THE BIOLOGICAL EVALUATION OF FOUR SPRAY ADDITIVES WITH *BLATTELLA GERMANICA* (LINNAEUS) AND *PERIPLANETA AMERICANA* (LINNAEUS)

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PREFACE

I have been interested in cockroach control since I began serving as an entomologist in the U. S. Army Medical Service Corps in 1955. In this position, I have been required to give technical assistance in the control of cockroaches and other insects in military housing and food serving establishments. Interest in additives used in pesticides was stimulated by reading several studies conducted at Oklahoma State University and by conversations with Dr. D. E. Howell, Dr. R. G. Price, and Dr. P. D. Sterling, Jr. A research project on additives, I felt, would better prepare me for future assignments in the U. S. Army and the results could be helpful in planning more effective insect control programs.

I wish to express my appreciation to: the U. S. Army for making this research project possible; Dr. D. E. Howell, Dr. R. R. Walton, and Dr. R. D. Morrison for their guidance and encouragement throughout this research; Dr. R. G. Price and Dr. J. H. Young for their suggestions and criticisms of the project; LTC N. E. Pennington, Z. B. Mayo, C. Bush, and Lt Cdr R. V. Peterson who helped in rearing of specimens and conducting research tests; and to my wife, Gladys, who aided in preparing the manuscript and who gave encouragement and understanding throughout the research project.

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INTRODUCTION

A large number of spray additives has been produced since the development of DDT usage in 1939. These additives may be classified as toxicants, surfactants, solvents, repellents, attractants, deodorants, or synergists. The toxicant is generally considered to be the main additive in an insecticide formulation. Research on toxicants has produced many new insecticides within the three major groups - chlorinated hydrocarbons, organophosphates, and carbamates. The bulk of publications on spray formulations has stressed the biological evaluation of the toxicant only.

The utilization of many different insecticides has required additives of variable composition to deliver the toxicant effectively to the insect. Additives, such as surfactants, may have adequate chemical and physical evaluation but may lack sufficient biological evaluation prior to their use in a spray formulation. Thus some additives have acted as repellents or as antagonists to insects when they were used with the toxicant. These undesirable properties of additives have resulted in less effective control of insects.

The picture seems even more confusing after reading pesticide formulations. The toxicant is listed as a specific percentage and remaining additives may be grouped and listed as inert ingredients.

Sterling (1966) biologically evaluated over two hundred different pesticide formulations for their repellent action on _P_. _americana_, American cockroach, and _B_. _germanica_, German cockroach. In addition, he tested the most promising repellents at three concentrations and after aging the one spray deposit for periods up to forty-two days. The research reported here is an extension of Sterling's research. A synergist (piperonyl butoxide), a repellent (R-11), and two surfactant additives (Triton X-155 and Volpa-3) were selected and evaluated as repellents for a 6-month period. White pine boards were treated at monthly intervals with additives and the repellency of these materials evaluated at 30-day periods.

At the end of the 6-month period, the effectiveness of diazinon alone and diazinon plus additive was evaluated. The purpose of this study was to simulate the deposits of additives which a restaurant or household might receive for cockroach control and to determine what effect these additives would have on the control of cockroaches.

REVIEW OF THE LITERATURE

The response of insects to repellents has been the subject of many excellent reviews. The methods of testing, types of materials, uses, and actions of repellents have been described by Dethier (1947, 1956), Shambaugh et al. (1957), Taylor (1960), Price (1963), and Jacobson (1966). Spray additives designed for purposes other than repellents or attractants are generally not included in these reviews. Sterling (1966) has listed a variety of spray additives, i.e., surfactants, synergists, perfumes, which repel both American and German cockroaches. This review will cover only the additives tested or the class of additives tested.

Biological Evaluation of Repellents

Goodhue (1960) tested 1000 materials for their repellency to German and American cockroaches. The best repellents were R-11, R-55, R-874, and R-949. The response of American cockroaches varied from 70% to 100% repellency with R-11. R-11 deposits were aged on glass, linoleum, masonite, and painted wood and were tested with German cockroaches. This chemical was generally less effective at 7 days than at 1 and 14 days. The porosity of the surface did not appear to be related to the results. The effectiveness of R-11 was improved by adding a synergist, MGK-264.

R-11 is being added to many insecticide formulations, especially cockroach sprays (Goodhue and Howell, 1960). This compound improves the performance of the spray by acting as both an agitator and a repellent. A common spray formulation is as follows: pyrethrin, 0.075%; R-11, 1.0%; piperonyl butoxide (PB), 0.15%; MGK-264, 0.25%; and petroleum distillate, 98.525%.

R-11 is also used as a repellent in livestock sprays to control house flies and stable flies. A formulation consisting of 0.025% pyrethrins, 1.0% MGK-264, and 98.7% petroleum distillates was tested alone and with 0.2% R-11 added on cattle. The mixture with R-11 gave better residual repellency, which built up after three applications (Goodhue and Howell, 1960). Roberts et al. (1963) tested a similar formulation against stable flies but reduced the concentration of R-11 (0.1%) and MGK-264 (0.62%). These authors found that pyrethrins alone gave better control than the mixtures of additives and pyrethrins. This discrepancy may be explained by the reduction in the concentration of repellent, R-11, and synergist, MGK-264.

Whiting (1960) compared the effectiveness of pyrethrins and PB mixture and R-11 as repellents to German cockroaches. The pyrethrin preparation was more repellent than R-11 for the first three weeks; however, R-11 performed better at five, seven, and nine weeks. At the end of nine weeks, R-11 repelled 26% compared to 7% for the pyrethrin preparation.

Cockroaches continue to be a problem in food and beverage manufacturing plants. Beer distributing and manufacturing plants in particular receive infested empty cases from customers. Mallis et al. (1961) treated beer cartons with an emulsion of 1% R-11 and 3% MGK-264. This treatment afforded 93.8% repellency from German cockroach invasions after one month and 64.2% repellency after six months.

Price (1963), using ten testing procedures, investigated 230 compounds against four household species of cockroaches. The repellents R-55, R-874, R-1116, R-1583, or R-1784 were superior to R-11 at concentrations from 1 to 25% and after aging for periods of 90 days. R-11 was sometimes more effective with increased age of the deposits to German cockroaches (three tests), but the difference was not as noticeable with American cockroaches. In wafer tests, 1% R-11 repelled 88% at 10 days and 100% at 20 days. Sterling (1966) indicated a slight reduction in repellency of R-11 after aging 1, 7, 21, and 42 days with both American and German cockroaches.

R-11 has received less evaluation as a control agent for plant insects. Wolfenbarger (1962) applied a foliar spray of R-11 to peas for leafminer control. The repellent was not effective in preventing or controlling leafminer infestations.

Biological Evaluation of Surfactants

The term surfactant is a coined word that designates surface-active agents. A surfactant is a compound that lowers the surface tension or interfacial tension, or both, of an emulsion. Surfactants may include emulsifiers, detergents, and wetting agents (Behrens, 1964). Bennet et al. (1968) have added to the above list, penetrants, dispersing agents, foaming agents, and protective colloids. The mode and action of surfactants are described by both authors. They emphasize their use on plants, with and without herbicides. The effects of surfactants with herbicides has received greater attention than with insecticides.

Surfactants were used early as contact insecticides (Siegler and Popenoe, 1925, Cory and Langford, 1935, and Ginsburg, 1935). In order to achieve maximum control, insects were either dipped or sprayed with the various materials. Sulfonated alcohols, sulfonated phenols, and sulfonated fatty acids were the general groups used most frequently. The thorough wetting of the insect's cuticle was found to be important in increasing mortality. The fatty acids were believed to penetrate the body wall and cause hemolytic action on the hemolymph and body cells.

Dills and Menusan (1935) examined some of the soaps in use and found that the water content varied 30 to 70% by weight in different brands. They concluded that the conflicting reports of experiments were probably due to the unknown water content. These same authors found that better potassium soaps and nicotine increased the effectiveness of the insecticide. The improved chemical quality of today's surfactants can be attributed to these early authors.

Turner et al. (1951) tested 30 polyethyleneglycol derivatives at 0.5% concentration with 0.04% nicotine on aphids. Toxicity of nicotine was increased with 19 of the surfactants, was unaffected by 6 others, and was decreased by the remaining 5. They labelled the increased toxicity of the surfactant-toxicant combination synergism and believed this synergism occurred because of increased penetration of the cuticle. Injection of the chemicals into insects did not result in synergism. The molecular weight and ethyleneglycol chain length were believed to influence the ability of the surfactant to penetrate the cuticle.

The concentration of a surfactant may influence its effectiveness with an insecticide. Hartzell and Wilcoxon (1960) reported that Triton X-155 spray of 2500 ppm was no more effective to plant mites than the control. The combinations of Triton X-155 at 2500, 5000, and

10,000 ppm and organophosphorous insecticides increased the toxicity of toxicants as additive concentration was increased, except in one case. Malathion exhibited a decrease in toxicity when the surfactant concentration was increased from 5000 to 10,000 ppm. No explanation was given for the increased or decreased activity.

Surfactants have been investigated to determine if they increase or decrease the residual life of an insecticide. Wolfenbarger et al. (1962, 1963a) tested several surfactants that showed no increase in residual of diazinon, parathion, or Dylox to leafminers. An increase in the concentration of surfactants did increase the toxicity of these insecticides with one exception. Triton X-100 insecticide combinations did not always give increased insect control. The addition of paraffin oils to surfactants enhanced the toxicity to aphids (Wolfenbarger, 1964). A combination of surfactants and oils with insecticides increased the initial and residual control of aphids.

Surface-active agents have been studied to determine if they prevent insect emergence from pupae. Bollworms were dipped into Triton X-155, Triton X-150, and Triton X-100 at 2% and 5% (Wolfenbarger et al., 1967). The adult emergence was reduced 89% or more at both concentrations. The contact and fumigant toxicity of surfactants was determined with aphids and beetles by Wolfenbarger and Holscher (1967). Within the nonionic group of surfactants, the toxicities varied from 100 to 15% when used at concentrations from 50 to 100%. Some nonionic emulsions showed selected toxicity to aphid species.

The evaluation of household insects with surfactants has received less attention than plant insects. Madden et al. (1946) tested aerosols of DDT with and without two wetting agents. Vatsol OT added to the aerosol increased the kill of mosquitoes but resulted in no effect on house fly mortality. Using a formula containing Nopco 1216, the mortality of both insects was much less than that obtained with the basic DDT formula.

Several authors have evaluated surfactants as larvicides. With the development of insecticides, surfactant-toxicant combinations proved to be more effective. More recently, insecticide resistance has become a major problem in mosquito control. A renewed interest has manifested itself in the development of surfactants as larvicides. Taylor and Schoof (1967) found household detergents and quaternary ammonium compounds were effective larvicides with Aedes aegypti larvae.

Mulla (1967a, 1967b) evaluated 120 aliphatic, fatty, and alkyl amines against both mosquito larvae and pupae. These compounds showed extreme ranges of toxicity from no biological activity at 200 ppm to an LC_{50} of 0.2 ppm on both larvae and pupae. The rapid biocidal activity of the materials was not due to lowering of surface tension, since other surfactants lower the surface tension better without killing mosquitoes. Biocidal action may be due to changes in the physical, chemical, or electrical properties of the cuticle, disruption of epidermal layer or anal gills, or interference of hormone and amino acid metabolism.

Maxwell and Piper (1968) tested 50 nonionic ethyleneoxide adjuvants on mosquito pupae. Some of the compounds were more effective in control of pupae than the reference insecticides, malathion and trichlorfon. Toxicity of these materials was primarily dependent upon the length of the ethyleneoxide chains.

Ebeling et al. (1967) added detergents, trisodium phosphate (TSP), and sodium alkyl sulfate to boric acid solutions for cockroach control. A more rapid kill was obtained with the sodium alkyl sulfate and boric acid, but it was not statistically significant. This same detergent significantly enhanced the insecticidal efficiency of sodium fluoride. TSP was slightly insecticidal when used alone but the boric acid and TSP combination decreased the effectiveness of the boric acid, changing it to sodium borate.

Insects exposed to dodecyl alcohols have exhibited highly unusual morphological and physiological effects (Pence et al., 1969). Earlier workers tested similar compounds derived from coconut oil without noting any unusual activity (Siegler and Popenoe, 1925). However, termites, German cockroaches, carpet beetles, and confused flour beetles showed progressive atrophy leading to loss of legs, albinism, wing deformation, impeded circulation of haemolymph, and in some cases, sterility. Cockroaches were observed to avoid residues, and repellent tests confirmed their repellent properties.

