



STUDY GROUP ON HEALTH HAZARDS FROM
 NEW ENVIRONMENTAL POLLUTANTS

Geneva, 30 September - 5 October 1974

INDEXED



RECENT ADVANCES IN
 CHEMICAL PEST CONTROL

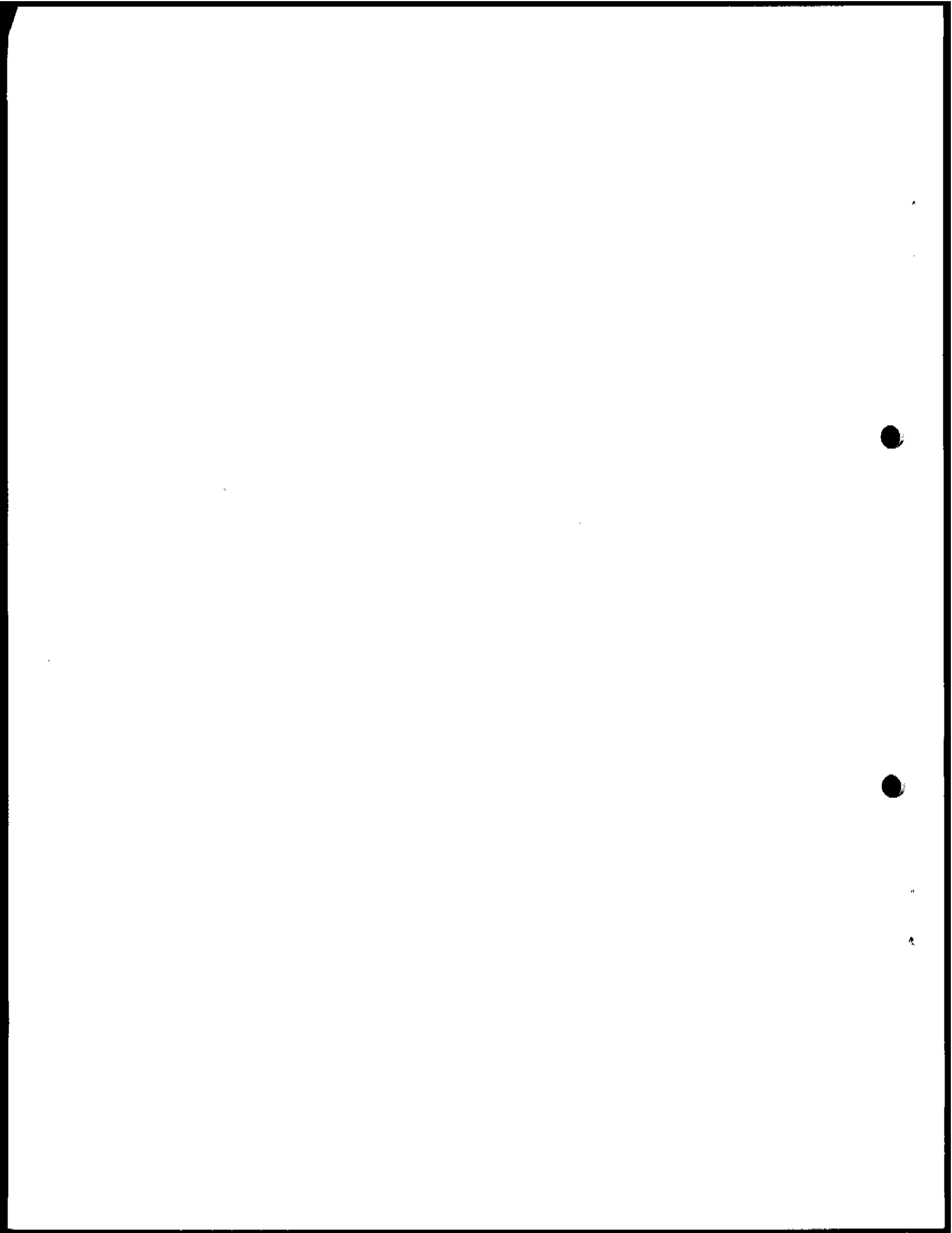
(Provisional agenda item 5.3)

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1. Introduction

The desirability for new methods of insect control has become increasingly evident with the realization of the consequences of misuse or over-use of a number of the more important pesticides e.g. chlorinated hydrocarbons and cyclodienes that have been used in enormous quantities (primarily DDT and related congeners). Concomittant with the awareness of the undesirable effects on the environment and of toxicity to mammals and of equal importance is the realization of the rapid acquisition of resistance by insects.

2. Conventional pest control agents

Although new and innovative methods are being sought for pest control, chemical pesticides at the present time are the basis of controlling public health disease vectors and will continue to be for the foreseeable future.¹ New pesticides are required and constantly sought for expanding the scope of vector control for those diseases not now adequately controlled and for possible use with vectors which have developed or may develop resistance to currently used materials. Development of new materials involves searching for chemicals with high specific activity on the target organisms and developing the correct formulation and methods of application.¹

Competition from the 500 or more first and second generation pesticides and the general concern with the quality of the environment, which is reflected in the more stringent requirements being adopted by many regulatory authorities and agencies, have had a significant impact on research and development within the agro-chemical industry.²

Recent legislation affecting the use of the persistent organo-chlorine insecticides is likely to stimulate efforts to find environmentally acceptable replacements more particularly among the organophosphate and carbamate group of compounds.

It is also probable, according to Daniel² that the industry will adopt the concept of "integrated control" which involves the optimization of all known methods of pesticide control and may significantly alter use patterns.³ The more likely results of this strategy could include²: (1) the use of more specific, less persistent pesticides; (2) selective use of organo-chlorine insecticides; (3) development and use of crop varieties resistant to insect attack. (The effectiveness of this approach must be viewed with some caution however since insects are highly adaptable and may adjust to resistant strains.) (4) changes in cultivation practices; (5) increased use of biological insect control including microbial and viral insect pathogens; (6) greater use of sterile insects; (7) increasing use of insect attractants, and insect growth regulators of both synthetic and natural origin such as juvenile hormones and ecdysones; (8) improvements in formulations or methods of applications; (a 50% reduction in the use of pesticides has, for example, been achieved in some instances, following the intervention of ultra-low volume (ULV) method of application), and (9) the use of new pyrethroids with improved stability. In addition it is believed that microencapsulation will also make a significant contribution to integrated control. Other practices that may be implemented include²: the storage of grain in an atmosphere of carbon dioxide; the use of pesticide-impregnated strips in storage buildings; improved methods for the detection of pests. (This could result in more effective control and require the use of relatively small amounts of pesticide.)

