THE EFFECT OF EIGHT TENSIDES ON THE TOXICITY
OF DIAZINON TOPICALLY APPLIED TO
THE MALE GERMAN COCKROACH

by

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Thesis submitted to the Graduate Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE
in
Entomology

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September, 1976
Blacksburg, Virginia
ACKNOWLEDGEMENTS

I wish to express my gratitude to the following people who have guided me during the course of this study: Drs. D. G. Cochran, E. C. Turner, and J. M. Grayson for their advice and constructive criticism of this manuscript; Dr. S. I. Ahmad for his help with the chemical aspects of this investigation; and to Dr. W. H Robinson, major professor, for his suggestions and guidance.

Special appreciation is extended to Ms. J. G. Knausenberger and to Mr. D. E. Simonet for their help with computer programming. I would also like to thank Mr. D. Hon for his helpful translation of an article published in Japanese.
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I. INTRODUCTION AND LITERATURE REVIEW

There are more than 200 chemicals registered and approved by the Environmental Protection Agency for use as insecticides. These materials are available in a variety of formulations, including dusts, granules, wettable powders, water emulsions, and others. Different formulations are necessary to meet the needs of a variety of different pests and pest control situations.

Insecticides designed to be used as water emulsions are very common. Their popularity is based on a number of factors. They are generally less expensive than oil base preparations, making them the most practical materials to use for many large scale crop applications. Water formulations are usually less phytotoxic than oil based insecticides and, in the case of home applications, are less damaging to finished surfaces. Safety is another factor favoring water emulsions. Unlike oil base preparations, they are not flammable. Because they are not as volatile as some other formulations, there is less danger from fumes. Water emulsions are safer than many other formulations because they will not penetrate human skin to any great degree.

Handling convenience is not the only advantage of water emulsions. Proper choice of an emulsifier may actually enhance the toxicity of an insecticide. If an emulsifier were found that enhanced the toxicity of an insecticide significantly, lower concentrations of the material might provide effective insect control while presenting less environmental hazard.
The following report concerns research conducted on water emulsion formulations. Eight different surface active agents, or tensides, were used to emulsify diazinon in order to determine the effect each had on its relative toxicity. All formulations were topically applied to the adult male German cockroach, Blattella germanica (L.). In addition, bioassays of other diazinon formulations were conducted for comparison.

Factors affecting the toxicity of insecticides.

Evaluation of any insecticide requires a basic understanding of the variables involved with the toxification process. A principal factor is the vulnerability of target species. Certain species are naturally more susceptible to some toxicants than other species, and some have developed resistance to several insecticides. The chemical structure of the toxicant is another important variable. A compound may be highly toxic in vivo yet not be stable enough in the environment to be of practical use. Such is the case with the oxygen analogs of many of the sulfur containing organophosphates. P=O is less stable than P=S, but is more toxic (O'Brien 1960). However, insect tissues usually oxidize P=S to the more toxic P=O (O'Brien 1960).

Once an insecticide comes in contact with an insect, several other variables affecting toxicity become important. These are absorption, distribution, the receptor site, storage and binding, metabolism, and excretion (Wilkinson 1973). Any formulation change affecting one or more of these variables will quite likely influence
the toxicity of the applied compound.

**Mode of action of insecticides**

Modern insecticides take advantage of several phenomena in order to bring about mortality. Some insecticides, such as the silica aerogels, are simply desiccants (O'Brien 1967). Others, such as rotenone, interfere with cellular respiration (Corbett 1974). The juvenile hormone insecticides inhibit the normal metabolic pathways in the maturation process. A new class of toxicants, the benzoyl phenylureas, has shown promise in the disruption of cuticle deposition among certain insect larvae (Mulder and Gijswijt 1963).

The majority of insecticides disrupt the nervous system. Evidence indicates that nicotine mimics acetyl choline (Corbett 1974), thus interfering with synaptic transmission. DDT apparently changes the permeability of the nerve membrane (Corbett 1974), causing disruption of axonic transmission. For other chlorinated hydrocarbons and the cyclodiene the mode of action remains unclear, but it is generally agreed that they attack the nervous system (Corbett 1974).

Of all the toxic compounds inhibiting the nervous system, the most is known about the organophosphates. Evidence indicates that these chemicals block synaptic transmission by competitive inhibition of acetyl cholinesterase (Corbett 1974). Diazinon (diethyl 2-isopropyl-6-methyl-4-pyrimidinyl phosphorothionate) is an organophosphate, or more precisely, a phosphorothionate. It is a broad spectrum insecticide and is currently one of the most widely used insecticides in the
United States (Matsumura 1975). When exposed to diazinon, an insect normally converts it to the more toxic form, diazoxon (Narahashi 1971)

The penetration of insecticides

The efficiency of contact insecticide formulations depends upon their ability to permeate the exoskeleton of the insect. Concise reviews concerning the structure and chemistry of the insect cuticle can be found in the works of Noble-Nesbitt (1970b), Hackman (1974), Locke (1974), and Neville (1975). The composition of the insect integument varies considerably between species. Basically, it consists of an epicuticular layer composed of lipoproteins, polyhydric phenols, and waxes overlying a protein-chitin complex known as the procuticle (Hackman 1974). Below the cuticle lies the cuticular epithelium which is separated from the haemocoel by the basement membrane (Locke 1974).

When considering the factors involved in the penetration of substances inside the insect, one must bear in mind that the cuticle is not a homogenous structure covering the entire insect. Articulating with it are numerous sensory receptors as well as spiracles. Within the cuticle are numerous pores and wax canals extending from the cuticular epithelium into the cuticulin layer (Ebeling 1974). Appendages and sclerites are joined by a less sclerotized, flexible material called the intersegmental membrane, or arthrodiol membrane (Neville 1975).

Experimental evidence over the years indicates that all of these structures may conduct insecticides through the cuticle. Insecticide
droplets applied to groups of setae and to the arthrodial membranes of cockroaches proved these structures to be the most permeable areas of the cuticle (Ebeling 1974). Wilcoxon and Hartzell (1931) showed that certain solutions of nicotine could penetrate the tracheae of the tomato hornworm, *Manduca quinquemaculata* (Haworth). The demonstration by Beament (1965) that water could be transported through the cuticle of the American cockroach, *Periplaneta americana* (L.), even at a gradient of 20 atm. osmotic pressure has strong implications for the transport of insecticides through the cuticle. Beament's work deserves special mention, for it shows that the insect cuticle is a dynamic, semipermeable membrane.

Beament (1965) observed that a drop of water placed on the cockroach thorax disappeared much faster than was possible by evaporation. Noting that Locke (1965) had observed minute pores in the outer epicuticle protein of several insects, Beament proposed a model for the transport of water through the cockroach cuticle. According to the model, water on the epicuticular grease layer alters the grease structure causing a micelle of grease to form and enter a water filled wax canal. This would leave an open channel for the surface water to enter. Beament stated that electrostatic interactions between the polar substituants of the grease molecules and the water molecules could create this phenomena.