The preceding references have discussed the use of surfactants in contact sprays or dips to increase or decrease toxicity. The repellency or attractancy of additive residues was not mentioned as influencing these results. Foster (1955) reported three emulsifiers, Atlas E-1276, Emcol 74, and Emcol 77, to be repellent to Japanese beetles. However, DDT combined with emulsifiers attracted more beetles than surfactants alone. Kadenatsii (1962) applied a 10-30% soap mixture and RV-5 to cows as a mosquito repellent. The residue of soap emulsion and RV-5 repelled more mosquitoes than dimethylphthalate.

Sterling (1966) listed 18 emulsifiers (including Triton X-155) that were repellent to either German or American cockroaches. These compounds were tested at three concentrations after aging for 1, 7, 21, or 42 days. The chemicals did not always show an increase in repellency response with an increase in concentration or a decrease in repellency upon further aging. Residues of Toximul-P plus diazinon were less toxic to German cockroaches than diazinon alone. This indicated that reduced control may be due to repellency. Surfactants have been reported by Jensen et al. (1961) which lower the toxicity of herbicides.

Biological Evaluation of Synergists

The biological evaluation of synergists has been well reviewed by Hewlett (1960), Metcalf (1967), and O'Brien (1967). The latter author defines synergism as the phenomenon which occurs when the toxicity of two compounds applied together is greater than that expected from the sum of their effects when applied separately. Antagonism is the opposite phenomenon of synergism. A successful example of synergism is the use of pyrethrin with PB synergist to control house flies (Chamberlain, 1950). The review presented here will be primarily restricted to biological evaluation of this synergist alone and in combination with diazinon and some other insecticides.

PB exhibits a wide range of biological activity when it is used with other insecticides. Pyrethrin and PB combinations were mentioned previously as effective repellents in control of cockroaches and flies. Tenet (1959) reported little advantage of PB as a synergist with pyrethrin for control of the cigarette beetle and recommended only 1% pyrethrin spray. Hadaway (1963) found a high degree of synergism when PB was combined with natural pyrethrins or carbamates on house flies. The range of activity of this synergist with these two groups of insecticides varies widely with species and methods of testing.

Hoffman et al. (1954) exposed house flies to residues of PB and organic phosphorous compounds. Synergism was noted with EPN, methyl parathion, coumaphos, and diazinon, but not with malathion. Hadaway et al. (1963) topically applied methyl parathion, malathion, and diazinon with the same synergist combinations. Antagonism was markedly exhibited with malathion but no marked synergistic activity was found with parathion and diazinon. Rai (1959) earlier obtained antagonism with malathion and PB. The discrepancies in these results show differences in experimental design. Hadaway's and Rai's improved technique of computing LD-50's and applying materials directly to the insect cuticle accounts for a more accurate determination of synergism or antagonism than Hoffman. In addition, topical applications reduce interference of repellent action and the uncertainty as to the amount of material on an insect.

Zschintzsch (1961) used Drosophilia melanogaster to evaluate parathion, malathion, and diazinon with PB. Low concentrations of these insecticides and PB generally produced synergism, but high concentrations of these materials produced antagonism. The diversity in organophosphorous compounds makes it difficult to generalize on joint action of a synergist and toxicant (Metcalf, 1967). The metabolism and detoxification by synergists seems to be accomplished by a variety of means. O'Brien (1961) names synergistic compounds modifiers or moderators. In the case of synergism, the modifiers inhibit a metabolizing system whose purpose is to detoxify some insecticide and thus the modifier performs as a synergist. The modifiers, whose major function is to toxify (activate) the toxicant, act as antagonists.

Less information is available on the effects of PB when it is used alone with insects. Chamberlain (1950) exposed house flies to 200 mg/sq ft deposits of the synergist for 60 minutes with no deaths occurring. Treated panels aged 14, 33, and 59 days and tested with house flies still exhibited no toxicity. Hadaway (1963) treated topically both mosquitoes and house flies with this compound with no toxic effect observed. The above tests were designed to prevent evaluation of the material as a repellent, rather than a toxicant.

Sterling (1966) tested the repellency responses of both German and American cockroaches to surfaces treated with PB. The material was applied to panels at 0.01, 0.1, and 1.0% concentrations and then aged for 1, 7, 21, and 42 days. The responses of German cockroaches showed a general decrease in repellency with the lowering of concentrations and the passing of time. American cockroach response did not always follow this same pattern of response. A decrease in concentration or increase in age of deposits sometimes increased the repellency response.

The effects of synergists on the development of insects has been studied with house flies (Hayashi, 1966). PB, safroxon, sulfoxide, and others were added to culture mediums at 0.03, 0.06, or 0.25%. Both sulfoxide and safroxon inhibited development of house fly larvae at the two lower concentrations, but PB showed no detectable activity at all three concentrations. Sulfoxide and safroxon were applied topically to last instar larvae. The percentage of pupation and adult emergence was decreased, thus showing a delayed toxicity to the house fly.

The residual life of PB deposits has been studied with various carriers, surfaces, and techniques. Chamberlain (1950) treated plywood with kerosene and synergist and determined the deterioration of deposits by biological evaluations with house flies. He found no deterioration after 65 days of aging deposits. Blinn et al. (1959) determined the half-life of PB on wheat by chromographic and colorimetric procedures. A wettable powder formulation had a half-life of 9.8 weeks on wheat.

Incho et al. (1953) treated kraft paper with an oil solution, emulsion, or wettable powder of pyrethrins and PB. Both chemical and biological evaluations indicated no loss in PB for a period of one year at the recommended doses for control of insects. Watters (1968) investigated biologically the residual activity of pyrethrin and PB mixture in kerosene. Glass, wood, and paper surfaces were fogged with the solution and biologically evaluated with grain beetles. Grooved and smooth plywood and filter paper treatments controlled beetles effectively for 11 months, while deposits on glass were ineffective.

The toxic effects of insecticides and their additives continue to be re-evaluated with respect to other animals. The long residual chemicals have received particular emphasis. Additives, i.e., PB, were once believed to be non-toxic to man and other animals at low dosages. Recently Epstein et al. (1967a, 1967b) and others have found that PB enhances the toxicity of various aerosol additives of Freon in white mice by synergistic action. The incidence of hepatoma was highest in male mice with combination of PB and Freons (24%) compared to control groups (4%), indicating synergistic hepatocarcinogenicity results.

However, this synergist may be of benefit by protecting animals from the effects of a toxicant by antagonism. Bond (1965) discovered that granary weevils were protected from the toxic effects of hydrogen cyanide by this material. The synergist may exert its effect by depressing oxidative metabolism.

Biological Evaluation of Miscellaneous Additives

The number of additives in pesticide formulations is increasing at an alarming rate (Hewlett, 1960). A survey of 2900 formulations used in North America was made in 1957. The results showed that 25.3% had at least two active ingredients in each formulation and one formulation had eight. The inactive ingredients in the formulations were not included in this survey. Their inclusion in the totals of additives would increase the total number of additives in use.

Oils are a common additive used in formulations to improve the delivery and the residue of insecticides. Hocking and Lindsay (1958) and Hocking (1961) found that Velsicol AR 50, Velsicol AR 55, and diesel oils were repellent to insects. The same oils were separated into light, medium, and heavy weights by fractional distillation and tested separately on insects. Olfactory responses indicated that repellency was inversely related to the boiling point and even the fractions with the highest boiling points were quite repellent. They concluded that additives tended to confound the purpose of pesticide formulations to kill insects, if these additives were repellent.

Wolfenbarger and Getzin (1963b) and Wolfenbarger (1964b) tested paraffinic and naphthenic and alkylate isoparaffinic oils alone or with insecticides. The paraffinic oils were superior in control of aphids and corn earworms. The same oils combined with insecticides increased the residual control of insects.

An investigation was made of the compatibility of two water repellents, a detoxicant for chemical warfare agents, and a fire retardant in combinations with deet, benzyl benzoate, or M-1960 in cotton uniforms (Markarian et al., 1968). The repellent performances of deet or benzyl benzoate were affected the least when combined with the non-repellent compounds. The mosquito repellency of M-1960 was decreased by both the fire retardant and the detoxicant. The tick repellency of M-1960 was improved by the detoxicant but was reduced by the fire retardant. All three of the insect repellents destroyed the effectiveness of the water repellent compounds.

Disinfectants have long been used around latrines to reduce odors. Field tests indicated that house flies were repelled by disinfectant mixtures of phenylphenols (Shambaugh et al., 1968). Various phenylphenols and related compounds were later evaluated as house fly repellents in the laboratory. The authors found three formulations of phenylphenol compounds to be effective fly repellents and have since patented them. One phenylphenol mixture was an effective fly repellent for seven days.

Smittle and Burden (1968) studied the effects of dieldrin, malathion, and diazinon formulated in lacquers for the control of German cockroaches. Lacquer-toxicant formulations were compared with formulations of emulsions, and solutions with a toxicant. All formulations containing dieldrin were ineffective against the dieldrin resistant strain. Residues of malathion in kerosene were superior to both emulsion and lacquer residues applied on either painted or unpainted plywood against normal cockroaches. All formulations proved less effective on enameled plywood than on unpainted plywood. Diazinon in lacquer was less effective than toxicant-solvent and toxicant-emulsion residues, when cockroaches were exposed for 15 minutes. However, when cockroaches were exposed 30 minutes and residues aged six weeks, diazinon in lacquer was more effective than diazinon in solutions.

Biological Evaluation of Insecticides with Repellent Properties

Insects are somewhat repelled by most insecticides. A toxicant by its very nature seems to be irritating to insects initially and later contacts with the material may be avoided by them. The repellency of an insecticide may be of such magnitude that control of a pest is reduced considerably. Barnhart (1943) studied the repellency of aqueous solutions of sodium arsenate, mercuric chloride, boric acid, borax, and sodium fluoride. German cockroaches were repelled by all solutions except boric acid. Bare (1945) reported that cockroaches were repelled by baits mixed with sodium fluoride but not by boric acid and borax baits.

The most extensive work to date on repellency of blatticides has been conducted by Ebeling et al. (1966, 1967, 1968a, and 1968b). The authors studied the repellency of the common cockroach insecticides in use today against German, American, brown-banded, and oriental cockroaches. A special box (choice box) was developed to give cockroaches a choice to enter an unattractive light area or an attractive dark area contaminated with insecticides. The four cockroach species were repelled by the following materials in descending order: Drione, Baygon, diazinon, chlordane, sodium fluoride, and boric acid. Boric acid exhibited so little repellency that cockroaches visited residual deposits readily and succumbed to its low toxicity. The order of toxicity was the same as that for repellency, Baygon exhibiting the highest toxicity and boric acid the lowest toxicity. The cockroaches were repelled by the more toxic materials and the extent of control was therefore reduced. These laboratory observations were also confirmed in mock-up kitchens and field trials.

Cockroaches were able to learn to avoid deposits of insecticides, even when applied in attractive dark areas. The learning of cockroaches by avoidance of pesticide deposits is known as "associate learning" and the retention of this associate learning was surprisingly longer than previously experienced. Cockroaches learned to habituate themselves by remaining in lighted areas. The combination of habituation and associate learning was believed to contribute to the insecticide-avoidance behavior pattern.

Flynn and Schoof (1966) developed a similar test chamber whereby cockroaches had a choice of contacting or avoiding insecticide deposits. The amount of treated surface could also be varied from a complete application to any degree desired. Baygon performed better than diazinon when cockroaches were forced to remain on toxicant residues; however, diazinon performed better than Baygon when cockroaches were given a choice of a treated and untreated surface.

Smittle et al. (1968) used a different method for testing the repellency of blatticides. A test consisted of two ice cream cartons, one treated with solvent and one treated with solvent plus an insecticide. Both cartons were placed in a large tub and repellency was resolved by comparing the number of German cockroaches in toxicant-treated containers to those in solvent-treated containers. Baygon and a pyrethrin mixture appeared to be repellent materials. Chlordane, diazinon, ronnel, and malathion exhibited too high mortality to determine their repellency. The authors believe that these materials were not so repellent that they failed to be effective blatticides.

An insecticide possessing both toxic and repellent action may be desirable in some insect control. The treatment of myiasis requires a material with these unique properties (Loeffler and Hoskins, 1946). The immediate kill of maggots in animal wounds may require their removal later from difficult areas. An ideal larvicide is one that has a delayed positive toxicity and a strong larval repellency, thus allowing the larvae time to crawl from the wound to the ground and die. A material with similar properties would be helpful in protecting food commodities. Incho et al. (1953) used pyrethrin mixtures to repel and kill stored grain insect pests. Insecticides with these properties may be useful in cockroach control.