It is useful to recall the previous use patterns of pesticides. For example in 1969, the Mrak Commission was told that synthetic organic pesticide production in the USA was increasing at the rate of about 15%/year.¹ In 1971 FAO² suggested that the annual rate of growth was in the region of 10% with the increase applying more to herbicides and fungicides than to insecticides. It is estimated that 50-75% of pesticides were produced

in the USA and for 1967, FAO estimated a global use of 1,300,000 metric tons, although this did not include the amounts used in some countries including the United Kingdom, France and China. In that year, primary chemical manufacturers in the USA produced 897,000 tons. The United States' President's Scientific Advisory Committee estimated that 120,000 tons were used in developing countries (excluding Latin America) and predicted that this would have to be raised by 7,000,000 tons if food production was to be doubled. Taking an estimate of total usage of 1,500,000 tons of pesticides in 1967 and the FAO estimated annual rate of increase (10%), the amount used in 1972 would be in the region of 2,400,000 tons and by 1975 will be more than double the 1967 figure.⁴

The production figure shown for pesticides by primary chemical manufacturers in the USA in 1967 included 13.4% fungicides, 32.1% herbicides and 54.5% insecticides (it did not include rodenticides and fumigants). Based on this production ratio and allowing for 5% annual increase for insecticides since the rate of growth is lower than for the other two categories, the amount of global insecticide production in 1972 was estimated to be in the region of 1 million tons.⁴

The contribution of DDT to the total production of insecticides has declined considerably. In 1967, US production alone (which is believed to be about half the world production, was 47,000 metric tons, a reduction of 27% below the previous year and 40% below the peak production year 1960-1963. The decline has continued, however at a slower pace due to the continued predominant requirement for DDT in public health which still has to be used for malaria eradication. Although more than 1000 million people now live in areas that have been freed from the endemic form of the disease, it is generally acknowledged that to maintain this achievement as well as to permit the extension of protection to the many millions of persons still exposed to infection will require the continued availability of DDT.⁵ The withdrawal of DDT from public use at this time could give rise to immense problems and expose large populations to outbreaks of endemic and epidemic malaria.

The other major use of DDT is for is for cotton growing and this too has slowed the discontinuance of DDT.

Examples of newer chemicals for use in some vector control include the organo-phosphorus compounds and carbamates for residual application for malaria control, organophosphorus compounds for larvicide application on mosquitoes and blackflies, organo-phosphorus compounds and synthetic pyrethroids for aerosol dispersal for rapid knockdown of insects, for epidemics of the arbovirus caused diseases (mosquito vectors) and for control of tsetse flies.

The WHO programme for evaluating and testing new insecticides has included a considerable number of compounds for use in public health practice. The testing starts with the laboratory investigation at Stage I and progresses through to the field epidemiological trials at Stage VII.⁶⁻⁸

Concomittant with the development of new insecticides is the ancillary requirements of evaluating and/or predicting their environmental biodegradability and toxicity. Booth⁹ recently described the usefulness of model ecosystems in insecticide development. The system is composed of a 10 gallon aquarium with sand, water and air and aquatic organisms. Sorghum is planted and grown on the terrestrial phase. The plants were treated with 5 mg of radiolabelled insecticide and salt marsh caterpillars were used to forage on the plants. At the end of 30-40 days, all of the organisms and water were examined for metabolite distribution. Concentration factors, i.e. the ppm in the tissue divided by the ppm in water, were recorded to determine if the compounds accumulated in the organisms. The present data compared the fate of DDT, DDT analogs, orthene, and carbofuran in the model

ecosystem. DDT accumulated in very large amounts in every organism with DDE contributing to a major portion of the total ^{14}C . Orthene could not be detected in any of the organisms, but an average of 41% was found in the water. Large amounts of polar metabolites were obtained from the origin. The DDT analogs ethoxychlor, methiochlor, and methylchlor were rapidly metabolized to polar compounds, showing concentration factors for fish of 1500, 0, and 840 respectively. Carbofuran was not detectable in any of the organisms that stayed alive but was found in crabs which died soon after application of the compound to the system (41-80% of the total ^{14}C). Carbofuran was also quite toxic to clams, daphnia and snails. All of the compounds were biodegradable when compared with DDT; this included orthene, carbofuran and the DDT analogs. The model ecosystem appears to be a useful tool in developing safe and biodegradable insecticides for the future.

Casida et al.¹⁰ studied the chemical composition of toxaphene with a view toward elucidating the possible biodegradable sites of the various toxaphene components. One billion pounds of toxaphene have been used for pest insect control without detailed knowledge of its chemical composition or metabolic fate. Examination of toxaphene by silica gel adsorption column chromatography and glc-ms revealed a complex mixture of at least 175 C_{10} -polychloro compounds made up of $\text{C}_{10}\text{H}_8\text{Cl}_{10}$, $\text{C}_{10}\text{H}_{18-n}\text{Cl}_n$ and $\text{C}_{10}\text{H}_{16-n}\text{Cl}_n$ derivatives where the chlorine number (n) is 6, 7, 8 or 9. It is likely that the $\text{C}_{10}\text{H}_{18-n}\text{Cl}_n$ compounds are polychlorobornanes and the $\text{C}_{10}\text{H}_{16-n}\text{Cl}_n$ derivatives are polychlorobornenes and/or polychlorotricyclenes. Individual components can be isolated by chromatography involving, in sequence, a partition column, an adsorption column, a second partition column, a second adsorption column then preparative GLC and crystallization. One toxic component is identified by X-ray crystallography as 2,5-endo, 2,6-exo, 8, 9, 10-heptachlorobornane. Another more toxic component is an octachloro compound. These two toxic components appear to contribute a major part of the acute toxicity of toxaphene to mice treated intraperitoneally. Studies with ^{36}Cl - and ^{14}C -toxaphene establish that in rats about half of the carbon-chlorine bonds are metabolically labile in the technical mixture and in each of seven subfractions of varying composition and toxicity. Two sets of structural features are of interest relative to the individual toxaphene components. An appropriate steric relationship between certain chlorines present in only a few constituents may determine the neurotoxic potency. Biodegradable sites such as chloromethyl and other groupings are probably present in most if not all of the toxaphene components. Techniques are now available to define more completely the structure, metabolic fate and environmental persistence of the toxaphene components.