**The cockroach cuticle.**

The chemical composition of the cuticle differs among insects. In a study of the cuticular lipids of the American cockroach, Gilby
and Cox (1963) found that this greasy material has a relatively high proportion (75%) of largely unsaturated hydrocarbons. Predominant among them is heptacosa-9, 18-diene. Another unusual aspect of this lipid layer is that it had a high concentration of free aldehydes (>8%). The fluidity of this lipid layer was attributed to the involatile liquid hydrocarbons and free acid fractions. The composition of the cockroach grease layer is consistent with the water transport model of Beament (Gilby and Cox 1963).

Little work has been done on the cuticle of the German cockroach. Ebeling (1974) simply notes that the epicuticular lipid is a "soft grease". It has been noted, however, in studies conducted on the German cockroach as well as other cockroach species, that the cuticular lipids undergo a change at 30°C, rendering the cuticle more permeable to water (Ebeling 1974, Ramsey 1935, Beament 1945).

Phase partition and the chemistry of penetrants.

Just as the chemistry of the cuticle effects permeability, so does the nature of the penetrant. Phase partition, or the ability of a substance to solubilize in both polar and nonpolar phases, seems to be an important factor in the permeation of the cuticle (Ebeling 1974). Webb and Green (1945) designed a series of experiments to test the carrier efficiency of various solvents to transport diphenylamine across the cuticle of the sheep ked Melophagus ovinus (L.). Using the time of death as criteria, they found that the majority of the better solvents had high rates of penetration through beeswax, high
partition coefficients between beeswax and water, high solubilities of diphenylamine in solution with the solvent and water, and low volatilities.

Olson and O'Brien (1963) found that the polarity of a molecule had a great effect on permeability of the cockroach cuticle. A polar material such as K$_2$HPO$_4$ penetrated much faster than the nonpolar toxicant DDT. Olson and O'Brien felt that their application procedure disrupted the grease layer of the cuticle, thus enabling the applied material to enter the procuticle without partitioning with the lipid layer. They were apparently unaware that the lipid layer also contains polar molecules.

The work of Burt and Lord (1968) and Burt et. al. (1971) also indicates that polarity plays a role in cuticle penetration. In studies conducted on the American cockroach, it was shown that a higher percentage of the relatively polar material, diazoxon, could penetrate the cuticle than could the lipophilic insecticide pyrethrin I.

The pH of a penetrant also seems to affect permeability. Topically applied free nicotine has been found to be more toxic to Culex pipiens pipiens L. larvae and to American cockroach adults than its ionized form, nicotine sulfate. Toxicity also increased with increasing pH. Both materials, when injected into the cockroach were equally toxic (Yamamoto 1965).
Principle mode of entry of insecticides.

Controversy over the principle mode of entry of contact insecticides has developed over recent years. Investigations in the past have implicated the haemolymph as the main transporter of most toxicants within the insect body (Ebeling 1974). Gerolt (1969) concluded that organic insecticides moved laterally within the cuticle and entered the insect via the cuticular lining of the trachea. He based his conclusions on the results of several different experiments:

1) Crystalized dieldrin and filter paper impregnated with several other insecticides were less toxic when placed inside the haemocoel of the house fly than when applied topically;

2) The site of application of topically applied dieldrin had a marked effect on relative knockdown time. Gerolt stated that this would not be the case if the toxicant were transported principally by the haemolymph;

3) Olive oil blocked the movement of several insecticides when topically applied to house flies, but not when injected;

4) In an experiment involving the isolated cuticle of Sarcophaga larvae, no \(^{14}C\) dieldrin was found to penetrate into a saline solution on the inner side;

5) Beeswax collars prevented the migration of crystalline dieldrin through the cuticle of the house fly and the German cockroach; up to 4000 times the lethal dose was applied in this manner without toxic effect;

6) \(^{14}C\) dieldrin migrated laterally in the cuticle of the locust Sci\textit{histocerca gregaria} Forskål;

7) Dry deposits of \(^{14}C\) dieldrin were able to migrate into the trachea of house flies. [Other workers conducting similar experiments were unable to duplicate this result (Ebeling 1974)].
Gerolt (1969) obtained his results primarily through the use of toxicants in their pure form. He did recognize, however, that certain solvents rendered the integument more permeable to insecticides.

Other investigators disagree with Gerolt's theory. Lateral spread of $^{14}$C DDT through the cuticle of the housefly, Musca domestica L., has been shown by Quraishi and Poonawalla (1969), but this chemical seemed to diffuse into the insect via arthrodial membrane, bases of setae, and other possible entry points. Quraishi and Poonawalla used benzene as a solvent for $^{14}$C DDT. In another study involving the house fly, Benezet and Forgash (1972) found that $^{14}$C malathion formulated in acetone penetrated rapidly through the cuticle into the haemolymph.

In an experiment which challenged Gerolt's theory Moriarit and French (1971) used topical doses of $^{14}$C dieldrin formulated in 1,4-dioxane on the American cockroach. They found no evidence of lateral migration of this material. Moriarit and French (1971) felt that the results of Gerolt (1969) were open to interpretation. They suggested that the $^{14}$C dieldrin found in other areas of the cuticle could have arrived there through the haemolymph. Moriarit and French also disagreed with Gerolt's (1969) explanation that toxicity was influenced by the loci of application. Gerolt, as previously stated, felt this was evidence supporting lateral migration. Moriarit and French believed that if a toxicant were transported through the haemolymph, one could expect to find a lessening of toxic effect as the distance between the site of action and the site of application
increased. This would be the result of dilution of the poison as well as metabolism and excretion. Ahmed and Gardiner (1968) had a similar explanation when they found that toxicity varied with the site of topically applied malathion to *Schistocerca gregaria* Forskål.

The significance of tracheal transport (Gerolt 1969) has also been challenged. Burt et al. (1971), using pyrethrin I on the American cockroach, found that tracheal injections of the chemical were somewhat less efficient than topical applications. They also concluded that the haemolymph was an important carrier of pyrethrin I after finding that spontaneous activity of the abdominal nerve cords of *P. americana* L. were stimulated when flooded with haemolymph containing pyrethrin I.

The conflicting results of the investigations of insecticide penetration seem puzzling. It is quite possible that truth may be found in all of the conclusions. There is probably no single explanation that will account for the transport of every insecticide formulation in all insects. The differences in the results obtained may be due in large part to the use of different toxicants and different solvents. When Gerolt (1969) concluded that $[^{14}\text{C}]$ dieldrin migrated laterally in the cuticle, he formulated in benzene, a solvent only slightly soluble in an aqueous environment. In this situation, it is plausible that dieldrin does migrate laterally within the cuticular lipids rather than enter the haemolymph. The results obtained by Morriarity and French (1971) came from experiments using topically applied dieldrin formulated in 1,4-dioxane. This material is miscible in
all proportions with water and could have carried dieldrin into the haemolymph, as Morarity and French concluded.