METHODS AND MATERIALS

Test Insects

Periplaneta americana, the American cockroach, and *B. germanica*, the German cockroach, were used in these laboratory investigations. A diazinon-susceptible American cockroach strain was established from colonies present in the Oklahoma State University Department of Entomology Insectary. A *B. germanica* laboratory strain was obtained from the U. S. Department of Agriculture, Insects Affecting Man and Animals Research Laboratory, Gainesville, Florida.

American and German cockroaches were reared in 20-gal garbage cans of plastic and metal, respectively. Ventilation was provided in the bottom part of the rearing chambers by cutting two 4 x 6 inch holes in the sides and covering the holes with 32-mesh plastic screening. The tops of the containers were covered with the same screening, thus providing additional ventilation. A thin film of mineral oil 2 inches wide was applied to the interior walls of the rearing containers near the top. The screened top and mineral oil prevented escape of cockroaches.

An apartment house as pictured in Fig. 1 was placed in each container for resting sites. Each house consisted of 14 plywood floors, placed one inch apart. Purina Dog Chow was placed on the top shelf in two half-pint containers. Water was provided by placing on the top shelf three large test tubes filled with water, stoppered with cotton. Additional water was provided by inverting two 1-quart cotton-stoppered bottles filled with water and wedged alongside the apartment house. The temperature was maintained from 75^0 to 85^0 F in the rearing room. Fluorescent lights were provided during the entire 24-hr day to give uniform lighting.

Processing of Test Animals

Cockroaches were removed from rearing containers by anesthetizing with CO_2 and quickly transferring all cockroaches to other containers. Lot sizes of 50 American or 100 German cockroaches were counted and sexed. Nymphs and adult male and female cockroaches were counted to the nearest equal lots to make the above total count. Specimens of each lot were transferred to 1-gallon ice cream containers. The lids of these containers were screened with 32-mesh plastic screen. Food and water were provided until the start of a test. Insects for a repellency test were sexed and counted in the morning prior to evening tests on the same day. Specimens used in a mortality test were processed approximately 20-24 hours prior to the start of a test. This schedule allowed cockroaches sufficient time to recover from the effects of CO_2.

Treatment of Test Panels

Repellent and mortality tests were conducted on No. 1 white pine plywood panels or boards 1/4 x 4 x 6 inches in size. Spray additives or toxicants were sprayed on boards at the rate of 1 gallon per 1000 ft. A spray chamber, as pictured in Fig. 2, was used to apply residuals. A Tee Jet Nozzle 8004E (Spraying Systems, Bellwood, Illinois) was fitted on the spray boom. Routine calibrations were made on the sprayer to insure proper delivery. Pressure of 30-35 psi was maintained during the spraying. Phillips Petroleum Soltrol 130, a long chain paraffin oil, was the solvent used to formulate all additives and the toxicant, except in case of Triton X-155. Acetone (5.0% by volume) was added to the oil to make this additive miscible.

Boards were placed on wire trays (1/8 x 36 x 20 inches) for ease in handling and storage. Panels were sprayed twice on each side and then turned so that ends and sides of boards received adequate coverage. Boards treated with different chemicals and concentrations were stored in separate stacks. Air circulation within a stack was facilitated by placing two 3/4 x 4 x 36 inch boards between each tray. Each stack was then covered with black plastic for protection against light and contaminates. All panels were stored at the same height and in the same area throughout the tests.

Test Conditions

Both mortality and repellency tests were conducted at 76^{o} - 82^{o} F. Fluorescent lighting was provided during the entire tests. Repellency tests were conducted in a Peet Grady Chamber. Mortality tests were completed in an area isolated from the repellent studies. This procedure helped prevent contamination of the repellent study area. All repellent studies were conducted during the evening from 1900 to 2300 hours. Mortality tests were started in the morning at 0900 to 1000 hours. This schedule helped to eliminate variation between tests.

Repellency Tests

A factorial experiment was used to study the effects of replicates, readings, concentrations, and time on repellency. The additives were selected on the basis of their unusual repellency and attractancy action in Sterling's (1966) studies. The following four chemicals were used:

1. Piperonyl butoxide, a-{2-(2-butoxyethoxy)ethoxy}-4,5-methylenedioxy-2-propyltoluene, a synergist;
2. R-11, 1,5a,6,9,9a,9b-hexahydro-4a(4H)-dibenzofurancarbox=aldehyde, a repellent;
3. Triton X-155, alkyl aryl polyether, an emulsifier manufactured by Rohm and Haus Company, Philadelphia, Pennsylvania; and
4. Volpa-3, polyoxyethylene oleyl ethers, an emulsifier manufactured by Croda, Inc., New York, New York.

All four chemicals were tested at concentrations of 0.01, 0.1, and 1.0%. Each concentration of a particular additive was applied to its random selected board at day zero and then reapplied to its respective board at 30, 60, 90, 120, and 150 days later. Boards used on a test date did not receive treatment on that date. They were removed randomly from wire trays for the day's tests.

A test consisted of two replicates. Four boards for each concentration and chemical were used for each replicate. Four readings were made on each board. This resulted in 16 readings per replicate and 32 readings per test. Replicates were spaced one week apart. Only one chemical was tested on a particular evening to prevent multiple chemical contamination of the test room. After panels were used in a test, they were always discarded. This procedure eliminated contamination from cockroach contact.

Repellent procedures were similar to those conducted by Sterling (1966). A turntable 30 inches in diameter and a repellent chamber 18 inches in diameter by 10 inches high were used in all tests (Fig. 3). The turntable was rotated 3 rpm by a Model 500, Electric Motiondizer, Yemco, Incorporated, Chicago, Illinois. This device helped to eliminate the effects of variations in temperature, humidity, and light on the cockroaches.

The repellent test chamber was placed on the turntable. The chamber bottom was constructed of 3/4-inch plywood. Four panels were placed vertically on the bottom in each test. The boards were located equidistant on the radii of the circle, approximately one inch from the chamber wall. Four finishing nails were installed to hold the boards upright and permit panels to be inserted and removed easily. The wall of the chamber was made of transparent lucite plastic. The interior wall was coated with a two-inch band of mineral oil near the top. A sheet of clear glass (1/8 x 20 x 20 inches) was placed on top of the chamber. Both the glass and mineral oil prevented escape of specimens.

The procedure for each replicate repellent test was to place four new boards in a clean repellent chamber. The bottom of each chamber was covered with new, white paper. The chamber was placed in the center of the turntable. Cockroaches were then lightly anesthetized with CO_2 (100 German or 50 American), divided into four equal batches, and placed between the four upright boards. The turntable motor was started and a ten-minute period was allowed for cockroaches to revive. Then a count was made of the number of cockroaches on each board and counts recorded separately for each board. Three additional counts were made 5 minutes apart. After each count, a bulb duster was used to knock all the cockroaches off the boards with a jet of air. Cockroaches were discarded after each test.

Residual Mortality Tests

Cockroach mortality tests were conducted upon conclusion of each 180-day repellent test. Boards, which were treated with additives and aged for six months, were then sprayed with 1.0% diazinon. Another group of panels was sprayed with 1.0% diazinon alone and this group served as a standard for comparison with the additive plus toxicant treated panels. However, the standard group panels had received monthly treatments with Soltrol 130 for six months. The solvent treatment was necessary to make the test more uniform, since the objective of this entire experiment was to determine if the degree of repellency of an additive could be correlated with the toxicity of the additive combined with an insecticide.

Boards were placed in test containers similar to those used by Sterling (1966). Ice cream cartons, one-half gallon in size, served as test chambers (Fig. 4). A panel was centered vertically in the bottom of the container and held upright by two thumb tacks inserted through the bottom. A nylon netting was secured over the top to prevent specimens from escaping. Cartons were placed on a revolving turntable as described previously.

Each chemical additive was tested on separate days. A typical test day consisted of the following types and number of boards tested: 0.01% additive plus 1.0% diazinon, two each; 0.1% additive plus 1.0% diazinon, two each; 1.0% additive plus 1.0% diazinon, two each; standard 1.0% diazinon, three each; and control (untreated), three each. A mortality test consisted of two replicates done on two different days. Both Americans and Germans were included in a day's test. Both standard and control were weighted with an extra board, since they were used in comparing larger numbers of boards.

Treated and untreated boards were placed in cartons just prior to start of a test. Diazinon-treated boards were aged for approximately 24 hours. Cockroaches were anesthetized with CO_2 and placed in equal numbers on both sides of the boards. Cartons were then placed randomly on the turntable and the motor started. Knockdown counts were made randomly every half-hour for the first 4 hours, hourly from 5 to 12 hours and a final count at 24 hours, a method suggested by Keller et al. (1956) and the Armed Forces Pest Control Board (1959). The 16 readings on the same container had the disadvantage of producing non-independent measurements. However, it produced satisfactory data with a minimum of cost. A cockroach was considered "down" if it did not show coordinated movement.

Vapor Mortality Tests

A vapor mortality test was initiated to determine if cockroaches in residual mortality tests were killed by direct contact with residuals or vapors of diazinon. Panels treated and aged for 6 months with Volpa-3 and R-11 were selected for this test. German and American cockroaches were tested separately.

A replicate, as described in the previous section, was conducted in the same manner, with the exception of placement and numbers of cockroaches. A 1.5 x 3.5 inch screened-mesh cylinder was hung inside the ice cream carton, approximately 1/2 inch from sides, bottom and inserted panel. American and German cockroaches were anesthetized with CO_2 and placed in a tube in lots of 10 or 20, respectively. Time mortality readings were made on a tube following the same schedule as residual mortality tests.

Statistical Analyses of Data

The data from each chemical were analyzed as a split-split-plot in time in which the concentrations were considered as main plots in a randomized block design. Each main plot was divided into seven sub-plots (dates). Each sub-plot contained four boards. Each board was read four times, thus giving a split-split-plot design over successive readings.

Data collected from residual mortality tests were analyzed by Finney's (1952) probit analysis using an IBM 360 computer program (BMD03S) by Dixon (1968).

RESULTS AND DISCUSSION

The results and discussion of different tests for the biological investigation of four common spray additives will be described in this part. Repellency and mortality tests will be reported individually with *P. americana* and *B. germanica*. Vapor repellency tests with the two species will be combined.

Repellency of Four Additives to *P. americana* Tested at Three Concentrations

The analyses of variance for the response of American cockroaches to residues of PB, R-11, Triton X-155, and Volpa-3 are exhibited in Tables 1, 2, 3, and 4, respectively. The F values for the main effects of concentration, time, and readings were highly significant (0.005 level) among all four chemicals except R-11, where significance with concentrations was at 0.01 level. The avoidance response (repellency) due to a chemical generally increased with increased concentration with the various exceptions to be noted later. These results agree in general with those obtained by Sterling (1966).

The significance of time was noted over the 6-month aging period. The repellency responses of American cockroaches to residues of PB, R-11, Triton X-155, and Volpa-3 are illustrated in Figs. 5, 6, 7, and 8, respectively. A general decrease in repellency, with some irregularities, was noted as treated panels were aged from 0-day to the 30-day period. From the 60-day period to the 180-day period, the repellency response appeared to increase, and in some cases was greater at

150 or 180 days than at 0-day. The general trend of repellency for 180 days is illustrated in Fig. 7. A curve is sketched above the bar graph for 1.0% Triton X-155. The general increase in repellency, after repeated applications of chemicals and aging of their deposits, indicated an additive effect.

The readings with additives were highly significant in all analyses of variances. The importance of readings was expected with the test method employed and chemicals used. Cockroaches were removed from panels after readings one through three by a jet of air. Cockroaches may then have been reluctant to crawl on the panels again. Irritation of sensory receptors by chemicals used may have inhibited return of the cockroaches. Sensitivity of receptors may have been reduced.

PB (Fig. 5 and Table 1) displayed the general trend of repellency as described earlier, except at 180 days. The reaction of American cockroaches at 180 days was a decrease in avoidance of deposits at 0.1% and 1.0% compared to 150-day results. However, the interaction of time and concentration was not significant. At 0.01% concentration the response at 180 days remained at about the same level as at the 150-day period. The repellency at both 150 and 180-day periods was higher than experienced at 0-day.

The response of specimens to R-11 (Fig. 6 and Table 2) was different from the reaction obtained with PB. The reaction to this material did not follow the trend of being less repellent as concentration decreased in two cases. After 30 days, the 0.01% dosage was more repellent than the two higher concentrations. At 180 days, the 0.1% deposits performed better as a repellent than the other two dosages. The F value for the time x concentration interaction was highly significant. The 1.0% R-11 deposits were most repellent at 120 days, while deposits of 1.0% PB were most repellent at 150 days.