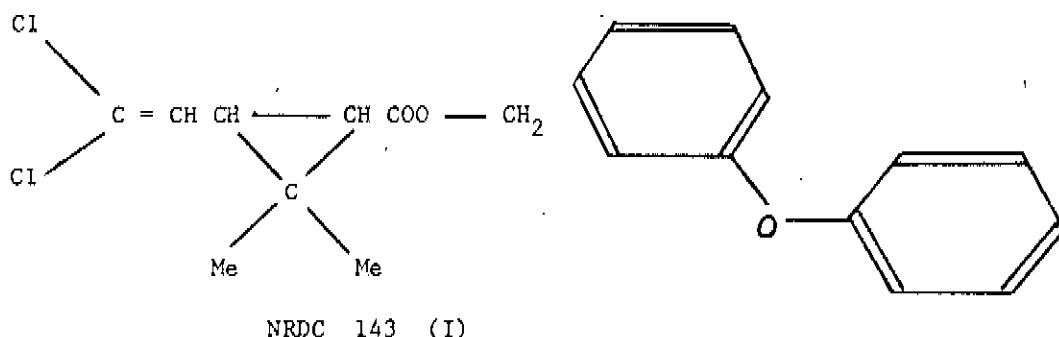
Mention was made earlier in this section of the decline in production of DDT accompanying a gradual replacement by organophosphorus and carbamate insecticides. One of the largest use categories of DDT is in the area of cotton farming. Its replacement in this use application has been primarily with methyl parathion, toxaphene and carbaryl (Sevin). An additional pesticide for cotton to replace DDT is a newly developed product by Ciba-Geigy called chlordimeform $\text{N}'-(4\text{-chloro-}o\text{-tolyl})\text{-N,N-dimethyl formamidin}$ which has been available in Europe and will be introduced into the US shortly. Studies with carbamate insecticides indicate the possibility of effecting a reduction in mammalian toxicity, while retaining high insecticidal activity. This has been achieved by N-acylation of the monomethyl carbamoyl moiety. The success of this approach clearly depends upon an understanding of the comparative biochemistry of mammals and insects.

What additionally can we expect in the way of new pesticides in the near future? In this regard it is informative to assess the relative pesticide research and development costs¹¹. In the United States, as of 1972, it was estimated that one new pesticide emerges for every 10,000 compounds tested; that the time from discovery to market is 8-10 years, and that the cost is in excess of 10 million dollars. This figure would undoubtedly be significantly higher as of 1974. It is also reasonable to assume that additional safety requirements for pesticides will become mandatory in many countries. These may include: 2 year (or longer) carcinogenesis studies on laboratory animals; mutagenesis and teratogenesis studies; toxicology on primates and humans and perhaps human

human metabolic studies as well. These additional tests might run the cost of the safety component package to over a million dollars per compound. The expected result may be a severely decreased number of candidate pesticides that would be available for national and international assessment.

Recently pyrethroids of very low mammalian toxicity have been developed including resmethrin (OMS-1206) β -5-benzyl- β -(furyl)methyl⁽⁺⁾ cis-trans-2,2-dimethyl- β -(2-methyl-propenyl)-cyclopropane carboxylate and bioresmethrin, which is its (+)-trans-isomer. These compounds have been suggested to provide an adequate margin of safety when used in the prescribed manner for applications such as the disinfection of aircraft. They are rapidly metabolized and detoxified so that no cumulative effects are believed to occur or be encountered.

A new synthetic pyrethroid NRDC-143⁽⁺⁾ has been recently synthesized at Rothamsted which may widen the range of application of these insecticides¹². It is 10-100 times more stable in light than previous pyrethroids, it is 5-30 times more active than dieldrin and 14-100 times more active than DDT, depending on the insect species. It also seems likely that it will be cheaper to manufacture since β phenoxybenzyl⁽⁺⁾-cis, trans-2,2-dimethyl- β -(2,2-dichlorovinyl) cyclopropane carboxylate is simpler to synthesize



than most of the other highly potent synthetic pyrethroids. It will be some four or five years before the new photostable pyrethroids are on the market. Although they seem to be only moderately persistent in the environment much testing needs to be done to determine their behaviour under practical pest control conditions.

Closely allied with the use of pyrethrins and pyrethroids is the use of other insecticide synergists. The chemical structure of a synergist for any given insecticide depends on the nature of the enzyme(s) responsible for its detoxification. Because of the overall importance of microsomal oxidation, attention has been focussed on materials that interfere with this system.

The best known and most important group of compounds with this property are derivatives of methylenedioxybenzene (1,3-benzodioxole). Of the many materials of this type evaluated as synergists only four, piperonyl butoxide, "sulfoxide", propyl-isome and Tropitol (figure 1) are of commercial importance. Piperonyl butoxide is the most important of these with an annual US production of about 800 pounds.