There is still much to be learned about the transport of materials through insect integuments. Further complicating the issue are the findings of Gerolt (1975) and Ahmed and Gardiner (1970). Gerolt (1975) provides evidence that the transport of materials through the integument may not be a simple physicochemical process, but rather an active, biological one. He suggests that the cuticular epithelium may be involved in this process. Results obtained by Ahmed and Gardiner (1970) suggest that the cuticle may actually activate some insecticides. Their conclusions were based upon an experiment indicating that the cuticle of *Schistocerca gregaria* converted malathion to malaoxon.

**Tensides and their application to insect control.**

Tensides, or surface active agents, have been indespensible aids in industrial processes for several decades. These diverse chemical additives are employed in the textile and dye industry, in ore floatation, paper processing, cosmetics, paints, and numerous detergents. They have also found their place in food processing and several pharmaceutical applications, as well as the pesticide industry.

The term "surface active agent", or "surfactant", encompasses a wide variety of substances noted for their wetting, foaming, cleansing, emulsifying, or dispersing properties. To describe all these properties under one term, a committee on nomenclature of the third
International Congress on Surface Activity at Cologne officially adopted the word "tenside" (Garrett 1972). So far the term has been slow to gain universal acceptance. Other widely used terms describing tensides (particularly those used in conjunction with pesticides) are defined below. These terms have been taken from Hensill and Hoskins (1935):

1) **emulsifying agent** - any substance which aids in the production or increases the stability of a dispersion of one liquid within another;

2) **defloculating agent** - any substance which facilitates the formation or increases the stability of a dispersion of a solid within a liquid;

3) **wetting agent** - any substance which increases the readiness with which a liquid makes real contact with a solid, i.e. wets it, if necessary by displacing a previous contaminant on the solid;

4) **spreader** - a material which increases the area that a given volume of liquid will cover on a solid or another liquid;

5) **sticker** - a substance that increases the firmness of attachment of finely divided material to a solid surface and is usually used with suspensions of solid insecticides.

Most tensides could be classified under several of these definitions.

General texts of the chemistry and practical application of tensides have been written by Osipow (1964), Elsworthy et al. (1968), and Garrett (1972). The following brief discussion concerns the chemistry of tensides in aqueous systems only.

Surface active molecules are amphipathic by nature, possessing both a polar and a nonpolar group. When a compound of this type is
added to water, the system must equilibrate to a state of minimum free energy (Elsworthy et al. 1968). At low concentrations, this is accomplished through a higher aggregation of surface active tensides at the air/water interphase where the nonpolar end of the molecule orients into the air. At still higher concentrations there is a tendency towards dimerization to achieve minimum free energy. Finally, when a certain critical concentration is reached, small packets of surface active molecules form, and are called micelles. At this point, called the critical micelle concentration (CMC), the nonpolar groups orient towards the inside of the packet and the polar groups orient towards the surrounding water molecules. Through the incorporation of nonpolar materials into micelles, emulsions are formed. Formulations at or above the CMC exist in dynamic equilibrium with the continual making and breaking of micelles (Elsworthy et al. 1968).

Emulsions are generally of two types: oil in water (O/W) where water is the continuous phase, and water in oil (W/O) where oil is the continuous phase (Osipow 1964). Most pesticide formulations are of the O/W type. Stability and particle size of water emulsions vary considerably and are a function of 1) water quality, 2) the emulsifying agent, 3) the material to be emulsified, and 4) the emulsification procedure. Particle size of emulsions generally ranges from 0.1 to 50 μ. Most commercial emulsions range between 0.1 to 10 μ (Osipow 1964).

The selection of a good emulsifier is highly important to the formation of a good emulsion. A useful method for choosing an
emulsifier is the hydrophile-lipophile balance (HLB) system designed by Griffin (Osipow 1964). The HLB system, which measures the relative polarity of emulsifiers, is composed of a scale ranging from 1 to 40. The more lipophilic the emulsifier, the higher the HLB number. The midpoint in the HLB system is about 10. When mixing emulsifiers, HLB numbers are additive (Osipow 1964).

The efficiency of a wetting agent at liquid-solid interphases can be measured by its ability to reduce surface tension, thus reducing the contact angle between the liquid and the solid. The wetting phenomenon is very important for agricultural sprays in order to achieve good foliar coverage.

Only a few workers have studied the wetting of insect cuticles. Wilcoxon and Hartzell (1931) devised a method for measuring contact angles of liquids on two beetle species. Their study showed a correlation between wetting efficiency and toxicity. Three wetting agents were used with 0.1% free nicotine against an aphid *Aphis rumicis* (L.). The formulation containing the superior wetting agent of the three was the most toxic.

Pal (1951) studied the wetting phenomena of 17 different liquids on 30 insect species. He found that the wetting abilities of each liquid varied considerably between species. Distilled water, for example, exhibited a smaller contact angle on the cuticle of *Periplaneta* sp. than for the cuticle of other insects. Pal noticed that irregularities in the cuticle surface, the presence of setae, and the age of the cuticle were important variables involved with the
wettability of the cuticle. The effects of water soluble tensides were also investigated by Pal. He found that all of them decreased surface tension, but the nonionic materials were superior wetting agents.

The chemical nature of tensides is very diverse. Classifying them into categories is difficult. Osipow (1964) chooses to divide them into two major classes: oil soluble surfactants and water soluble surfactants. The oil soluble surfactants are further divided into long chain polar compounds, fluorocarbon compounds, and silicones.

Most of the tensides used with insecticides are of the water soluble variety. Osipow divides this group into anionic, cationic, nonionic, and amphoteric tensides. The anionic tensides include the saponified fats, commonly called soaps, and many synthetic detergents such as sodium dodecyl benzene sulfonate, the common ingredient in household detergents (Osipow 1964). The cationic tensides are composed of long chain primary, secondary, and tertiary amines or quaternary ammonium compounds (Osipow 1964). The latter are very effective as disinfectants. The nonionic tensides can be classified as fatty alkanoamides, ethylene-oxide derived compounds or sugar esters. Many emulsifying and dispersing agents are formulations from this group. Amphoteric compounds, by nature, are pH dependent. Formulations from this group are used both as detergents and as conditioners in certain shampoos (Osipow 1964).
Tensides and biological systems.

Tensides are known to have a marked effect on biological membranes, yet the mechanisms involved in their action are poorly understood. The destructive nature of many of the cationics to cell membranes has been well documented (Cutler and Drobeck 1970). Florence and Gillan (1975) studied the effects of several tenside-drug formulations on the goldfish, Carassius auratus (L.). They found no simple correlation between HLB values and penetration of gill tissues. This was attributed to steric influences. Florence and Gillan also found that drug absorption decreased at tenside concentrations above the CMC.