Triton X-155 showed the greatest repellency of any additive tested against American cockroaches (Fig. 7 and Table 3). This material was the most repellent of four additives at 0-day (4.6 cockroaches per panel) and also the most repellent at the 180-day period (3.3 cockroaches per panel) on the 1.0% deposits. The response of cockroaches at this dosage was also the most uniform of any additive tested during the 180 days. Slight irregularities were noted in the response of cockroaches to 0.01 and 0.1% residues of Triton X-155. These inconsistencies were most noticeable between 60 and 150 days and will be commented on later.

The reaction of American cockroaches to Volpa-3 (Fig. 8 and Table 4) was similar in some respects to the emulsifier, Triton X-155. The main differences were responses recorded at 0 and 180 days. At 0-day, the 0.1% concentration was more repellent to insects than the 1.0%. At 180 days, the additional treatment did not increase the repellency response at the 1.0% dosage from the previous test period. The interaction of concentration and time was significant during the 180-day period. The results of the 90-day period will be discussed below in more detail. Volpa-3 was the second best repellent based on the mean numbers of specimens per panel (4.0) at 150 days.

The replicates were also significant in tests with Volpa-3. The inspection of the raw data showed the greatest differences in replicates occurring at the 90-day test period. Average counts per plate increased by two to three specimens in the second replicate (10 April 1969), compared to the first replicate (3 April 1969). A corresponding increase in cockroach activity was also observed with the increased plate counts. Cockroaches failed to settle down quickly after entering the test chamber. This abnormal activity of cockroaches lowered the repellency for all three concentrations at the 90-day period compared to the 60-day period. It was anticipated that the additional treatment applied to 90-day deposits would increase repellency.

This same cockroach behavior was also noted in 90-day tests with Triton X-155. Triton X-155 was tested on 14 April 1969 (first replicate) and increased activity of specimens was noted. Specimens tested a week later at replicate two displayed normal activity. The mean panel counts dropped 1-2 specimens at the second replicate. The above data suggest that there is seasonal influence occurring during mid-April and this influence increases the activity of cockroaches resulting in lower repellency. Earlier testing in 1968 further supports this seasonal behavior. Preliminary testing of techniques using PB and R-11 resulted in failure to duplicate replicates during 6 to 24 April 1968. The failure to reproduce results was initially believed to be due to poor techniques. Increased activity of cockroaches was noted for this period of testing but the abnormal activity subsided after 24 April.

The data for PB and R-11 also indicate seasonal variations in testing of repellents. Experiments conducted on 15-29 September 1968 showed an increase in repellency at 120-day period. R-11 at 120-day test period displayed lower repellency than at 150-day test period. Differences in replicates were greater at 120-day than for other test periods; however, replicates were not significant. The lower repellency response of cockroaches was accompanied by sluggish and general decreased activity of specimens. The decreased motion of insects was noted in both the test chamber and rearing chambers. The results with PB indicate less seasonal influence on repellency. The activity of cockroaches during the period of seasonal changes is believed to start at low level, to increase to a peak level and then to disappear gradually. The PB was tested during the early period of low level activity, thus cockroaches responded to materials with lesser degree of repellency.

The seasonal changes in insects noted when evaluating their responses to repellents are not a new phenomenon. Sterling (1966) tested repellents in summer and fall and experienced the same seasonal variations in response around mid-September. He noted increased repellency reaction and decreased activity of cockroaches in his tests. The increased activity of cockroaches during spring and summer is not well known. The yearly changes in cockroach activity show similarity of activity to that of many plant insects. Plant insects decrease activity in the fall prior to diapause and increase their activity in spring after diapause. The seasonal activity of cockroaches may be influenced by photoperiod or possibly unknown factors. Light is one factor that increases in intensity in the spring and decreases in intensity in the fall. Light is an element believed to be important in inducing hormonal activity affecting the circadium rhythm in cockroaches. The seasonal changes in intensity of light could also inhibit or activate certain hormones to affect cockroach activity.

The changes in repellency responses over 180 days are influenced by changes in the basic chemical as a result of aging and decomposition. No attempt was made to evaluate these deposits by chemical analysis. The additive effect experienced by repeated applications of repellent materials on the same panel is not a new one. Goodhue and Howell (1960) noted that R-11 after three applications built up repellency. The response of insects to chemicals is generally explained as increased or decreased repellency. The use of the word attractancy may have explained some of the irregularities where a material increased in concentration but insects responded with less repellency.

Repellency of Four Additives to *B. germanica* Tested at Three Concentrations

Tables 5, 6, 7, and 8 show the analyses of variance for the response of German cockroaches to surfaces treated with PB, R-11, Triton X-155, and Volpa-3, respectively. The F value for main effects of readings, time, and concentration were again highly significant as generally recorded earlier for American cockroaches. The readings were highly significant for the same reason as posed earlier.

The response in German cockroaches showed a higher F value for concentration than recorded for Americans. The German specimens were generally more sensitive to concentrations of chemicals than the other species. The repellency responses of German cockroaches to various concentrations of PB, R-11, Triton X-155, and Volpa-3 during 180 days are illustrated in Figs. 9, 10, 11, and 12, respectively. A decrease in repellency was generally noted with similar decrease in concentrations with a few exceptions.

The influence of time was quite similar to that noted with *P. americana.* Repellency response with *B. germanica* was high at 0-day period and then repellency decreased after 30 days of aging. After the 30-day test, the repeated applications at each period produced an additive effect which increased repellency. At the end of 180 days, both

Triton X-155 and Volpa-3 deposits were more repellent than at 0-day. The curve of additivity generally followed the outline as shown on Fig. 7, but in one case an upswing occurred prior to 180 days, i.e., Fig. 10 with R-11.

The results with PB (Table 5 and Fig. 9) exhibited very few exceptions to the general trend of responses with various concentrations and time. The 0.1% concentration was the most repellent of the three tested at 30 days, but it decreased in repellency at 60, 90, and 120 days. Concentration x time was also significant. Time x readings interaction was highly significant. The periods of high repellency, i.e., 0 and 180 days, may have contributed to this significance. Cockroaches were fewer in number on a panel at this period, and the smaller number plus increased repellency facilitated further removal and punishment.

R-11 (Table 6 and Fig. 10) demonstrated the greatest repellency of the four additives to specimens at 0-day test period with both 0.1% and 1.0% dosages. The 0.01% concentration showed the least repellency of materials at both 0-day (11.9 per panel) and at 30-day period (13.8 per panel). The upswing of the additive curve occurred as mentioned previously at both 150 and 180 days with corresponding loss in repellency. The interaction of concentration x time indicated these results were significant.

The response of _B. germanica_ to Triton X-155 indicated a seasonal influence at the 90 and 120-day test periods. The repellency responses decreased during those periods, but should have increased due to additive effect. The influence of changes in season seemed to affect German cockroaches for a longer period than American cockroaches. The increased activity of German cockroaches started in mid-April and subsided in mid-May. The greatest differences between replicates were observed during this period and the main effect of replicates was significant. The bar graphs in Fig. 11 illustrate the decreased repellency at 90 days and to a lesser extent at 120 days.

The 1.0% deposits of Volpa-3 were the most repellent of any chemical at 180 days (Fig. 12), however, R-11 had the same mean number of cockroaches per panel (2.5) at 0-day. The response of specimens to Volpa-3 was similar to those obtained with Triton X-155. The seasonal influence had less effect with this material since only one test was conducted during the critical period of 15 April to 15 May. Replicates were significant at the 0.01 level and the greatest differences between replicates were noted at 120 days. A decrease in repellency was observed for this period.

The seasonal variances of responses to PB and R-11 tested during late summer and early fall were similar to those described earlier for _P. americana._ The 120-day test period for both R-11 and PB showed the greatest differences among replicates during any periods. An increase in repellency response was most noticeable for R-11 in Fig. 10. The increase in repellency associated with seasonal changes was less noticeable with German cockroaches than with American cockroaches. The greater sensitivity of _B. germanica_ to repellent materials may explain this difference.

The increased activity of both species in the spring and early summer and decreased activity noted in the late summer and fall was related with corresponding decreases and increases in repellency responses. The activity of the insects during the late summer and fall appears to be about the same for both species, but the spring and early summer activity period appears to be slightly longer for German cockroaches. Wright and McDaniel (1969) studied the monthly abundance of both species in buildings. The abundance figures were based on number of times cockroaches were observed in buildings by month. These numbers may then be correlated with the activity of the cockroaches. The German cockroaches were observed to be present more in the fall month of September than in the months of January through May. Cockroaches may have been seen more in the fall because they were more sluggish as observed in repellent tests here. Likewise, some may associate increased abundance with increased activity. The American cockroaches were observed in about equal numbers of times for each month. Those observations on *P. americana* could not be correlated with seasonal changes in repellent tests conducted here. However, a more careful study may indicate seasonal fluctuations of activity for both American and German cockroaches.

Mortality Responses of *P. americana* to Residues of Four Additives and 1.0% Diazinon

The results of mortality tests with 1.0% diazinon alone and combinations of 1.0% diazinon and PB, R-11, Triton X-155, and Volpa-3 are illustrated in Figs. 13, 14, 15, and 16, respectively, for *P. americana*. The LT-50 of diazinon alone varied between two test periods of July (Triton X-155, 5.4 hours and Volpa-3, 4.9 hours) and November (PB, 6.1 hours and R-11, 5.7 hours). The lower mortalities which were obtained in the fall month compared to the summer month are quite common in insecticide tests with insects. Diazinon tested alone provided a standard for comparison with additive plus toxicant.

The mortality line for 0%, 0.01%, 0.1%, and 1.0% PB and 1.0% diazinon showed a slight deviation at 0.01% concentration (Fig. 13). The combination of 0.01% PB plus diazinon (LT-50 of 5.6 hours) was more toxic to American cockroaches than diazinon alone (LT-50 of 6.1 hours). The remaining tests of additive plus toxicant were less toxic than the standard, indicating higher repellency to insects. Sterling (1966) applied PB treatment once in combination with 1.0% diazinon and aged the deposits for various periods. Readings of mortality were made only at 12 and 24 hour periods, thus not recording enough data to compute the LT-50. One treatment after aging one day caused only a one percent decrease in mortality in a 1.0% PB plus toxicant versus toxicant alone at 12 hour reading. The results here indicated that almost one hour longer was required to obtain an LT-50 with diazinon plus 1.0% PB than diazinon alone. Repellency responses exhibited in Fig. 5 for the 180-day test period also support mortality data. An increase in repellency seems to be correlated with a decrease in mortality.

Tests with R-11 and toxicant were quite different from PB. The mortality at .01% dosage decreased from the standard then increased at 0.1% and then decreased at 1.0% of R-11 (Fig. 14). The 0.01% dosage of R-11 and toxicant was more toxic than diazinon alone. The 0.1% R-11 plus diazinon (LT-50 of 7.7 hours) was less toxic than 1.0% R-11 plus diazinon (LT-50 of 6.5 hours) indicating greater repellency for the lesser concentration. The repellency response on Fig. 6 showed close correlation between results of repellency and mortality tests. The 0.1% R-11 surfaces were more repellent to American cockroaches than the 1.0% R-11 surfaces.

The mortality response lines for both Triton X-155 (Fig. 15) and

Volpa-3 (Fig. 16) were almost linear. An increase in concentration of an additive caused a corresponding decrease in toxicity. The 1.0% Triton X-155 plus toxicant produced an LT-50 of 7.7 hours in insects, a lower mortality than any of the other additives at this concentration. The results of repellency tests indicated the 1.0% Triton X-155 dosage to be the most repellent also. The repellency responses of Triton X-155 (Fig. 7) and Volpa-3 (Fig. 8) followed a similar straight line curve at 180 days for three concentrations as did the mortality curve.

A lower mortality of American cockroaches was obtained by exposure to deposits of emulsifiers and diazinon. The emulsifiers could act as antagonists with the toxicant. The antagonism is believed to be primarily the result of repellent action. During mortality tests, the numbers of cockroaches on boards decreased with increased concentrations of additive. However, other actions could influence the results. The emulsifier may inhibit a metabolizing system in an insect whose primary role is to activate the toxicant. The additives applied monthly or over a six-month period may very well leave complex deposits of active ingredients and decomposition products. These residues could mask or cover toxicant to prevent its pickup by insects. The decomposing of diazinon by additives or decayed chemicals is also a remote possibility during the 24 to 48 hour aging and testing period. The decreased toxicity of additives and toxicant may be caused by all or combinations of these and other factors working together at the same time.