In recent years other groups of compounds have been shown to possess similar activity to the 1,3-benzodioxoles. These include several series of aryl propynyl ethers, propynyl oxime ethers and propynyl phosphonate esters as well as a variety of other materials such as the benzylthiocyanates. N-alkyl compounds such as SKF 525-A, and Lilly 18947 have long been known as drug potentiators in mammals and as this property results from

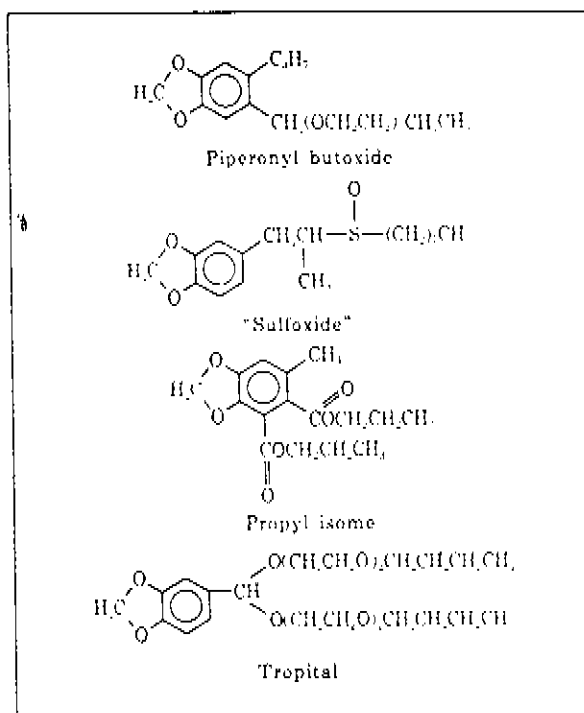


Figure 1: Methylenedioxy synergists of current commercial significance

microsomal enzyme inhibition it is not surprising that in the free-base form these materials are also active as synergists for insecticides. More recently there has been considerable interest in the activity of compounds with nitrogen-containing rings and indeed some of these, including the 1,2,3-benzothiadiazoles and the 1- and 4(5)-substituted imidazoles (figure 2) are some of the most potent microsomal enzyme inhibitors known.¹³

From the practical standpoint the use of insecticide synergists has several obvious advantages. Since they enhance the efficacy of an insecticide with which they are combined they have the ability to reduce the amount of the material required for insect control. This would clearly be beneficial in reducing the possible environmental hazards associated with insecticide usage and would also serve to reduce the cost of applying expensive materials.¹³ The increased cost of developing and registering these more sophisticated chemicals must ultimately be borne by the consumer, so that the economic advantages to be gained from the use of synergized formulations might become a more significant factor in the future. At the present time the commercially available synergists are themselves expensive and in limited supply and it is only with the pyrethroids that their use is economically justified. If new types of relatively cheap, effective, safe and environmentally sound synergists or new processes for known synergists can be developed, they may be of considerable use for agricultural pest control.¹³ It should be recognized that in addition to blocking detoxication in insects the synergists also have the unfortunate property of stabilizing potentially hazardous foreign compounds in man and other animals. Thus although synergists in general have a low acute mammalian toxicity *per se*, their properties will have to be carefully evaluated. The present of residues of stable synergists in the environment would be a justifiable cause for concern although it was stressed by Wilkinson¹³ that there is little or no hazard associated with the present usage patterns of synergists because the levels used are small and the materials used are all rapidly degraded by mammals.

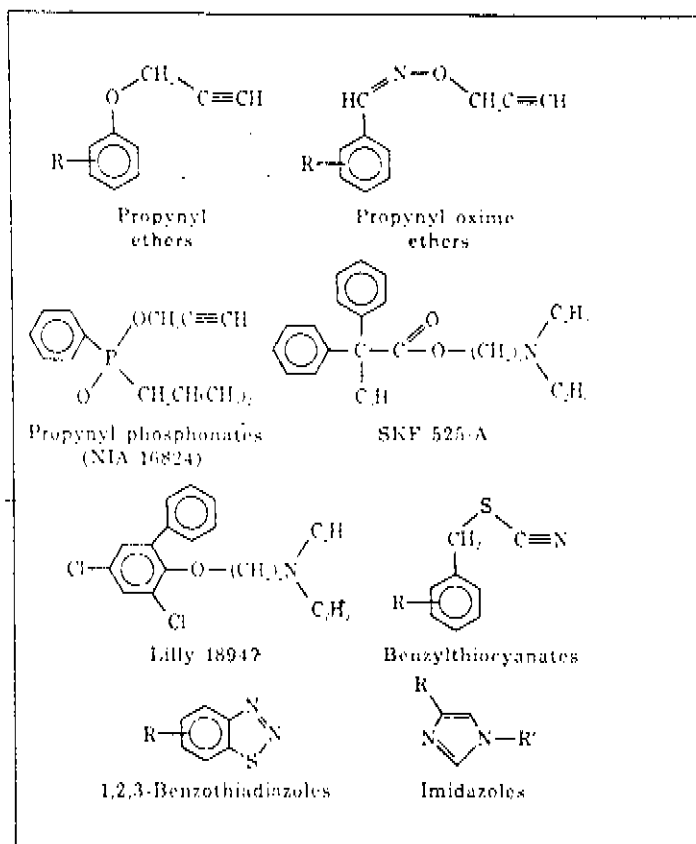


Figure 2: Compounds exhibiting synergistic activity

The prospect of reduced pesticidal dosages through controlled release is a promising aspect of new pest control methods. In recent years, a remarkable number of methods of achieving this control have been proposed and have been reviewed by Allan et al¹⁴ in terms of the problems and opportunities forcing polymer and pesticide technologies. It has been shown in small scale testing that controlled release combinations, in comparison with free pesticides provide either an extended period of protection at equivalent levels of application, or the same period of protection at greatly reduced levels of application. Both of these alternatives tend to reduce the environmental hazards associated with the continued use of conventional pesticides.

Penwatt Corporation in the US is now marketing, under an experimental label, a product consisting of methyl parathion-containing microcapsules dispersed in water, in a flowable formulation for release by ordinary ground or air equipment³. It is claimed that this polyamide encapsulated product is effective for a longer time in the field, yet safer to handle than ordinary emulsifiable concentrates.

3. Juvenile hormones and ecdysones

Insect juvenile hormones and compounds which mimic their effects have received much attention in the last several years as possible insect control agents. Insect hormone studies can be traced to their early beginnings with the work of Kopec¹⁵ who first suggested that insect molting was controlled by hormones.