Only a few investigations have been made concerning tenside effects on the permeability of the insect cuticle. Wigglesworth (1945) demonstrated increased water loss from the cuticle of Rhodnius sp. after treatment with several tensides in chloroform. Beament (1945) used a more theoretical approach to study the effect of various tensides on the permeability of beeswax films. Like Wigglesworth (1945), Beament found some materials more effective than others in altering the permeability to allow increased evaporation of water. Beament stated that the ability of a tenside to enhance transpiration across an insect epicuticle is dependent on: 1) the amount by which the tenside disturbs the wax; 2) the permeability of a layer of the tenside; 3) the effect of wax molecules on this permeability; and 4) the ability of the tenside to dissolve or penetrate the cement layer, when it is present.
An experiment conducted by Ebeling and Reierson (Ebeling 1974) approached the problem in a way directly opposite from Wigglesworth (1945) and Beament (1945). They showed that American cockroaches immersed in a 0.1% solution of the tenside, Triton X-100, absorbed far more water than did those insects immersed in water alone.

The use of tensides as insecticides and as additives to insecticides.

Surface active agents, or tensides, have been used in conjunction with insecticides for many years. Their value as emulsifying, wetting, and dispersing agents has been well documented. In general, however, they have not been employed as toxic agents or to increase the toxicity of other substances. Although many tensides have been shown to have insecticidal properties, few workers have conducted research in this area.

Many surface active substances have been shown to have insecticidal activity when used alone. Soaps were often employed as insecticides before modern insecticides came into use, particularly as contact poisons against aphids (Martin 1948). Probably the most complete study of the insecticidal value of fatty acids and their soaps was conducted by Dills and Menusan (1935). Using the rose aphid, Macrosiphum rosae (L.), and the bean aphid, Aphis rumicis L., they determined that capric and lauric acids were the most potent fatty acids when used as contact insecticides. When potassium soaps were prepared from the fatty acids, oleate, caprate and laurate were the most toxic.
In a study of several emulsifiers and other tensides, Wolfenbarger (1957) found that Triton X-160, (a mixture of alkyl aryl polyether alcohols and organic sulfonates) could be used as an effective contact spray against certain insects. His study showed that a 1:100 dilution of Triton X-160 compared favorably with several commonly used insecticides in the control of the cabbage aphid, *Brevicoryne brassicae* (L.), on turnips and brussel sprouts and the avocado red mite, *Oligonychus yothersi* (McGregor), on avocado leaves. Another study conducted by Wolfenbarger (1962), failed to demonstrate any toxicity of tensides towards the larvae of the leafminer, *Liriomyza munda* Frick, within their mines.

In a laboratory experiment conducted by Wolfenbarger and Holscher (1967), several commercial tensides were found effective in controlling the cabbage aphid, *Brevicoryne brassicae* (L.), green peach aphid, *Myzus persicae* (Sulcer), a weevil, *Sitophilus zeamais* Motschulsky, red flour beetle, *Tribolium castaneum* (Herbst), and the cowpea curculio, *Chalcodermus aeneus* Boheman. Each tenside was tested by placing the insects in a petri dish containing a piece of filter paper saturated with one of ten tenside concentrations ranging from 10 to 100%. The tensides were emulsified with a 1% concentration of Triton B-1956.

Three nonionic tensides were used against the cabbage aphid. Treatments using each of these materials resulted in LC$_{50}$'s at 4% concentration or less. These were Triton X-100 (Ethoxylated isoctyl phenyl polyethoxyethanol), Triton AF-100 (Polyethylene glycol ether of nonylphenol), and Triton B-1956 (modified phthalic glycerol alkyd resin).
Three nonionic tensides were also tested against the cowpea curculio. All resulted in LC$_{50}$'s at 2% concentration or less. These were Retzanol M-139 (polyoxyethylene thioether), Retzloff L-775 and Retzloff 50 (both composed of mixed fatty acids and oxyalkylated dinonyl phenols).

Twenty-five tensides were tested against the green peach aphid. LC$_{50}$'s for these compounds were found to be 14% concentration or less. These were the anionic tenside Triton GR-7 (sulfonated alkyl esters), the nonionic tensides Retzonol M-139 and Pronon 505 (Ethoxylated alkyl polyethylene ethanol), and the cationic tenside Catanac SN (stearamido propyldi-methyl-B-hydroxyethyl ammonium nitrate).

Forty-five tensides were tested against the weevil Sitophilus zeamais in the study conducted by Wolfenbarger and Holcher (1967). Fourteen resulted in LC$_{50}$'s at 12% or less. Twenty-six tensides were tested against the red flour beetle, five of which demonstrated LC$_{50}$'s at 10% concentration or less.

Wolfenbarger and Holscher noted that several of the tensides were selective against the four different insects tested.

Certain tensides have also been shown to control the bollworm, Heliothis zea (Boddie), tobacco budworm H. virescens (F.), and pink bollworm Pectinophora gossypiella (Saunders). Experiments conducted by Wolfenbarger, Lukefahr and Lowry (1967) showed that cotton bolls dipped in 3 or 5% concentrations of Triton X-150 (ethoxylated alkyl phenol blends with organic sulfonates), or Triton X-100 and Triton X-155 (ethoxylated alkyl phenols), reduced diapausing pink bollworm
larvae by 89%. These workers also demonstrated that ethoxylated tridecyl alcohol containing a mole ratio of 15:1 of ethylene oxide to alcohol, and Retzolate 58 (ammonium salts of ethoxylated alcohol sulfates) were effective against the bollworm and tobacco budworm when used as foliar sprays.

One of the most promising insecticidal uses of tensides is as mosquito larvacides. Taft and Strandtmann (1945) showed that the cationic compound alkylmethybenzylammonium chloride at a 1:100,000 dilution, would kill the eggs, larvae and pupae of the Southern house mosquito Culex pipiens quinquefasciatus Say. Aedes aegypti (L.) larvae, they noted, were somewhat more resistant.

A more recent study conducted by Mulla (1967) showed that several tensides, in the range of 100-200 ppm, were highly effective as mosquito larvacides. Among the most effective chemicals were the cationic beta amines and beta diamines. Mulla also noted a correlation between toxicity and chemical structure of tensides. He found that the beta amines with long carbon chain lengths were more toxic to fourth instar C. pipiens quinquefasciatus than beta amines with short carbon chains.

Most studies of the insecticidal properties of tensides involved commercial preparations. Formulations of these materials are generally complex mixtures of various tensides and are often trade secrets. Correlations between chemical structure and toxic properties have therefore been difficult to make. A study conducted by Maxwell and Piper (1968) evaluated fifty non-ionic tensides whose chemical
structure was known. The pupae of the southern house mosquito were subjected to various concentrations of tensides. Failure of the adults to emerge was the criterion for mortality. Maxwell and Piper noted that some of the tensides were as effective as reference insecticides. Dinonyl phenolethylene oxide with a ratio of 4-6 moles ethylene oxide per mole of alkylphenol was the most effective compound, having an LC$_{50}$ of 1-2 ppm.