The increased cockroach mortality experienced with lower concentrations of PB and R-11 was believed to be due primarily to the reduced repellency of residues. The increased toxicity to insects may also be caused in part by synergistic action. Synergistic action is well known in insects treated with PB and toxicants. The explanation of decreased kill when the synergist is increased in concentration is more difficult to explain. At lower synergist concentrations, the insect may be repelled less or even attracted to residues of toxicant and synergist. At higher concentrations of the synergist, insects are repelled more from the toxicant and synergist mixture. The over-all effects of additives on cockroach control will be discussed later.

Mortality Responses of *B. germanica* to Residues of Four Additives and 1.0% Diazinon

Figures 17, 18, 19, and 20 illustrate the mortality responses of German cockroaches to residues of 1.0% diazinon and various concentrations of PB, R-11, Triton X-155, and Volpa-3, respectively. Comparisons of the above combinations were always made with the standard, 1.0% diazinon alone. Seasonal variations of LT-50 for the standard were quite similar in German cockroaches when compared to earlier tests with American cockroaches.

The mortality responses of *B. germanica* to 1.0% diazinon plus 0%, 0.01%, 0.1%, and 1.0% PB deposits were similar to those mentioned earlier for *P. americana.* The mortality at 0.01% PB plus 1.0% diazinon was lower than the mortality with the standard. The repellency responses of German cockroaches also resembled a straight line response obtained at concentrations 0.01%, 0.1%, and 1.0% in mortality tests. *B. germanica* responded quite differently to R-11 plus toxicant (Fig. 18). The LT-50 for German cockroaches increased from 5.9 hours for diazinon alone to 7.9 hours for diazinon plus 0.1% R-11, but then decreased to 6.7 hours when 1.0% R-11 was added. The highest concentration appeared to be less repellent than the next lower concentration. The repellency response at 180 days for R-11 (Fig. 10) paralleled the mortality response line and thus supports the influence which repellency action plays in increasing or decreasing mortality in insects.

Both emulsifiers, Triton X-155 (Fig. 19) and Volpa-3 (Fig. 20) displayed a straight line mortality response with the exception of a slight deviation for Triton X-155. Repellency response curves, which may be visualized on the bar graphs for three concentrations of Triton X-155 (Fig. 11) and Volpa-3 (Fig. 12), also show a straight line reaction after 180 days. The highest LT-50's of any additive plus toxicant was recorded with 1.0% Volpa-3 (8.1 hours) and 1.0% Triton X-155 (8.1 hours). Even though both additives had the same LT-50 of 8.1 hours, Volpa-3 had the lowest mortality based on LT-50 ratios. The ratio of the standard (1.0% diazinon) to 1.0% Volpa-3 plus diazinon was higher at 1:4 than the ratio of standard to 1.0% Triton X-155 plus diazinon at 1:1. The responses of both German and American cockroaches were in fairly close agreement on both mortality and repellent tests. American cockroaches generally displayed lower LT-50's than German cockroaches. An LT-50 of 7.7 hours was the highest recorded for _P. americana_.

The influence of additives in both repellent and mortality tests indicated that these materials could reduce control of cockroaches in the laboratory. The evaluation of reduction of cockroach control in the field by repellent additives will have to await further tests. Spray additives, i.e., emulsifiers, may limit the amount of time an insect will remain on a toxicant by repellent action. Ebeling (1967) reported that German cockroaches exposed only five minutes to

15 mg/sq ft of diazinon increased the KD-50 to greater than 48 hours compared to KD-50 of 22 minutes under continuous exposure. Cockroaches were able to recover from five minutes of exposure to toxicant and learned to avoid it.

The spot application of blatticides, i. e., diazinon, gives cockroaches a choice of contacting a treated or untreated surface. Cockroaches will avoid surfaces free of irritating deposits. The monthly applications of both emulsifiers and toxicants may cause a build-up of materials which increase insect repellency response. The placement of these spot applications in cracks and crevices does not allow easy removal by normal washing and cleaning. The effects of six monthly applications indicate an additive effect on repellency of materials tested here.

Pest control operators have reported a general decrease in cockroach control with spot application of the newer organophosphorous and carbamate insecticides compared with the older chlorinated hydrocarbon insecticides. The failure of these new materials may be due to the additives used in the spray mixture. The additives themselves may be repelling insects from picking up lethal deposits of the toxicant. The increasing reports of insecticide resistance may also be partially associated with spray additives which repel insects from toxic residues. It is imperative that future spray additives be developed which do not elicit repellency responses in insects. Additives may be developed which actually attract insects. The inclusion of such additives into spray formulations would increase the effectiveness of the toxicant. Future formulations of insecticides should be based on more basic research on individual additives before mixing and testing them in the final spray.

Vapor Mortality Tests with R-11 and Volpa-3 plus 1.0% Diazinon Using Two Species of Cockroaches

The results of vapor mortality tests with American cockroaches was zero mortality for 24 hour test period for 1.0% diazinon alone and 1.0% diazinon in combination with three concentrations of Volpa-3 and R-11. The negative results indicate that specimens in screened cages were not killed by toxic fumes of the toxicant. Insects were killed in normal tests primarily by contact with treated panels.

In parallel tests with German cockroaches, mortality was observed in screened cages within mortality test chambers. The results are listed in Table 9 for R-11 plus diazinon and Volpa-3 plus diazinon. Vapor mortality was not recorded until five hours in the test chamber, and then only in two concentrations. The mortality increased from 2.5% at five hours to a high of 17.5% at 12 hours. Vapors of diazinon did not appear to affect the mortality of *B. germanica* during the first five hours.

The delayed toxicity to cockroaches indicated that screened cages may have been gradually coated with diazinon deposits. Diazinon has a high vapor pressure of 1.4 x 10^{-4}mm Hg at 20° C (Metcalf, 1955). The diazinon could have vaporized from treated panels and redeposited itself on screen cages nearby. This type of action is common with this chemical. These smaller deposits had less effect on the larger American cockroaches but may have helped increase mortality in German cockroaches. The mortality in cages after five hours was believed due to residues of diazinon on the screen surface with the possibility of some influence by vapors. The toxicity to insects of additives at 0, 0.01, 0.1, and 1.0% and 1.0% diazinon was about equal at 12 hours. Therefore, the effect of diazinon vapor or redeposited residues should influence the LT-50's in a uniform manner.

SUMMARY AND CONCLUSIONS

Repellency of Four Spray Additives to *P. americana* and *B. germanica* Tested at Three Concentrations

These tests were conducted as factorial experiments with replicates, concentration, time, and readings being investigated. In both American and German cockroach tests, the differences in readings, time and concentrations were highly significant at the 0.005 level for PB, R-11, Triton X-155, and Volpa-3, with the exception of R-11 where concentrations with American cockroaches were significant at the 0.01 level. The readings showed an increase in repellency after three of the four counts. A loss in repellency to insects was generally noted as chemicals were aged from 1 to 30 days. The further applications of additives at 30-day interval for 150 days resulted in a general increase in repellency to specimens on later tests. The increase in response was generally additive after 30 days. At 150 or 180-day tests, all concentrations of materials were more repellent than at 0-day except R-11 at 1.0% concentration. Increasing the concentration of additive generally increased the avoidance of treated surfaces by specimens, with some exceptions. The largest value of significance was found in differences in concentrations. German cockroach F values were higher than those for American cockroaches, suggesting greater sensitivity to chemicals.

The results with PB to both species indicated differences in repellency responses over the 180 days of testing. American cockroaches followed the trend of being less repelled as the concentration decreased. German cockroaches did not follow this trend completely, because at 30-days the 0.1% concentration of PB was more repellent than the higher concentration of 1.0%. The repellency response of P. americana generally increased up to 120 days, but at 150 and 180 days a decrease was observed at the two higher concentrations. B. germanica followed the general trend of response of increasing repellency after the 30-day period except with the 0.1% concentration. At this dosage, the reaction response did not decrease below the 30-day level until the 180-day period. Interaction of concentration x time was significant for the test period.

R-11 exhibited the greatest repellency to German cockroaches among additives and species at 0-day with both 0.1% and 1.0% concentrations, but the least repellency at the 0.01% dosage at 0-day and 30-day period. These differences in responses are supported by the highest F value for concentrations. Both species tested showed a higher avoidance of 0.1% dosage than the 1.0% after 150 days. P. americana was repelled more by 0.01% PB than the two higher concentrations after 30 days. The influence of time on repellency did not follow the general course of increasing repellency after the 30-day period. This was most noticeable at the 1.0% dosage, where repellency response to both species decreased after the 120-day period. These differences of concentrations x time were highly significant.

Triton X-155 was the most repellent chemical to P. americana both at the beginning and at the end of the test. This additive was the second best repellent at the end of test with B. germanica. The repellency response of both species decreased as the concentrations of materials were decreased. After the 30-day period, the repellency response generally increased with each application of chemicals except where seasonal influences altered the results.

The 1.0% residues of Volpa-3 repelled more German cockroaches than any other concentration of additive at 180 days. The repellency response of German cockroaches was quite uniform with increasing concentrations of additive, but American cockroaches responded with higher repellency at 0-day with the 0.1% dosage than the 1.0%. The effect of repeated doses after 30 days increased the repellency generally until 180 days except where seasonal influences lowered or raised responses. At 180 days, *P. americana* were repelled less from 1.0% deposits than they had been at 150 days.

Both species were more active during the spring and early summer and were less active or sluggish during the late summer and fall. The repellency responses were likewise decreased during the spring-summer and increased during the summer-fall. Differences between replicates were highest during these periods. Replicates were significant with R-11, Volpa-3, and Triton X-155. The period of seasonal influence began in mid-April for both species, lasting about 30 days for *B. germanica* and 15 days for *P. americana.* In the summer-fall period, both species were more sluggish from approximately mid-September to 1 October.

Mortality Responses of *P. americana* and *B. germanica* to Four Additives and 1.0% Diazinon

PB, R-11, Triton X-155, and Volpa-3 plus diazinon residues were less toxic to German and American cockroaches at all concentrations than standard 1.0% diazinon, except at the 0.01% dosage with PB and R-11 plus toxicant. The higher concentration of additives usually resulted in larger LT-50's. This lower kill as dosage of additive was increased indicated that cockroaches were repelled from the toxicant by the additive. Visual observations during mortality tests confirmed this. The results of repellency tests conducted at 180-day period could be correlated with the mortality test results at all three concentrations (0.01, 0.1, and 1.0%).

The amount of mortality with or without additives plus toxicant varied with species and among materials. The standard varied in LT-50's during the test periods, resulting in higher LT-50's during the fall than in the summer. The mortality response lines of both American and German cockroaches were similar with PB. The line for R-11 varied with species. The mortality to specimens at 0.01% R-11 plus diazinon increased from the standard, then decreased at 0.1% and then increased at 1.0% with American cockroaches. With German cockroaches, the mortality to specimens decreased from the standard to the 1.0% diazinon plus 0.1% R-11 and then increased with 1.0% R-11. The LT-50 for 1.0% R-11 plus toxicant was about equal to 0.01% R-11 plus toxicant.

Both species demonstrated similar straight line responses to Triton X-155 with a minor variation at 0.01% with German cockroaches. The lowest kill of American cockroaches was obtained with 1.0% Triton X-155 and toxicant. This dosage of additive also gave the highest avoidance response in repellent tests. With German cockroaches, Triton X-155 at 1.0% was slightly more toxic than Volpa-3. _P. americana_ and _B. germanica_ both produced straight mortality lines with Volpa-3. The 1.0% of Volpa-3 plus toxicant was the least toxic of any combination of additive plus toxicant to German cockroaches. A lower mortality was generally obtained with additive plus toxicant in German cockroaches compared to American cockroaches.

Vapor mortality tests with R-11 and Volpa-3 plus 1.0% diazinon were negative for the 24-hour test period, with P. americana. These results indicated that mortality of American cockroaches in residue tests was primarily due to contact with the toxic residues. German cockroach vapor tests with R-11 and Volpa-3 plus toxicant indicated that mortality was due to contact with residues up to the fifth hour. After five hours, the diazinon appeared to redeposit itself on the screened test chambers and accounted for 17.5% mortality at 12 hours. The mortality response seemed to be almost equal at 0%, 0.01%, 0.1%, and 1.0% additive plus toxicant at 12 hours.

Additives to be used in insecticide formulations will have to be carefully studied in the future. Additives can repel insects from the toxicant, thus resulting in lower control of insects, as indicated here. The difficulty in cockroach control and insecticide resistance may be due to repellency of toxicants or additives with toxicants.