In insects, the processes of growth and development are controlled by three primary hormones, e.g. the brain hormone, the molting hormone and juvenile hormones. The brain hormone (BH) is produced by the neurosecretory cells of the brain, transported via axons to the corpus cardiacum, and released from there. The brain hormone stimulates the release of a second hormone known as the molting hormone (MH) or ecdysone. The molting hormone initiates the development of a new cuticle and the maturation of the tissues in the insect prior to ecdysis or the casting off of the old skin. In immature insects, a third hormone, the juvenile hormone (JH) is also released into the blood along with the molting hormone and suppresses or inhibits the full maturation of adult tissues.

A new concept of the biochemical mechanism of hormone action was developed from the observation of Clever and Karlson¹⁶ that ecdysone can induce puffing in salivary gland chromosomes and recently it has been shown that some puffs in *Drosophila* respond to juvenile hormone.¹⁷ The biochemical significance of puff induction is that puffs are the site of RNA synthesis or, in other terms, the site of active transcription of DNA into RNA. Combination of the Jacob-Monod model of gene expression¹⁸ with the experimental findings on puff induction led to a scheme of the mechanism of hormone action as proposed by Karlson¹⁷. In this scheme, the hormone is visualized as a specific inducer of transcription of specific genes, e.g., the production of messenger RNA and, in consequence, of specific proteins which can set in motion the steps of morphogenesis. Experiments on the action of ecdysone and juvenile hormone on isolated cell nuclei from insect tissues (epidermis and fat body) appeared to lend strong support to the theory on the mechanism of hormone action at the level of the cell nucleus¹⁷.

Juvenile hormone and its mimics have been heralded as third generation insecticides by Williams^{19,20} who listed their possible attributes as including : specificity, high activity, safety, and possible circumvention of resistance.

It is generally acknowledged that successful pest control by insect growth regulators requires that they possess at least the following properties : high potency in pest insects; moderate field stability without undue persistence; selectivity for target pest organisms; and structural simplicity for economically feasible synthesis. During the last several years, a large number of chemical structures with juvenile hormone activity have been synthesized and investigated²¹⁻³³. Reviews of insect juvenile hormone analogs and their structure-activity relationships have been published by Slama³⁴ and Menn and Beroza³⁵.

Direct comparison between current insecticides and insect growth regulators is undoubtedly difficult, particularly when complex long-term effects on the total ecosystem are considered. For example, simple ID₅₀ (Inhibition Dose) values are not a satisfactory basis for making a comparison between the two categories of control agents. However, such values do provide an accurate basis for making structure-activity correlations within each group of chemicals, e.g., the potency of a number of alkyl 3-7-11-trimethyl-2,4-dodecadienoates.

Any potential use of a juvenile hormone (or any agent) in insect control requires an understanding of its metabolism and environmental degradation. One possible method of application of these materials would be spraying which would leave a thin film on the plant foliage and hence the compounds would be exposed to heat, sunlight and oxygen from the air as well as climatic conditions. They could also be adsorbed on the plant surfaces and absorbed through the cell.

The mammalian metabolism and environmental degradation of the juvenoid 1-(4'-ethylphenoxy)-3,7-dimethyl-6,7-epoxy-trans-2-octene (ethyl epoxide) and related compounds (its diene precursor (ethyl diene); its 6,7-diol and 2,3,6,7-diepoxy) was studied by Gill et al.³⁶). Major identified products formed from the ethyl epoxide were :

the ethyldiol and α -hydroxyethyldiol on incubation with mouse, rat, or rabbit liver microsome - NADPH systems and algae; compounds resulting from ether cleavage in living mice; the ethyldiol, ethyldiepoxyde, and ethylphenol on exposure to sunlight or residual deposits or in aqueous solution. Comparable studies with the ethyldiene, ethyldiol and ethyldiepoxyde led to tentative identification by primarily cochromatography and deuratization techniques of over 25 metabolites and photoproducts. The ethyl epoxide was transformed at varying rates in the systems examined by epoxide hydration, 2,3-epoxidation, α or β -oxidation of the ethyl group, and ether cleavage.

The major pathways for metabolism in mammals and algae and for photoalteration of the ethyldiene, ethyl epoxide, ethyl diepoxyde, and ethyldiol involve 6,7-epoxidation and -hydration, 2,3-epoxidation and -hydration with or without cyclization, oxidation of the ethyl side chain, to a greater extent at the α than at the β position, and ether cleavage.

In living rats and mice, the phenyl-labelled ethyl epoxide is rapidly metabolized and the metabolites are excreted without persistent storage in tissues. Orally administered ethyl epoxide is likely to undergo rapid acid-catalyzed hydration to the ethyldiol in the stomach. Conjugation is also important in ethyl epoxide metabolism in rats and mice, as well as insects ³⁶. A major portion of the ethyl epoxide dose undergoes ether cleavage in rats ³⁷, and mice whereas under in vitro conditions ether cleavage does not occur due possibly to competing reactions. Ether cleavage in vivo is probably initiated by oxidation at the activated O-methylene position. O-dealkylation might be further facilitated by preliminary oxidation of the ethyl to the aceto, carboxy, and carboxymethyl groups. However, O-dealkylation in mice can occur prior to oxidation of the side chain since ethylphenol is excreted.

The ethyl group of the ethyl epoxide is extensively metabolized in living rats yielding carboxymethyl, carboxy, and 4-hydroxyphenyl derivatives. ³⁷ A recent study with mice confirmed the presence of hydroxybenzoic and hydroxyphenylacetic acids as in vivo urinary metabolites of the ethyl epoxide but it did not detect the hydroxydiene and -diol, carboxytetraol, and ethyl-2,3-diol-6,7-epoxide reported as rat metabolites by Hoffman et al ³⁷. The urinary and fecal metabolites of mice do not include any one of the carboxy-methyldiene and -diol, the carboxydiene and -diol, and the hydroxydiene, hydroxy epoxide and hydroxydiol, compounds that would result from extensive oxidation or cleavage of the ethyl moiety while retaining the ether linkage. In analogy with metabolic studies on other alkylbenzenes, the complete scission of the ethyl group in the ethyl epoxide is an unanticipated reaction.

The ethyl substituent is preferentially oxidized by the microsome-NADPH system at the activated benzylic methylene group with subsequent oxidation of the α -hydroxyethyl compound, catalyzed more effectively by soluble than microsomal enzymes, to the aceto derivative. Only small amounts of the α -hydroxyethyl compounds are found and the acids resulting from their further oxidation are not detected.