Certain tenside-insecticide combinations have been shown to increase the toxicity of the insecticide used. In their study on fatty acids and their soaps, Dills and Menusan (1935) found that pure soap in combination with nicotine sulfate increased the toxicity of the nicotine sulfate. The order of toxicity for the combination was similar to that of the soaps alone--oleate, laurate and caprate.

Turner et al. (1951) studied the effects of thirty polyethylene glycol derivatives as well as several other tensides on the insecticidal properties of nicotine. The studies included spray tests applied to Aphis rumicu (L.) on nasturtiums and injection tests involving milkweed bugs, Oncopeltus fasciatus (Dal.). In agreement with Dills and Menusan (1935), they found that sodium oleate increased the toxicity of nicotine sulfate. Of the thirty polyethylene glycol derivatives studied, seventeen increased the toxicity of nicotine sulfate. Five of these compounds (polyethylene-glycol-1000 monolaurate, 400 and 600 monolaurate, polyethyleneglycol-400 monolaurate and octaethyleneglycol monododecyl ether) increased the toxicity tenfold or more. Six of the compounds did not alter the toxicity of nicotine sulfate.
Five reduced the toxicity, including Triton X-100 [decaethylene glycol mono-p-(1,1,3,3-tetramethylbutyl) phenyl ether], a material also noted for decreasing the toxicity of parathion in studies conducted by Wolfenbarger (1962). Fifteen of the ethyleneglycol derivatives were injected with alkaloid nicotine into the milkweed bug. None increased toxicity.

Tenside-insecticide combinations applied to the larvae of the leafminer, Liriomyza munda Frick were not superior to insecticidal applications alone, according to Wolfenbarger (1962).

Ten commercial tensides were used with parathion and five were used with diazinon. Three tensides were found to reduce the toxicity of parathion. They were plyac, Triton B-1956, and Triton X-100.

A study by Matteson and Taft (1964) involved the testing of 87 tensides added to the systemic insecticides phorate and Zectran TM. Phorate was added to a plant nutrient solution at 100 ppm and Zectran at 50 ppm. The systemic activity of phorate was not effected by any of the tensides. Three tensides, all quarternary ammonium chlorides, enhanced the systemic activity of Zectran. These were Arquad C-50, Ahco DD-50, 50 and tridecyldimethylbenzlyammonium chloride, 50.

Butler (1974) tested nine commercial tensides with malathion EC topically applied to the black carpet beetle, Attagenus megatoma (F.). All of the tensides increased mortality after 72 hours. In her study, 0.2 ul of the insecticide and tenside mixture (0.1% malathion, 0.55% tenside) was applied to the pronotum of each insect. After 72 hrs., applications containing Regulaid (polyethylene polypropanol propylene
glycol) resulted in 93% mortality, Tween 80 (polyethylene sorbitan monolaurate), 84%, Multi Film X-77 (alkylypolyethoxylate and Na⁺ salt of alkylsulfonated alkylate), 83%. Topical applications using malathion EC without an adjuvant resulted in only 6% mortality after 72 hrs.

Two recent studies have provided more direct information concerning enhanced cuticular penetration of insecticide-tenside systems. In the work conducted by Ebeling and Reierson (Ebeling 1974), male American cockroaches were dipped in solutions containing 2% NaF alone, or in 2% NaF and 0.1% Triton X-100. The spiracles of some insects were blocked with fingernail polish while those of other insects were left open. There was no mortality after 24 hrs. among the insects to which NaF alone was applied. The addition of Triton X-100 had a definite synergistic effect on the NaF, resulting in 87% mortality after 24 hrs. Blockage of the spiracles reduced the toxicity of the tenside enhanced NaF, but did not prevent toxication.
II. MATERIALS AND METHODS

Rearing and handling of the insects.

Adult male German cockroaches, *Blattella germanica* (L.) from two colonies of the V.P.I. "normal-strain" reared at the Department of Entomology, V.P.I.&S.U., Blacksburg, Virginia, were used in this study. Nymphs from both colonies were randomly mixed and placed in 4 gal. aquaria fitted with a stack of masonite panels separated by approximately 2 cm. The insects were maintained at approximately 24°C, and were provided with water and commercial dog food. Only adult male cockroaches taken no later than 2 wks. after their final molt were used in the bioassays. Care was taken not to use teneral adults.

The insects were trapped in plastic tubes (3.5 cm. dia., 10 cm. l.) by means of a vacuum powered aspirator. Each tube had a cheesecloth screen at one end and was sealed at the other end by a rubber stopper. All cockroaches were immobilized by cold treatment. This was accomplished by placing the plastic tube "traps" in 1 gal. battery jars which were placed in an ice bath. This method cooled the traps to approximately 2°C, the highest temperature that would allow efficient handling of German cockroaches. During a normal treatment procedure, the cockroaches were kept at 2°C for 2-3 hrs. A preliminary study showed that a 24 hrs. exposure to this temperature produced no apparent ill effects. Cold treatment was preferred to CO₂ anesthesia because of the deleterious effects of CO₂ reported by
Brooks (1956) and Brady and Sternburg (1964). According to Busvine (1971), cold treatment has been shown to be one of the safest methods available.

Chemicals.

Technical diazinon (Ciba-Geigy, 87%), was evaluated with 8 different tensides formulated in an \( H_2O \) emulsion. A listing of these materials can be found in Table I. Technical diazinon in dimethyl sulfoxide (DMSO), Stoddard's Solvent (a refined kerosene), and Stoddard's Solvent with piperonyl butoxide were evaluated for comparison. Each time the Stoddard's Solvent was used, diazinon was initially dissolved in reagent grade acetone making the final formulation 10% acetone. In addition, bioassays using a commercial emulsifiable concentrate, Diazinon 4E (Ciba-Geigy, 47.5%), and Diazinon 4E plus two tenside additives, were also conducted. These formulations are listed in Table II.

All of the tenside formulations emulsifying technical diazinon were formed by the invert emulsion procedure recommended by Osipow (1964). All of the tensides in liquid form were used at 0.5% concentration by volume. This concentration was above the critical micelle concentration. Formulation by weight was not used because several of the tensides are complex mixtures and precise molecular weights were not known. Hexadecyltrimethyl ammonium bromide, here designated as HAB, was the only tenside in solid form used. It was formulated at 0.06% by weight, an amount well above reported critical
micelle concentrations (Mukerjee and Mysels 1971). All tenside formulations produced fairly stable emulsions. Little sedimentation was noted after 24 hrs. Triton X-100 and Regulaid generally formed clear emulsions. The other materials were rather "milky" in appearance. HAB formed the least stable emulsion, having had more sedimentation than the rest. All formulations were the result of serial dilutions from a stock concentration. All were made up in 100 ml volumes except those in DMSO (25 ml volumes). Tenside concentrations were held constant throughout the serial dilutions.

The treatment procedure.