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APPENDIX

Table 1. Analysis of variance for the response of _P_. _americana_ to surfaces treated with piperonyl butoxide

Source	DF	MS	F
Total	671		
Replicates	1	.10	
Concentrations	2	485.44	282.23***
Error (a)	2	1.72	
Times	6	106.54	41.29***
Conc. x Times	12	3.88	
Error (b)	18	2.55	
Readings	3	64.85	20.14***
Conc. x Readings	6	1.98	
Times x Readings	18	2.91	
Conc. x Times x Readings	36	2.45	
Error (c)	441	3.22	
Plates in Conc. in Times in Reps.	126	2.25	

*Significant at the .05 level of probability
**Significant at the .01 level of probability
***Significant at the .005 level of probability

Table 2. Analysis of variance for the response of P. *americana* to surfaces treated with R-11

Source	DF	MS	F
Total	671		
Replicates	1	9.05	
Concentration	2	275.36	79.35**
Error (a)	2	3.47	
Times	6	94.43	35.90***
Conc. x Time	12	34.98	13.30***
Error (b)	18	2.63	
Readings	3	20.21	6.12***
Conc. x Readings	6	5.19	
Times x Readings	18	2.69	
Conc. x Times x Readings	36	2.46	
Error (c)	441	3.30	
Plates in Conc. in Times in Reps.	126	3.28	

Table 3. Analysis of variance for the response of *P. americana* to surfaces treated with Triton X-155

Source	DF	MS	F
Total	671		
Replicates	1	6.68	
Concentrations	2	774.43	281.61***
Error (a)	2	2.75	
Times	6	136.21	30.34***
Conc. x Times	12	3.79	
Error (b)	18	4.49	
Readings	3	51.81	18.64***
Conc. x Readings	6	2.12	
Times x Readings	18	2.33	
Conc. x Times x Readings	36	2.49	
Error (c)	441	2.78	
Plates in Conc. in Times in Reps.	126	1.61	

Table 4. Analysis of variance for the response of *P. americana* to surfaces treated with Volpa-3

Source	DF	MS	F
Total	671		
Replicates	1	32.60	22.03*
Concentrations	2	275.34	186.04***
Error (a)	2	1.48	
Times	6	130.49	17.90***
Conc. x Times	12	17.89	2.45*
Error (b)	18	7.29	
Readings	3	65.88	22.26***
Conc. x Readings	6	.90	
Times x Readings	18	3.93	
Conc. x Times x Readings	36	1.69	
Error (c)	441	2.96	
Plates in Conc. in Times in Reps.	126	1.74	

Table 5. Analysis of variance for the response of *B. germanica* to surfaces treated with piperonyl butoxide

Source	DF	MS	F
Total	671		
Replicates	1	.38	
Concentrations	2	916.12	2955.23***
Error (a)	2	.31	
Times	6	90.35	32.38***
Conc. x Times	12	22.80	8.17***
Error (b)	18	2.79	
Readings	3	226.57	68.24***
Conc. x Readings	6	.93	
Times x Readings	18	9.94	2.99***
Conc. x Times x Readings	36	4.90	
Error (c)	441	3.32	
Plates in Conc. in Times in Reps.	126	5.15	

Table 6. Analysis of variance for the response of _B_. _germanica_ to surfaces treated with R-11

Source	DF	MS	F
Total	671		
Replicates	1	2.63	29.22*
Concentrations	2	1150.06	12,778.44***
Error (a)	2	.09	
Times	6	244.05	49.30***
Conc. x Times	12	84.65	17.10***
Error (b)	18	4.95	
Readings	3	210.96	70.09***
Conc. x Readings	6	4.40	
Times x Readings	18	3.39	
Conc. x Times x Readings	36	3.40	
Error (c)	441	3.01	
Plates in Conc. in Times in Reps.	126	5.08	

Table 7. Analysis of variance for the response of *B. germanica* to surfaces treated with Triton X-155

Source	DF	MS	F
Total	671		
Replicates	1	5.01	19.27*
Concentrations	2	422.68	1625.69***
Error (a)	2	.26	
Times	6	75.27	22.60***
Conc. x Times	12	4.05	
Error (b)	18	3.33	
Readings	3	338.81	157.59***
Conc. x Readings	6	.69	
Times x Readings	18	2.50	
Conc. x Times x Readings	36	1.03	
Error (c)	441	2.15	
Plates in Conc. in Times in Reps.	126	3.41	

Table 8. Analysis of variance for the response of _B_. _germanica_ to surfaces treated with Volpa-3

Source	DF	MS	F
Total	671		
Replicates	1	47.15	109.65**
Concentrations	2	1297.40	3017.21***
Error (a)	2	.43	
Times	6	188.31	31.28***
Conc. x Times	12	8.99	
Error (b)	18	6.02	
Readings	3	516.28	286.82***
Conc. x Readings	6	8.16	4.53***
Times x Readings	18	2.94	1.63*
Conc. x Times x Readings	36	2.11	
Error (c)	441	1.80	
Plates in Conc. in Times in Reps.	126	3.64	

Table 9. Vapor and residue mortality of spray additives plus 1.0% diazinon to *B. germanica*

Concentration of Chemical(s)	Percent of Cockroaches Knocked Down After Indicated Hours							
	5	6	7	8	9	10	11	12
0.01% Volpa*	0	2.5	5.0	10.0	10.0	12.5	15.0	15.0
0.1% Volpa	2.5	5.0	7.5	12.5	12.5	12.5	12.5	15.0
1.0% Volpa	0	2.5	2.5	5.0	7.5	10.0	12.5	12.5
1.0% Diazinon	0	2.5	5.0	7.5	7.5	12.5	12.5	17.5
0.01% R-11	2.5	5.0	7.5	10.0	12.5	12.5	15.0	15.0
0.1% R-11	0	2.5	2.5	5.0	7.5	7.5	10.0	10.0
1.0% R-11	0	5.0	7.5	7.5	7.5	10.0	10.0	15.0
1.0% Diazinon	0	2.5	5.0	7.5	10.0	12.5	15.0	15.0