The ethyldiene is epoxidized to the ethyl epoxide in all microsome-NADPH systems examined. In the rat system, the ethyldiene is poorly metabolized relative to the ethyl epoxide and ethyldiol, whereas in housefly ³⁶, and mouse systems there is extensive modification of the ethyldiene. Epoxidation of the 2,3-double bond, which occurs to a greater extent with the ethyl epoxide, than with the ethyldiol, converts the epoxide to the diepoxyde and both the ethyl epoxide and ethyldiol to the ethyltetrahydrofuran diols and other cyclic ethers which probably arise via the 2,3-epoxy-6, 7-diol ³⁸.

The ethyl epoxide undergoes rapid enzyme-catalyzed hydration to the ethyldiol. The epoxide hydratase activity of rat liver acting on the ethyl epoxide can be compared with that of guinea pig liver acting on styrene oxide ³⁹⁻⁴¹ as follows: similar tissue distribution; the most active subcellular fraction is the soluble with the ethyl epoxide and the micro-

comes with styrene oxide; compounds that inhibit hydration of styrene oxide do not inhibit ethyl epoxide hydration of microsomes. These differences could result from one or more of the following: species specificity; the presence of more than one epoxide hydratase in the microsomes (Oesch, 1973; Oesch et al., 1971a); the involvement of different hydratases or hydration mechanisms with highly lipophilic trisubstituted epoxides (the ethyl epoxide) than with monosubstituted epoxides (styrene oxide). Additional species and substrate specificity studies are necessary to clarify these speculations.

Enzymatic hydration of the ethyl diepoxide gives rise to a number of cyclic products similar to those formed on aqueous acid treatment, indicating that an electrophilic site on the enzyme catalyzes formation of an epoxydiol which subsequently undergoes intramolecular cyclization. No tetraols are detected as ethyl epoxide or diepoxide metabolites in rat liver microsomes, yet the acetotetraol is an *in vivo* metabolite of the ethyl epoxide in rats³⁷, and mice. Thus, the ethyl diepoxide is not a likely intermediate in formation of the acetotetraol.

In regard to the effect of the juvenoids on algae, the growth of algal cultures at 27° was delayed at the following juvenoid concentrations (parts per million):

Chlorella, 0.1 for ethyldiene, 0.2 for ethyl epoxide, and 10 for ethyldiol;
Chlamydomonas, 1 for ethyldiene and 10 for ethyl epoxide, ethyldiol and two for aliphatic diene esters. Considerably higher juvenoid concentrations (2-100 fold) are needed to block growth. Thus, these compounds delay the time before algal growth enters the log phase but not the growth rate within the log phase. The potency of the ethyl epoxide and ethyldiene is increased about tenfold when Chlamydomonas cultures are held at 5° for 48 hours before inoculation into the treated medium.

Inhibition of algal growth was suggested by Gill et al⁴² to be unlikely to limit the use of phenyl geranyl ether juvenoids in control of mosquito larvae since they block insect development at much lower levels. However, there are conceivable situations of low populations and slow growth rates where application of an inappropriate juvenoid could lead to a detrimental shift in the composition of a phytoplankton community.

The ethyldiene and ethyl epoxide are both rapidly degraded by algae giving a variety of products including ethyl- and acetophenols. Chlamydomonas carry out rapid oxidation and then hydration of the 6-7-double bond. The 2-3-double bond is relatively resistant to oxidation as evidenced by the low levels of the ethyl diepoxide and ethyltetrahydrofurandiols formed.

One photoproduct, the ethyl-6-hydrox-2-ene, may undergo oxidation to the corresponding keto analog since comparable ketones form on photooxidation of trisubstituted olefins (pyrethroids) and isomerization of trisubstituted oxiranes (citronellol epoxide)^{43,44}

The ethyl epoxide undergoes significant photoepoxidation to the ethyl diepoxide. A similar epoxidation occurs with the C₁₈-juvenile hormone on UV exposure⁴⁵. The cyclic diols and ethers other than the ethyltetrahydrofurandiols probably arise by photoepoxidation of the ethyl epoxide and then hydration of the ethyl diepoxide rather than 2,3-epoxidation of the ethyldiol since they form in larger amounts from the diepoxide and are not photoproducts of the ethyldiol.

Photodecomposition of the ethyl epoxide and particularly the ethyl diepoxide gives larger amounts of the ethyltetrahydropyrandiols I and II than of the ethyltetrahydrofurandiols.

Ethyl epoxide hydration to the ethyldiol is the major route of photo-alteration in aqueous media; however, on silica gel oxidation of the 2,3-double bond also occurs to yield an unidentified cyclic monohydroxy derivative. In aqueous media, the ethyl epoxide and

ethyldiol give the *cis*- and *trans*-ethyltetrahydrofurandiols as the only identified cyclic products, the ethyldiol being a likely intermediate in conversion of the ethyl epoxide to the ethyltetrahydrofurandiols.

The ethyl epoxide and ethyldiol in aqueous media and the ethyl epoxide on silica gel when sensitized with xanthen-9-one undergo benzylic oxidation. The α -hydroxy-ethyl compound is presumably the intermediate in formation of the aceto compound. Photochemical ether cleavage forming phenols, which occurs with many of the test compounds and conditions, results either by photolysis of the carbon-oxygen bond or photooxidation at the activated α -carbon giving the unstable α -hydroperoxide as an intermediate.

The ethyl epoxide, ethyldiene, ethyl diepoxide, and ethyldiol undergo rapid and extensive modifications with loss of morphogenic activity in each of the biological and photochemical systems examined. This limited persistence is advantageous in minimizing environmental pollution but detrimental in the practical use of juvenoids in pest insect control. The groupings that are most labile to degradation are the ethylphenyl and epoxide moieties, the 2,3-double bond, and the ether linkage. Substitution of any of these groupings with others more resistant to metabolism and photodecomposition might yield compounds of increased effectiveness, but also the pollution potential of such compounds.