Approximately 0.17 ul of each formulation was applied to the metacoxal area of each cockroach. This volume was delivered by means of a 0.5 ml syringe fitted with a 27 ga. needle ground to a blunt tip. The syringe was driven by a micrometer screw microapplicator. Twelve insects were used for each concentration. Five different diazinon concentrations were used for each formulation type. In addition, control treatments were conducted using the appropriate formulation components minus diazinon.

The entire treatment method proceeded as follows. First, 12 male cockroaches were caught in each of the plastic tube traps and immobilized as previously described. Next, the insects were gently placed on a small aluminum tray lined with filter paper which rested on a cold plate. The insects were then aligned with a camel's hair brush and treated with the microapplicator. Finally, they were placed in
1 qt. wide mouth jars greased at the rim with petroleum jelly and covered with cheesecloth. Each jar was supplied with a water vial and a pellet of commercial dog food. Subsequent to treatment, all of the jars were placed in an environmental chamber maintained at 25°C, 12 hrs. light, 12 hrs. dark. The relative humidity ranged from 74-82%.

All of the treatments were made at approximately the same time each day. Mortality checks were made at 2, 4, 8, 24, and 48 hrs. after treatment. Mortality was found to stabilize at 48 hrs., consequently this time period was used to compute the response curves. Three replications were conducted for all of the formulations except for diazinon in Stoddard's Solvent, in Triton X-100 and in Regulaid. Four replications were made for these materials. At least one replication for each formulation bioassayed was conducted on a separate day.

**Statistical analysis.**

All data were analyzed by computer program. The 1976 Statistical Analysis System (SAS'76) program entitled "Probit Procedure" was followed (Barr et al. 1976). Fiducial limits for the LD_{50}'s were calculated at the 95% confidence level from the computed variance using the following formula (Busvine 1971):

\[ m_1, m_2 = m \pm 1.96 \sqrt{V} \]
where $V$ equals the variance for the $LD_{50}$, $m$ equals the $LD_{50}$, and $m_1$ and $m_2$ are the upper and lower limits for the $LD_{50}$. 
TABLE I. Tensides used to emulsify diazinon.

<table>
<thead>
<tr>
<th>Tenside</th>
<th>Chemical components</th>
<th>Manufacturer</th>
<th>Type*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-Film&lt;sup&gt;TM&lt;/sup&gt;</td>
<td>alkylarylpolyethoxy ethanol, free fatty and combined fatty acids, Glycol ethers, dialkyl benzenedicarboxylate, isopropanol.</td>
<td>Colloidal Products</td>
<td>N</td>
</tr>
<tr>
<td>Bio-88&lt;sup&gt;TM&lt;/sup&gt;</td>
<td>alkyl polyethoxy ethanol, free fatty acids, isopropanol</td>
<td>Colloidal Products</td>
<td>N</td>
</tr>
<tr>
<td>HAB</td>
<td>hexadecyltrimethyl ammonium bromide</td>
<td>Eastman Kodak</td>
<td>C</td>
</tr>
<tr>
<td>Igepal CO-630&lt;sup&gt;TM&lt;/sup&gt;</td>
<td>nonylphenoxypoly (ethylenoxy) ethanol</td>
<td>General Aniline and Film Corp.</td>
<td>N</td>
</tr>
<tr>
<td>Multi-Film X-77&lt;sup&gt;TM&lt;/sup&gt;</td>
<td>alkylarylpolyethoxy-ethylene glycols, free fatty acids, isopropanol</td>
<td>Colloidal Products</td>
<td>N</td>
</tr>
<tr>
<td>Regula&lt;sup&gt;TM&lt;/sup&gt;</td>
<td>Polyethylenepolypropoxy-propoxy-propanol, dihydroxypropane</td>
<td>Colloidal Products</td>
<td>N</td>
</tr>
<tr>
<td>Triton X-100&lt;sup&gt;TM&lt;/sup&gt;</td>
<td>condensation product of ethylene oxide with analphenol</td>
<td>Rohm and Haas</td>
<td>N</td>
</tr>
<tr>
<td>Tween 80&lt;sup&gt;TM&lt;/sup&gt;</td>
<td>Polyethylene sorbitan mono-oleate</td>
<td>Atlas Chemical Division</td>
<td>N</td>
</tr>
</tbody>
</table>

*<sup>N</sup> = nonionic  
C = cationic
TABLE II. Formulations used in the bioassay of diazinon against the German cockroach.

<table>
<thead>
<tr>
<th>Tenside emulsifier</th>
<th>Insecticide</th>
<th>Solvent(s)</th>
<th>Concentration of diazinon (ug/insect)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Bio-Film</td>
<td>Technical diazinon</td>
<td>de-ionized distilled water</td>
<td>0.04-0.13</td>
</tr>
<tr>
<td>2) Bio 88</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) HAB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4) Igepal CO-630</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5) Multi-Film X-77</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6) Regulaid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7) Triton X-100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8) Tween 80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9) none</td>
<td>Diazinon 4ETM</td>
<td>de-ionized distilled water</td>
<td>0.07-0.17</td>
</tr>
<tr>
<td>10) Igepal CO-630</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11) Multi-Film X-77</td>
<td>Technical diazinon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12) none</td>
<td>Tech. diaz. + pip. butoxide</td>
<td>Stoddard's Solvent*</td>
<td>0.03-0.10</td>
</tr>
<tr>
<td>13) none</td>
<td>Technical diazinon</td>
<td></td>
<td>0.02-0.10</td>
</tr>
<tr>
<td>14) none</td>
<td></td>
<td>DMSO**</td>
<td>0.01-0.07</td>
</tr>
</tbody>
</table>

*Stoddard's Solvent, #3 grade (a refined kerosene) + 10% reagent grade acetone.

**dimethyl sulfoxide.
III. RESULTS

The LD$_{50}$ value for each formulation tested is recorded in Table III, along with the corresponding fiducial limits. The probit regression line is presented along with the fiducial limits for the LD$_{50}$ in Figures 1-14. The equation for each line is also reported along with the Chi-square probability. The Chi-square values indicate that there was no significant heterogeneity among the insect populations tested at the 5% level.

None of the tensides in water controls proved toxic when used without diazinon. Stoddard's Solvent alone, Stoddard's Solvent plus 0.5% piperonyl butoxide, and DMSO alone were also non-toxic when applied according to the methods previously described.

Five of the tensides evaluated produced emulsions with diazinon that were comparable to diazinon in Stoddard's Solvent. These were Triton X-100, Multi-Film X-77, HAB, Tween 80, and Bio-Film. The LD$_{50}$'s for the emulsions using Igepal CO-630, Regulaid, and Bio 88 were significantly higher than the LD$_{50}$ for diazinon in Stoddard's Solvent.

All of the tensides used to emulsify technical diazinon seemed to perform better than the commercial emulsifiable concentrate Diazinon 4E. Two of the tensides, Triton X-100 and Multi-Film X-77, produced emulsions having LD$_{50}$'s significantly lower than that for Diazinon 4E.

