. Label. Compounds Radiopharmaceuticals, 18, 471-473 (1980).

6. J.R. Busvine, "A critical review of techniques for testing insecticides" Commonwealth Agricultural Bureau, 2nd edition, Slough, Eng. (1971).

7. H.H. Shepard, "Methods of testing chemicals on insects" Vol. I, Burgess Pub. Co., Minn. (1958).

8. J.E. Casida, "Mixed function oxidase involvement in the biochemistry of insecide synergists" J Agr Food Chem 18, 753-772 (1970).

9. F.E. Ahmed, R.W. Hart and N.J. Lewis, "Pesticide induced DNA damage and its repair in cultured human cells" Mutat. Res. 42, 161-174 (1977).

10. K.Y. Hall, F.B. Daniel, N.J. Lewis, S.M. D'Ambrosio and R.W. Hart, "Halogenated hydrocarbons induce DNA damage, repair and mutagenesis" Abstracts of the 145th Amer. Assoc. Adv. Sci. meeting p. 120 (1979).

11. S.M. D'Ambrosio, K.Y. Hall, R.W. Hart and N.J. Lewis, "Pesticide induced DNA damage" Fed. Proc. 38, 539 (1979).

12. N.J. Lewis, F.D. Cazer and N. Ekwuribe, "Facile micromolar synthesis of ^{14}C-pesticide derivatives and metabolites" Abstracts of the 7th regional meeting of the Amer. Chem. Soc., Col., Ohio (1979).

13. Girardi, F.C. Jensen and H. Koprowski, "SV-40 induced transformation of human diploid cells: Crisis and recovery" J. Cell Comp. Physiol. 65, 69-84 (1965).

14. F.E. Ahmed, N.J. Lewis and R.W. Hart, "Pesticide induced ouabain-resistant mutants in chinese hamster V79 cells" Chem. Biol. Interact., 19, 364-374 (1977).

15. S.M. D'Ambrosio, F.B. Daniel and R.W. Hart, "Cellular repair of DNA damage induced by 7,12-dimethylbenz(a)anthracene and its fluoro analogs in vitro" In: Polynuclear Aromatic Hydrocarbons, edited by P.W. Jones and P. Leber, pp. 793-803 (1979).

16. J.D. Regan, R.B. Setlow and R.D. Ley, "Normal and defective repair of damaged DNA in human cells: A sensitive assay utilizing the photolysis of bromodeoxyuridine" Proc. Natl. Acad. Sci. USA, 68, 708-712 (1971).

17. F.B. Daniel, L. Wong, C. Orivec, F.D. Cazer, A. Wang, S.M. D'Ambrosio, R.W. Hart and D.T. Witiak, "Biochemical studies on the metabolism and DNA-binding of DMBA and some of its monofluoro derivatives of varying carcinogenicity" In: Polynuclear Aromatic Hydrocarbons, edited by P.W. Jones and P. Leber, pp. 804-815 (1979).

18. W.L. Carrier and R.B. Setlow, "Paper strip method for assaying gradient fractions containing radioactive macromolecules" Anal. Biochem., 43, 427-432 (1971).

19. S.M. D'Ambrosio, F.B. Daniel, R.W. Hart, F.D. Cazer and D.T. Witiak, "DNA repair in syrian hamster embryo cells treated with 7,12-dimethylbenz(a) anthracene and its weakly carcinogenic 5-fluoro analog" Cancer Lett., 6, 255-261 (1979).