Pawson et al ⁴⁵ studies the environmental stability of juvenile hormone-I (figure 3). This compound is a mixture of 8 *cis/trans* isomers, e.g. 2-*cis*, 6-*cis*, 10-11-*cis/trans* and 2-*trans*, 6-*trans*, 10,11-*cis/trans*. No attempt was made to duplicate closely field conditions, but instead, a thin film of pure actual compound was placed on a glass surface and exposed to UV light supplied by a 275W-Hanovia sun lamp. Under these conditions, more than 95% of the starting material disappeared after 24 hours. The activity of the synthetic juvenile hormone I after exposure to sunlight for 16 hours was approximately one-tenth that of the unexposed material.

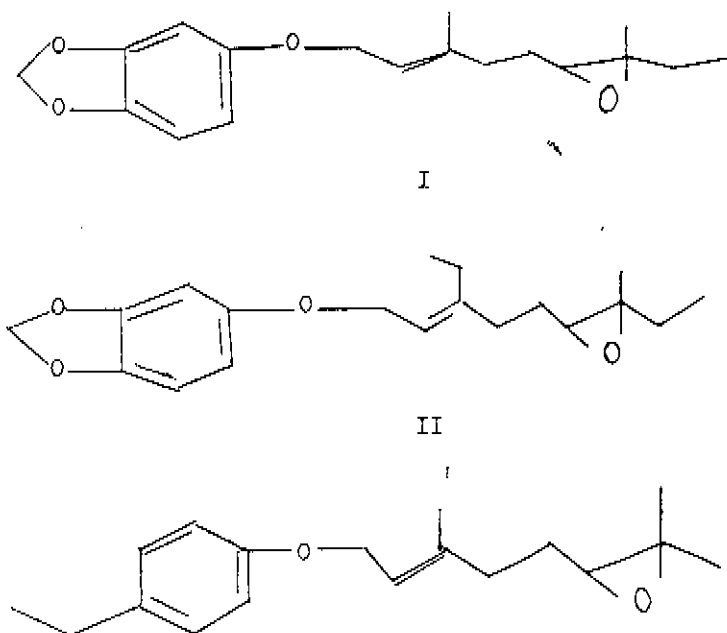


Figure 3: Juvenile hormone mimicking agents

Similar decomposition studies were carried out with the juvenile hormone mimicking agent II (figure 3). Under ultra violet exposure, 50% of the starting material had disappeared after 16 hours and 90% after 96 hours. After 40 hours, the activity of the decomposition material (as assayed in the *Tenebrio* screen) was less than one-tenth that of the original material. The four isomers of the JH-mimicking agent II also exhibited similar stabilities when subjected to ultra violet exposure as above.

Another factor of critical importance in assessing the potential use of a juvenile hormone mimicking agent in the field is the effect of the material on the plant itself. When JH-mimicking agent II (4-isomers and a mixture) were evaluated on sugar beets, corn, wheat, soybean, cotton and tomato, no significant differences between the isomers and the mixture were observed. Plant injury was the same in all cases, typified by slight necrosis.

An interesting observation has been reported by Bowers,⁴⁶ who found that a number of insecticide synergists such as piperonyl butoxide, sesamin, and sesamol had activity which mimicked that produced by JH. Most of these same synergists have been shown to improve the efficacy of certain insecticides by inhibiting microsomal oxidases which would normally act in detoxification processes⁴⁷⁻⁴⁹. Then, later, based on results with *Tenebrio* and *Oncopeltus* Bowers found that JH activity increased in terpenoid ethers were synthesized into methylenedioxyphenyl and benzene ring systems. However, if methylenedioxyphenyl and benzene ring systems are retained in some JH structures, they may be suspected of causing the same effects as their particular synergist analogs on nontarget organisms such as other invertebrates, vertebrates, and man.

Mayer et al⁵¹ recently reported several JH analogs, e.g. 4-(6,7-epoxy-3,7-dimethyl-2-monyl)oxy-1,2-(methylenedioxy) benzene (I), its ethylhomolog (II) and 6,7-epoxy-1-(p-ethylphenoxy)-e, 7-dimethyl-2-octene (III) to be *in vitro* inhibitors of rat liver microsomal oxidases (e.g. aniline hydroxylase). Cytochrome P-450 binding for all three compounds was observed to be of the type I Category. The results of Mayer et al⁵¹ thus provided additional evidence that JH analogs react with mammalian tissues and are thus not completely insect specific. The methylene dioxyphenyl- containing JH analogs do resemble insecticide synergists in their reactions with microsomal systems. Although they do not appear to have any significant acute toxicity where mammals are concerned, the possible long-term effects such as induction could constitute a concern.⁵¹

In the initial section on insect hormones as potential pest control agents, much stress was placed on the juvenile hormone analogs, it is nevertheless of importance to consider the ecdysones as well. An attractive feature of the potential use of ecdysones and related substances for insect control is that they are already widespread in the environment.

In addition to the ecdysones identified from insects and crustaceans approximately forty ecdysteroids, including the two major insect ecdysones, alpha-ecdysone and 20-hydroxy ecdysone,^{52,53} have been isolated and characterized from plants.⁵⁴⁻⁵⁹

It would appear likely that the next few years will see increased testing of various ecdysone-based substances for use as agents of insect control.⁶⁰⁻⁶³ In addition, we can anticipate a search for agents which block ecdysone synthesis, which compete with ecdysones for binding sites in tissues and which prevent brain hormone from initiating ecdysone production.⁶⁰

4. Sex Attractants (Pheromones)

Sex attractants have been proposed for many years as one of the potential tools to be developed to help modify and reduce insecticidal treatments. Progress however has been relatively slow in the past, primarily because of the time and expense involved in identifying the pheromone structures.