The two tensides added to Diazinon 4E, Igepal CO-630 and Multi-Film X-77, did not enhance toxicity. They were, if anything, antagonistic.
Diazinon dissolved in Stoddard's Solvent and piperonyl butoxide, and diazinon dissolved in DMSO were significantly more toxic than any of the water emulsions or diazinon in Stoddard's Solvent alone.
TABLE III. LD$_{50}$ values for 14 formulations of diazinon used against the male German cockroach.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>LD$_{50}$ (ug/insect)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(fiducial limits)</td>
</tr>
<tr>
<td>1) Bio-Film + diazinon</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>(0.08-0.12)</td>
</tr>
<tr>
<td>2) Bio 88 + diazinon</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>(0.09-0.17)</td>
</tr>
<tr>
<td>3) HAB + diazinon</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>(0.08-0.12)</td>
</tr>
<tr>
<td>4) Igepal CO-630 + diazinon</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>(0.09-0.14)</td>
</tr>
<tr>
<td>5) Multi-Film X-77 + diazinon</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>(0.08-0.10)</td>
</tr>
<tr>
<td>6) Regulaid + diazinon</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>(0.10-0.15)</td>
</tr>
<tr>
<td>7) Triton X-100 + diazinon</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>(0.06-0.09)</td>
</tr>
<tr>
<td>8) Tween 80 + diazinon</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>(0.08-0.12)</td>
</tr>
<tr>
<td>9) Diazinon 4E</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>(0.12-0.21)</td>
</tr>
<tr>
<td>10) Diazinon 4E + Igepal CO-630</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>(0.13-0.25)</td>
</tr>
<tr>
<td>11) Diazinon 4E + Multi-Film X-77</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>(0.09-0.61)</td>
</tr>
<tr>
<td>12) Diazinon in Stoddard's Solvent</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>(0.06-0.08)</td>
</tr>
<tr>
<td>13) Diazinon + piperonyl butoxide in</td>
<td>0.05</td>
</tr>
<tr>
<td>Stoddard's Solvent</td>
<td>(0.04-0.06)</td>
</tr>
<tr>
<td>14) Diazinon in DMSO</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>(0.03-0.05)</td>
</tr>
</tbody>
</table>
Figure 1. Response of normal-strain male German cockroaches to diazinon emulsified with Bio-Film™.
\[ y = 3.90x + 8.50 \]

Probability > \( \chi^2 = 0.67 \)

\( \text{LD}_{50} = 0.13 \mu g/\text{insect} \)

**FIGURE 2.** Response of normal-strain male German cockroaches to diazinon emulsified with Bio-88™.
$y = 4.15x + 9.17$
Probability > $\chi^2 = 0.34$
$L_D_{50} = 0.10 \mu g/\text{insect}$

**FIGURE 3.** Response of normal-strain male German cockroaches to diazinon emulsified with hexadecyltrimethylammonium bromide.
**Figure 4.** Response of normal-strain male German cockroaches to diazinon emulsified with Igepal CO-630™.
FIGURE 5. Response of normal-strain male German cockroaches to diazinon emulsified with Multi-Film X-77™.

\[ y = 4.66x + 9.86 \]

\[ \text{Probability} > \chi^2 = 0.89 \]

\[ \text{LD}_{50} = 0.09\mu g/\text{insect} \]
FIGURE 6. Response of normal-strain male German cockroaches to diazinon emulsified with Regulaid™.
$y = 3.08x + 8.54$

Probability $> \chi^2 = 0.54$

$LD_{50} = 0.07 \mu g / \text{insect}$

**Figure 7.** Response of normal-strain male German cockroaches to diazinon emulsified with Triton X-100TM.
FIGURE 8. Response of normal-strain male German cockroaches to diazinon emulsified with TWEEN-80™.
FIGURE 9. Response of normal-strain male German cockroaches to Diazinon 4E \(^\text{TM}\).
\[ y = 4.32x + 8.24 \]

Probability > $\chi^2 = 0.59$

\[ LD_{50} = 0.18 \mu g/\text{insect} \]

**FIGURE 10.** Response of normal-strain male German cockroaches to Diazinon 4E™ + Igepal-CO-630™.
$y = 2.26x + 6.42$

Probability > $\chi^2 = 0.60$

$LD_{50} = 0.24 \mu g/insect$

**Figure 11.** Response of normal-strain male German cockroaches to Diazinon 4E $^\text{TM}$ Multi-Film X-77 $^\text{TM}$. 
$y = 4.24x + 9.91$
Probability $> \chi^2 = 0.92$
$LD_{50} = 0.07 \mu g/insect$

**FIGURE 12.** Response of normal-strain male German cockroaches to topical applications of diazinon in Stoddard's Solvent.
\[ y = 2.70x + 8.43 \]
Probability $> \chi^2 = 0.55$
\[ \text{LD}_{50} = 0.05 \mu g/\text{insect} \]

**FIGURE 13.** Response of normal-strain male German cockroaches to diazinon + piperonyl butoxide in Stoddard's Solvent.
FIGURE 14. Response of normal-strain male German cockroaches to diazinon in DMSO.

\[ y = 2.37x + 8.42 \]

Probability \( > \chi^2 = 0.19 \)

\[ \text{LD}_{50} = 0.04 \mu g / \text{insect} \]
IV. DISCUSSION

0-100% mortality resulted from a rather narrow concentration range of diazinon for all of the formulations tested. Therefore it is debatable whether the difference in performance between the tensides is biologically significant or not. Correlation between chemical composition and performance of the tensides is not possible because most of them were complex mixtures of various organic compounds (Smith 1975).

The performance of the tenside emulsions was probably the result of several different phenomena related to surface activity. All of the tensides used in this study are good wetting agents. By lowering the surface tension of water, these tensides would have allowed the emulsion to enter the cuticle more freely. The importance of wetting in relation to insecticide toxicity was shown by Wilcoxon and Hartzell (1931).

The ability of the tenside-insecticide emulsion system to partition between the polar and nonpolar components of the insect cuticle was probably also an important factor influencing the performance of the materials studied. The importance of phase partition to the permeation of insect cuticles (previously reviewed) was shown by Webb and Green (1945).

The literature shows at least one investigation demonstrating increased cuticle penetration using one of the tensides used in this study. Ebeling and Reierson (Ebeling 1974) showed that a 0.1% solution
of Triton X-100 increased the permeability of the American cockroach cuticle to water.

None of the tenside emulsions employed in this study were more toxic than the Stoddard's Solvent formulation of diazinon. Quite likely, this oil base preparation dissolved certain components of the cuticle wax layer, disrupting the cuticle enough for the formulation to penetrate. The tenside emulsions, probably disrupted the cuticle wax through emulsification and detergent action. This disturbance apparently did not disrupt the cuticle enough to enhance the toxic action of diazinon. Lack of a dramatic synergistic effect may have been due to the nature of the cuticular grease layer, a rather unique feature of the cockroach.