*All additive treatments contained 1.0% diazinon

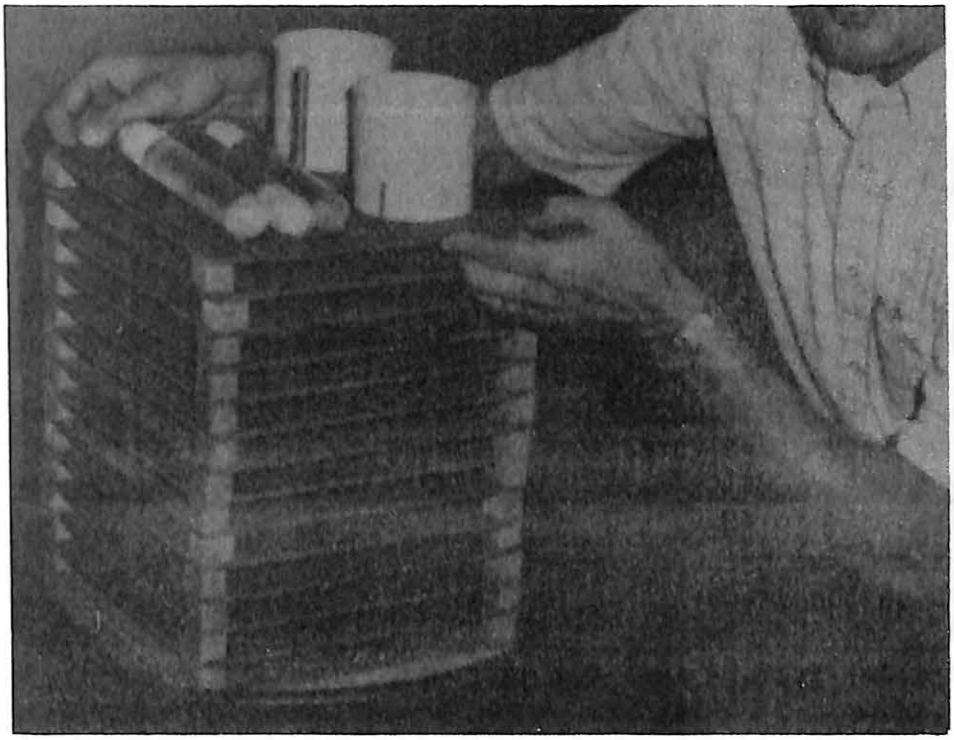

Figure 1. Cockroach rearing chamber and apartment house

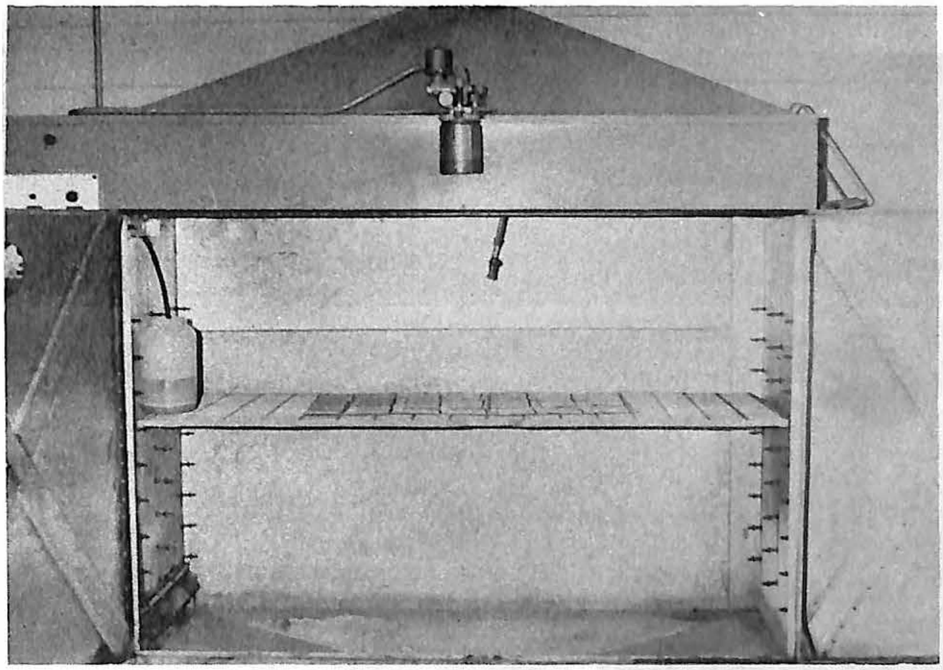

Figure 2. Spray chamber used for applying residual deposits

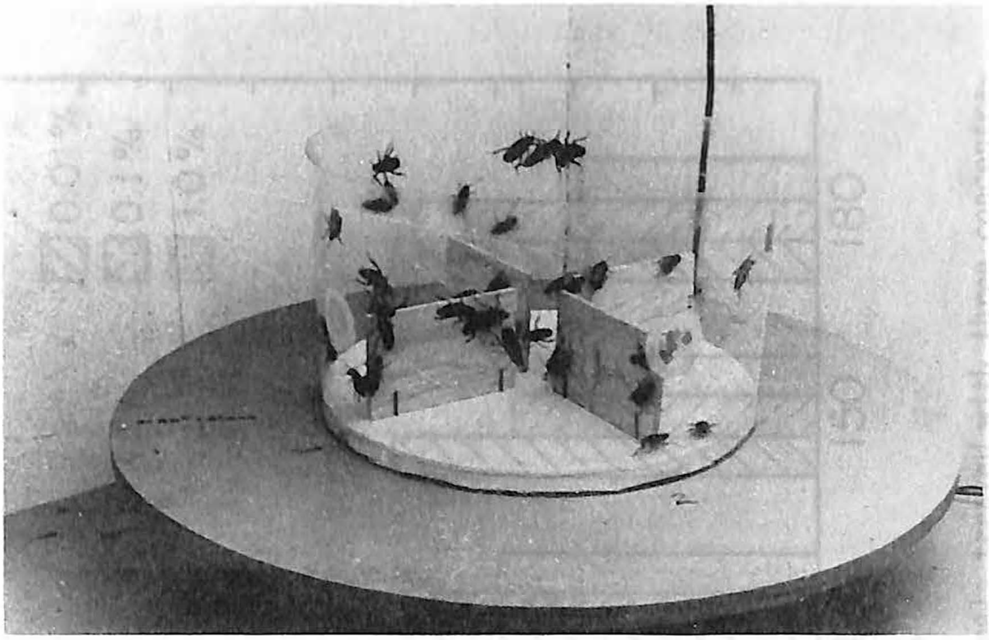

Figure 3. Cockroach repellent test chamber located on a turntable

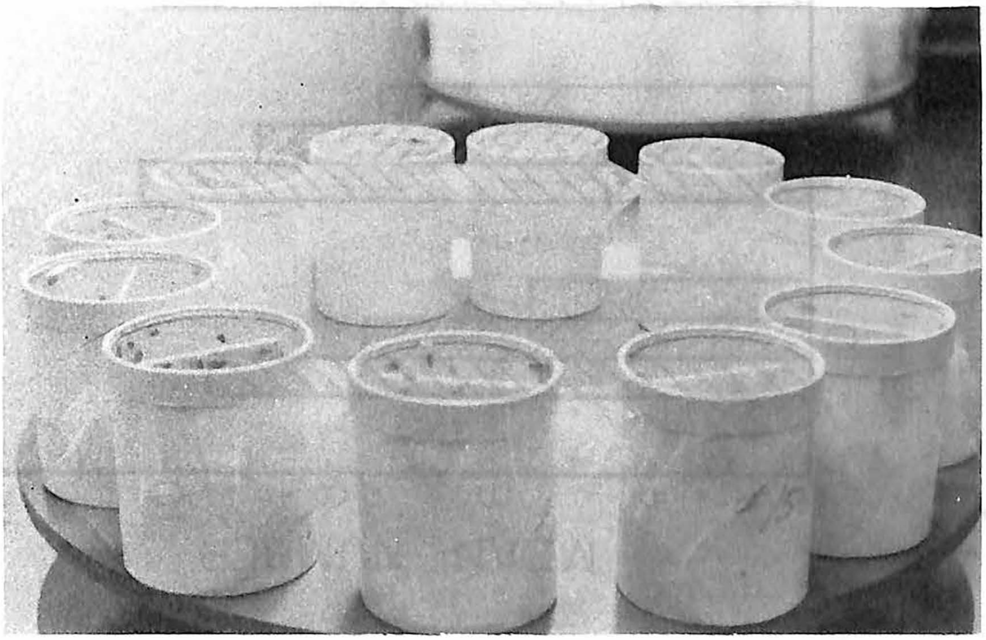

Figure 4. Cockroach mortality test chambers located on a turntable

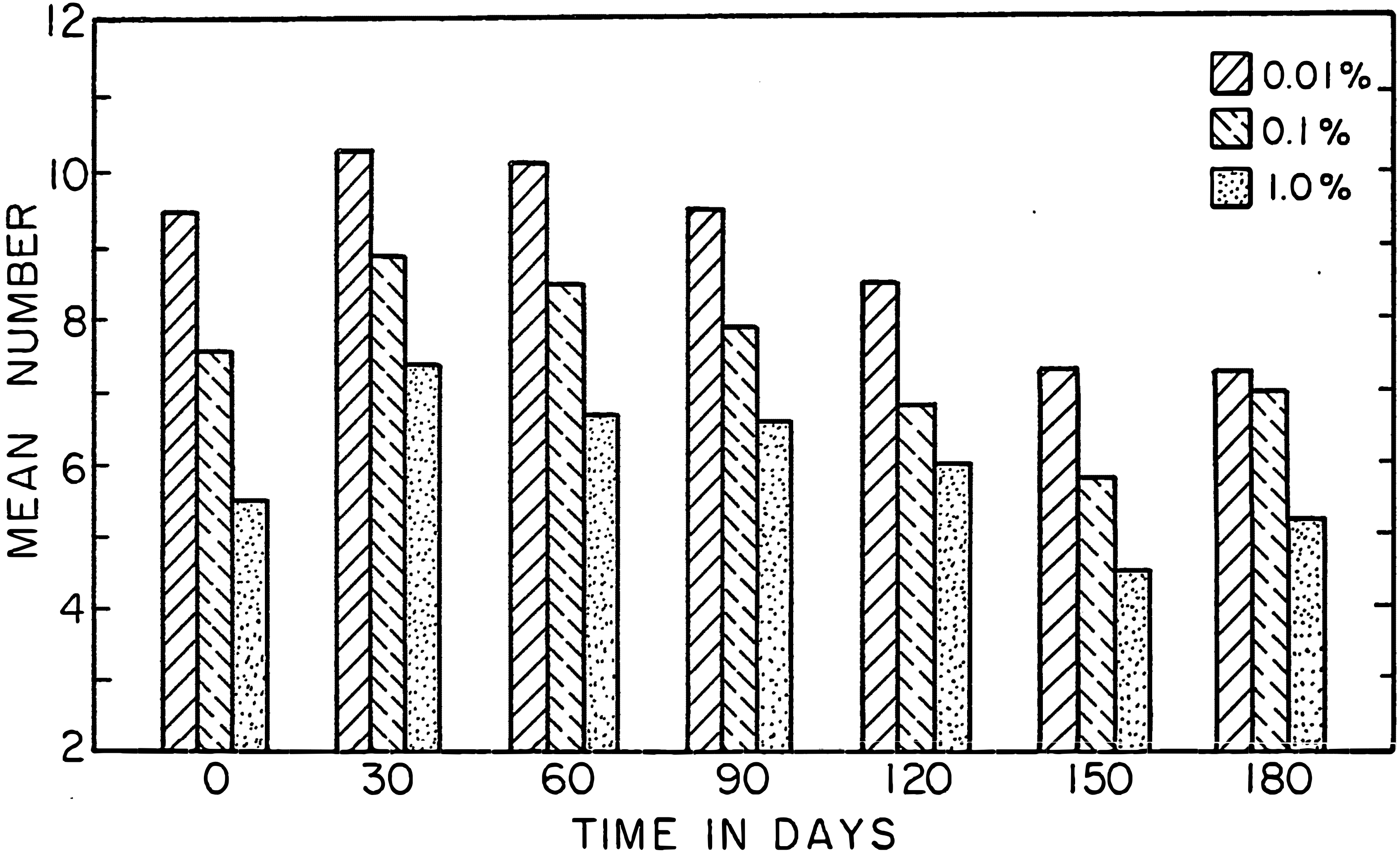

Figure 5. Mean number of _P. americana_ found on plywood panels treated with three concentrations of piperonyl butoxide

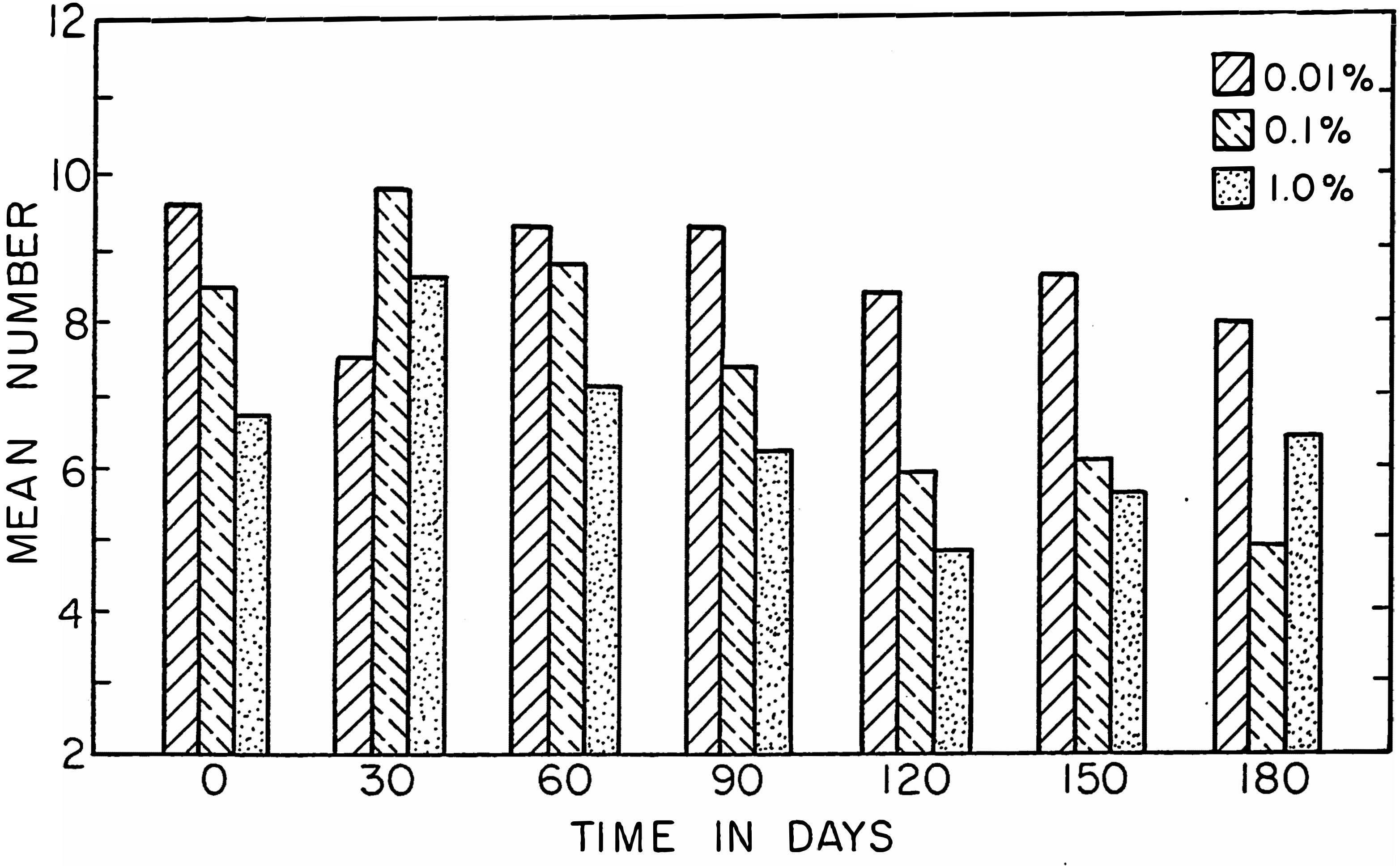

Figure 6. Mean number of *P. americana* found on plywood panels treated with three concentrations of R-11

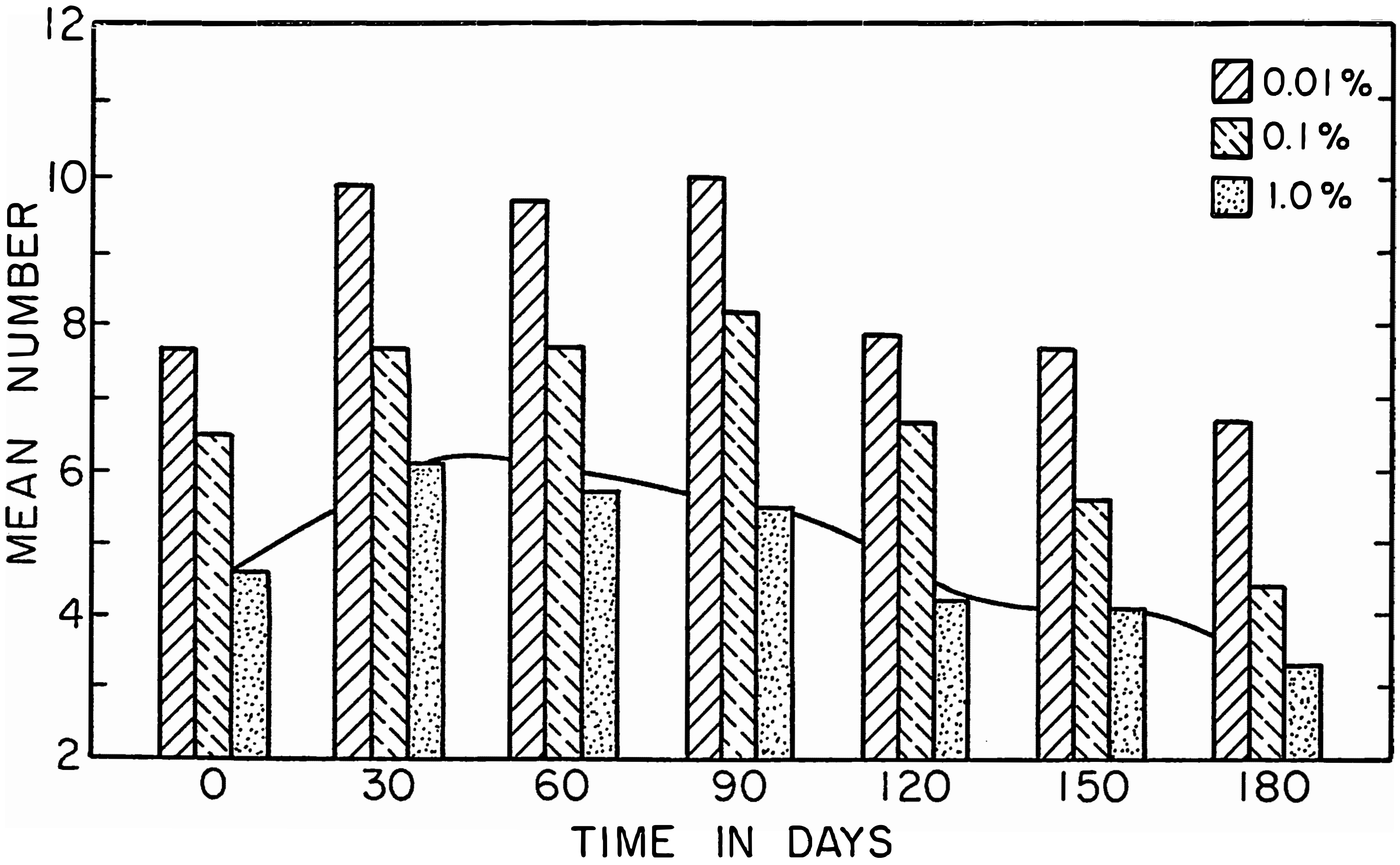

Figure 7. Mean number of P. americana found on plywood panels treated with three concentrations of Triton X-155