During the past 5 years, the list of insect sex attractants pheromones identified has increased steadily, and effective attractants are currently available for at least 35 species of economic importance. ⁶⁴ However, recent developments in insect pheromone research have also indicated that many oversimplifications have been made in the past. ⁶⁵ For example, behavioural responses formerly attributed to one single substance have been shown to be governed by a mixture of compounds (sometimes closely related synergistic isomer). Some pheromones appeared to be much less species-specific than was supposed. It has also become more difficult to use sensible classifications for pheromones or semiochemicals in general. ⁶⁵ These various points can be illustrated with semiochemicals isolated and partly or completely identified by TNO, Delft in recent years and include : trail substances and attractants for a termite, a spring tail and an ant; the alarm pheromane of aplids; aggregation and sex pheromones for cockroaches and sex pheromones of Lepidoptera. ⁶⁵

Another example of recent developments in pheromone research is in the potential control of dendroctonus bark beetles, which represent the most destructive force affecting the coniferous forests of North and Central America. ⁶⁶

5. Chemosterilants

Another sex-based method of insect control is the sterile-male release technique, pioneered by Knipling of the WSDA. In this approach billions of male insects are reared, then sterilized (in the reproductive sense) via radiation, although alkylating agents and other chemosterilants are also used. The sterile males are released in insect-infested areas, there to compete with normal males for the attention of the females. Since the eggs fertilized by the sterile males do not develop and of the ratio of sterile to normal males is high enough, the population of that particular insect falls off drastically, sometimes to the vanishing point.

It is generally acknowledged that since chemosterilants are biologically active chemicals, a very cohesive, well-coordinated, interdisciplinary approach is required in chemistry, biochemistry, biology, genetics and pest control sciences.

Initial studies with chemosterilants in pest control primarily centered about the use of the aziridinyl alkylating agents.

The testing of other classes of compounds for activity as insect sterilants, includes phosphoramides ⁶⁷, S-triazines ⁶⁸, dithioburets ⁶⁹ and dithiazolinium salts ⁷⁰ have also been described.

Recent chemical research has increased the number of active chemosterilants to over 1000, and the classification by structural types now includes : aziridines, N-mustards, alkanesulfonates, anti-metabolites, non-alkylating phosphorus amides, melamines, diaminotriazines, boron and tin compounds, dithiazoles, antibiotics, and miscellaneous agents. ⁷¹

The degradation and residues of the chemosterilant aziridinyls have been reviewed by Fishbein. ⁷²

The rates of absorption, degradation and excretion of ³²P-Metepa in mosquitoes ⁷³, houseflies ^{73,74} in mice ⁷³, screwworm fly ⁷⁵, stable fly ⁷⁵; the metabolism, distribution and residues of ³²P-Thiotepa following topical application to the German cockroach (Blatella germanica) ⁷⁶, housefly (Musca domestica) ⁷⁶, stable fly (Stomoxys calcitrans) ⁷⁶ and ball weevil (Anthonomus grandis) ⁷⁶ have been reported.

All aziridine chemosterilants are apparently extremely susceptible to moisture and acidic conditions⁷⁷⁻⁷⁹. (For example at pH 3.0 degradation of TEM is complete and occurs almost immediately, whereas minor degradation occurs in buffered or unbuffered solutions at pH 7.5). Thermally stable non-alkylating derivatives of tretamine and TEPA, e.g. Hemel [(2,4,6-trisdimethylamino)-1-triazine] and Hempa (hexamethylphosphoric triamide) developed by Ching et al⁸⁰, are effective chemosterilants for houseflies (*Musca domestica*).

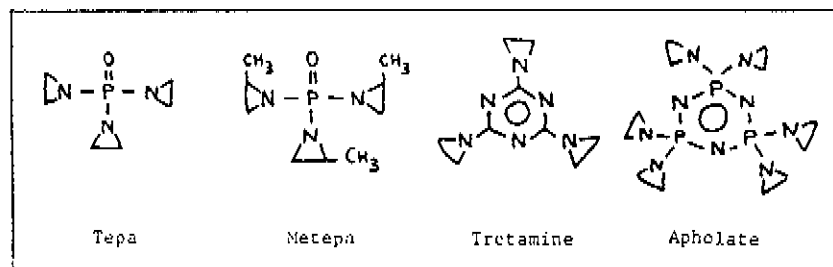
The use of volatile chemosterilants, applied in the vapour phase, can avoid the problems of non-uniformity of treatment. A closed, circulating fumigation system containing chemosterilant vapours at atmospheric pressure has been widely used by Terry et al⁸¹. This closed circulating system has the following advantages: (1) it is safe to the operator; (2) the treatment is rapid; (3) it is economical in the use of chemosterilants; (4) the insects are easily treated; and (5) the system could easily be scaled up for mass treatment.

Beyond the mechanics of an eradication programme *per se* and of primary consideration in terms of potential environmental hazards is the question of the toxicity of a number of compounds which have reached the field testing stage. Here a major concern is the mutagenicity of a number of the alkylating aziridinyl chemosterilants. For example, the mutagenic activity of TEM (tri-ethylenemelamine) has been demonstrated in mice⁸²⁻⁸⁶, *Drosophila*⁸⁷⁻⁹¹, *Musca Domestica*⁹², screwworm fly, *Chochliomyca hominivorax*⁹³ and the agent has also been shown to induce chromosome aberrations in mice^{83, 94-99}, *Drosophila*^{100,101} cultured human leukocytes^{102,103}, *S. typhimurium*¹⁰⁴ and *E. coli*¹⁰⁵.

The mutagenicity of TEPA has been demonstrated in the parasitic wasp *Habrobracon*¹⁰⁶ and in the dominant lethal test in mice⁸⁴, the mutagenicity of Metepa in mice (dominant lethal test)^{84,107} and teratogenicity in the rat¹⁰⁸; the induction by Apholate of dominant lethal mutations in mature sperm and gonial cell death in *Musca domestica*¹⁰⁹ the mutagenic activity of Thiotepa in mice (dominant lethal)⁸⁴, screwworm fly *Cochliomyia hominivorax*¹¹⁰ as well as its induction of chromosome aberrations in human chromosomes¹¹¹ have all been reported.

Of the chemosterilants tested, compounds with nitrogen containing heterocyclic groups, expressed highest mutagenic activities. TEPA was considered one of the most interesting compounds, with highest mutagenic index in *Culex*. Figure 1 shows the structures of some aziridinyl chemosterilants tested extensively in insects.

Figure 1



Source - Borkovec, A. B. 1969, Alkylating Agents as Insect Chemosterilants, Ann. N.Y. Acad. Sci., Vol. 163, Art. 2, 865.

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