The results of this study indicate that the commercial emulsifiable concentrate of diazinon was not as effective as the other emulsion formulations tested. While the diazinon EC was the easiest material to formulate (it formed an emulsion spontaneously) it was probably less efficient in partitioning between the nonpolar and polar factions of the cockroach integument and/or less efficient in wetting the cuticle.

The quality of the emulsions prepared may also have had a bearing on performance. Triton X-100 formed a translucent emulsion with diazinon, an indicator of fine particle size (Osipow 1964). This formulation had the lowest LD$_{50}$ of the tenside formulations evaluated, 0.07 ug/insect. Emulsion quality alone cannot account for the performance of all the formulations, however. Regulaid also produced a
translucent emulsion but did not form one of the better preparations. HAB formed the poorest emulsion of the tensides evaluated, yet it produced one of the more toxic formulations. Although care was taken to prepare each emulsion in an identical manner, uniformity of each preparation could not be assured.

The cationic tenside HAB may have functioned differently from the non-ionic tensides. This is suggested by the toxic properties of several other cationic compounds previously described. HAB usually functions best at pH 12 (Ahmad 1975). Diazinon is unstable in strongly alkaline conditions. Consequently, the pH of the HAB formulation was not altered.

The addition of Igepal CO-630 and Multi-Film X-77 to the commercial diazinon EC did not enhance toxicity, contrary to what Butler (1974) found for the tensides she added to malathion EC when applied topically to carpet beetles. Because Butler used a different insect and an insecticide structurally quite different from diazinon, a meaningful comparison between her results and the results of this study cannot be made.

The addition of the pyrethrin synergist, piperonyl butoxide, to diazinon in Stoddard's Solvent definitely enhanced the toxicity of diazinon. This result is consistent with the findings of Hoffman et al. (1954). In a study conducted on DDT resistant house flies, they found that applications of diazinon plus piperonyl butoxide (1:5) resulted in 100% mortality at a concentration of diazinon where no mortality normally occurred when this insecticide was used alone.
Piperonyl butoxide does not synergize all organophosphates. Rai et al. (1956) noted that it antagonized malathion. The synergistic effect piperonyl butoxide has with several insecticides is attributed to its ability to inhibit insect microsomal mixed-function oxidases (Agosin and Perry 1974).

Diazinon dissolved in DMSO proved to be the most efficient formulation in this study. This material is highly soluble in water and in several organic solvents and is known for its unique penetrating properties in living systems (Leake 1966). Olinger and Kerr (1969) found that DMSO increased the \( LT_{50} \) value for carbaryl, had no significant effect on the \( LT_{50} \) and \( LT_{95} \) values for malathion, and decreased the \( LT_{50} \) and \( LT_{95} \) values for endosulfan in topical dose studies conducted on 4th instar larvae of the Mexican bean beetle, Epilachna varivestis Mulsant. Apparently DMSO is more efficient with some insecticides than with others.

Several practical applications for DMSO may be found in the future. It has been studied as a carrier for insect repellants on humans (Leake 1966) and may be useful in animal systemic formulations. Investigations probing the value of DMSO in plant systemics has been disappointing, however (Olinger and Kerr 1969). Because DMSO can carry substances deeply and rapidly through the human skin (Leake 1966), highly toxic compounds would be extremely dangerous if prepared with it and would be of little practical value.

All the formulations used in this study have been evaluated by means of topical dose treatments. While several of the diazinon
emulsions proved to be as effective as diazinon in Stoddard's Solvent using this technique, this does not necessarily mean that they will perform as well in practical applications such as surface residue treatments. Additional studies need to be conducted to determine this.

The LD\textsubscript{50}'s calculated from this study are somewhat lower than those reported by other workers who have conducted topical dose experiments with diazinon on German cockroaches. Fisk and Isert (1953) reported an LD\textsubscript{50} of 0.33ug per female cockroach for diazinon formulated in 20% acetone and 80% Deo-Base (a refined kerosine). This difference is not unexpected, considering the weight difference between the male and female of the species. The average adult female from the V.P.I. normal-strain weighed 0.090g. The average male weighed 0.054g.

Kitagaki et al. (1972) reported an LD\textsubscript{50} of 0.472ug per male German cockroach for diazinon formulated in pure acetone. This higher value is probably the result of the different solvents used in their investigation and this one.
V. SUMMARY

The effect of 8 tensides on the toxicity of diazinon were investigated by means of topical dose applications to male adult German cockroaches, *Blattella germanica* (L.). Other diazinon formulations were evaluated for comparison. These were Diazinon 4E, Diazinon 4E plus Igepal CO-630, Diazinon 4E plus Multi-Film X-77, diazinon in DMSO, diazinon in Stoddard's Solvent, and diazinon in Stoddard's Solvent plus piperonyl butoxide.

Five of the tensides formed emulsions that were similar in toxicity to diazinon in Stoddard's Solvent. These were, in order of decreasing LD$_{50}$'s, Bio Film, Tween 80, hexadecyltrimethyl ammonium bromide (HAB), Multi-Film X-77, and Triton X-100.

All of the tenside emulsions were more effective than Diazinon 4E. Emulsions formed with Triton X-100 and Multi-Film X-77 had LD$_{50}$'s significantly lower than the Diazinon 4E formulation. The two tensides added to Diazinon 4E seemed to decrease effectiveness.

DMSO and piperonyl butoxide were the two most effective additives to diazinon. Both were synergistic with respect to diazinon in Stoddard's Solvent.

The results from this investigation indicate that several of the tested formulations may prove effective in cockroach control. Additional studies of these preparations under practical conditions need to be made.
VI. LITERATURE CITED


Ahmad, S. 1975. Personal communication.


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VII. VITA

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THE EFFECT OF EIGHT TENSIDES ON THE TOXICITY OF DIAZINON TOPICALLY APPLIED TO THE MALE GERMAN COCKROACH

by

William H. Candler, Jr.

(ABSTRACT)

The effect eight different surface active agents (tensides) have on the toxicity of diazinon was evaluated through topical dose treatments applied to male German cockroaches Blattella germanica (L.). A commercial emulsifiable concentrate of diazinon was also evaluated with and without tenside additives. In addition, diazinon in Stoddard's Solvent, diazinon in Stoddard's Solvent plus piperonyl butoxide, and diazinon in DMSO were evaluated for comparison.

All of the tenside formulations had lower LD₅₀'s than diazinon EC. Triton X-100, hexadecyltrimethyl ammonium bromide, Multi-Film X-77, Tween 80, and Bio-Film formed emulsions that were comparable in toxicity to diazinon in oil (Stoddard's Solvent). DMSO and piperonyl butoxide formed the most effective preparations with diazinon, having LD₅₀'s significantly lower than all the other formulations tested.

Results of the bioassays indicate that several of the formulations might be effective for use in cockroach control.