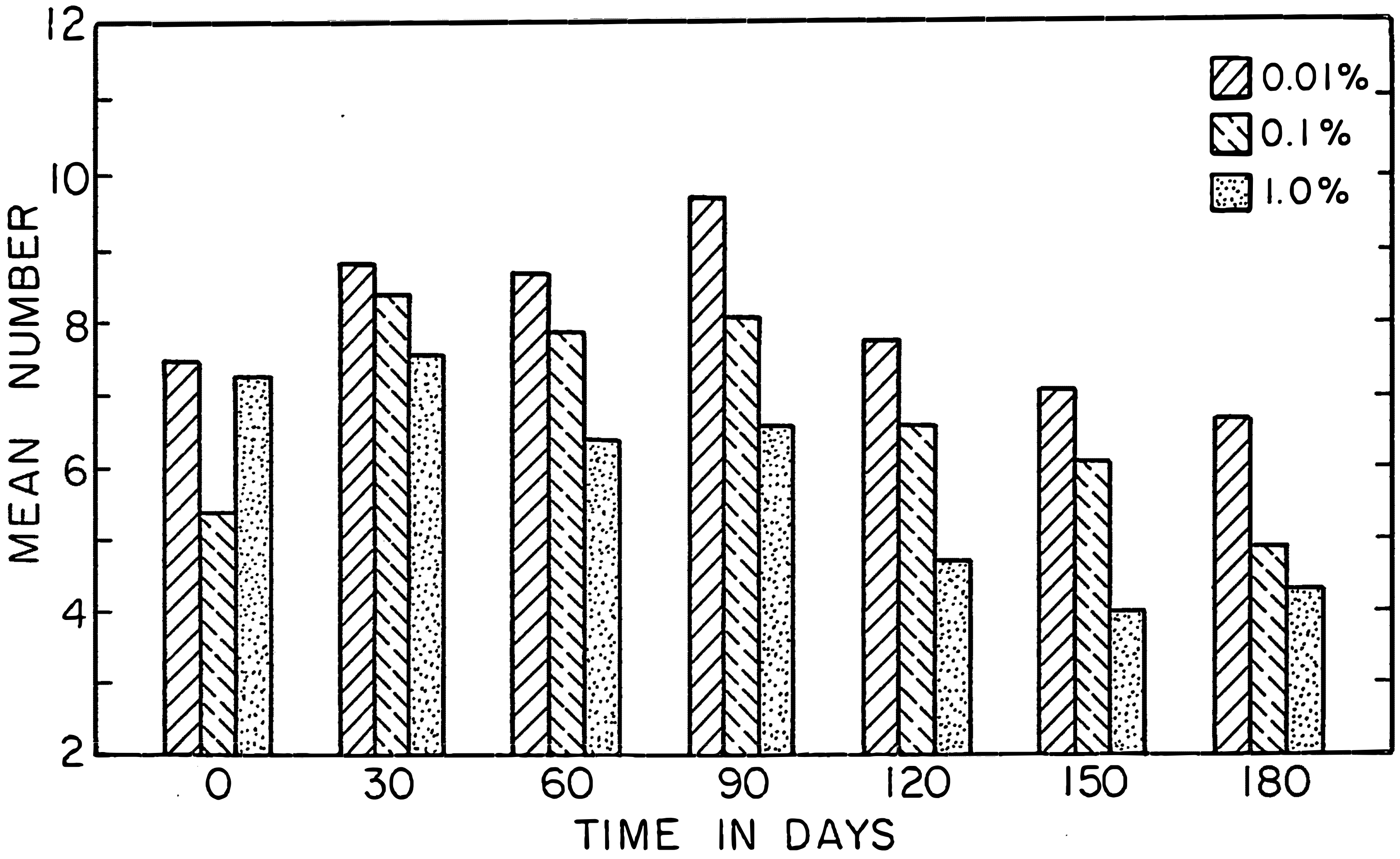

Figure 8. Mean number of P. americana found on plywood panels treated with three concentrations of Volpa-3

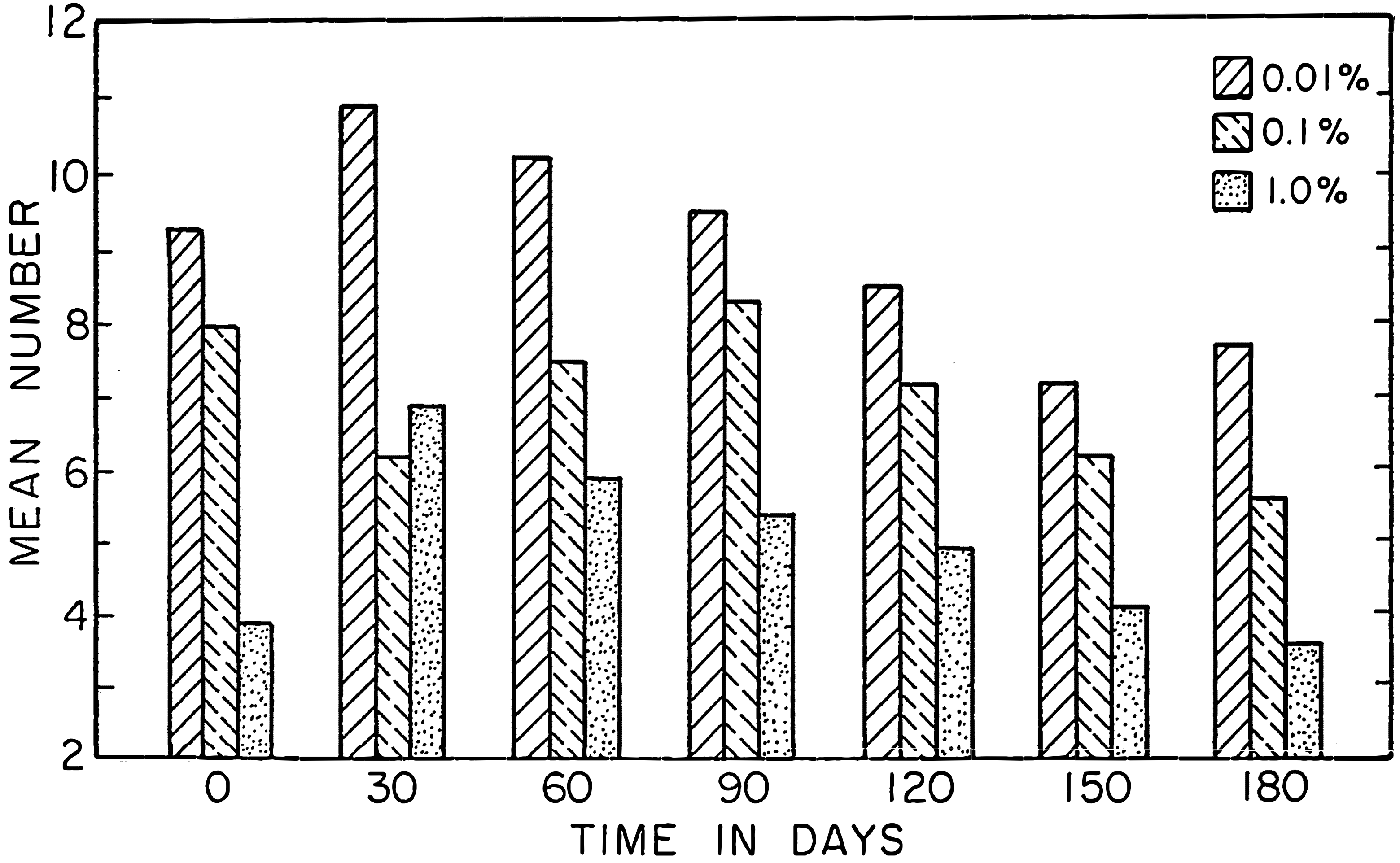

Figure 9. Mean number of B. germanica found on plywood panels treated with three concentrations of piperonyl butoxide

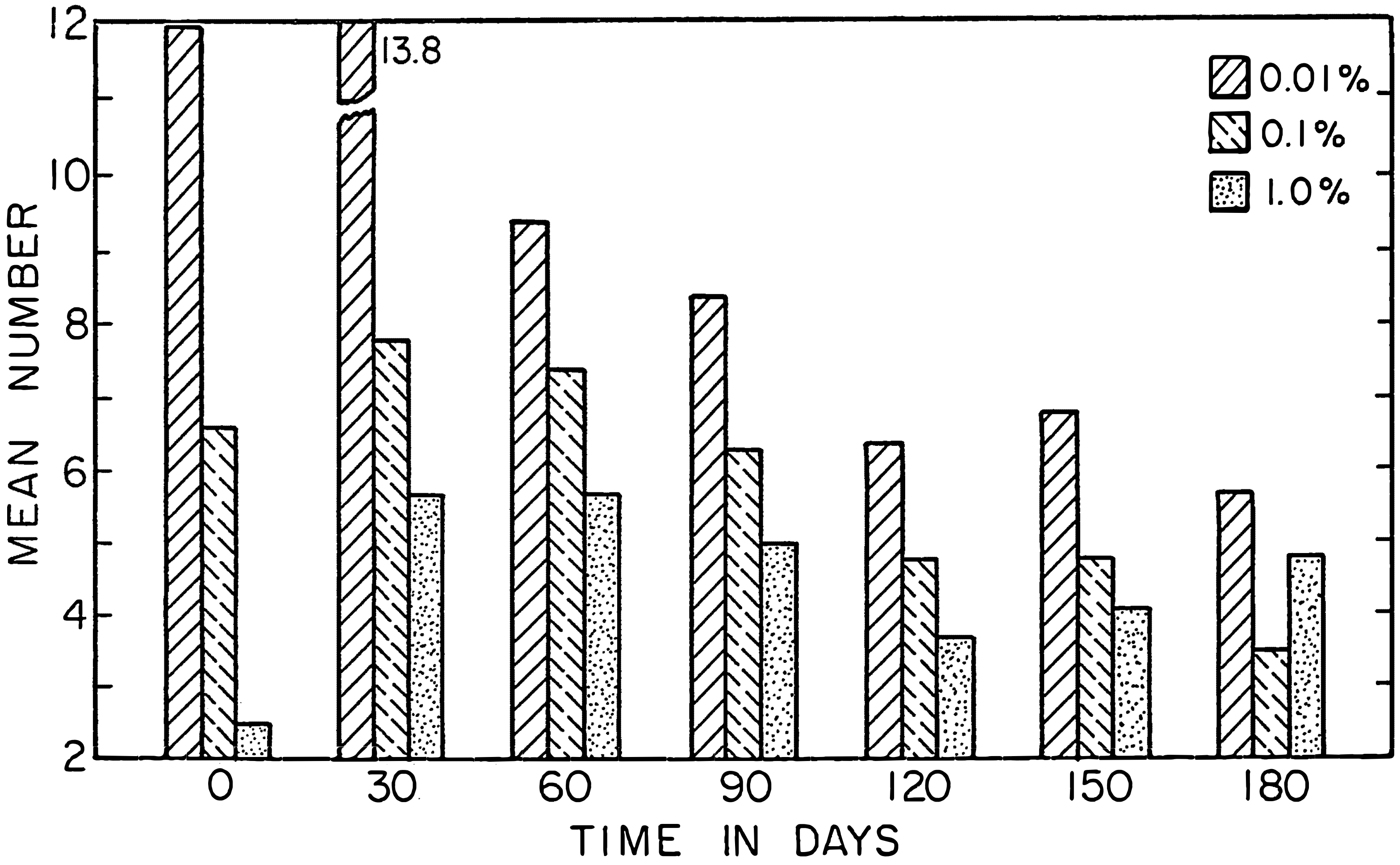

Figure 10. Mean number of B. germanica found on plywood panels treated with three concentrations of R-11

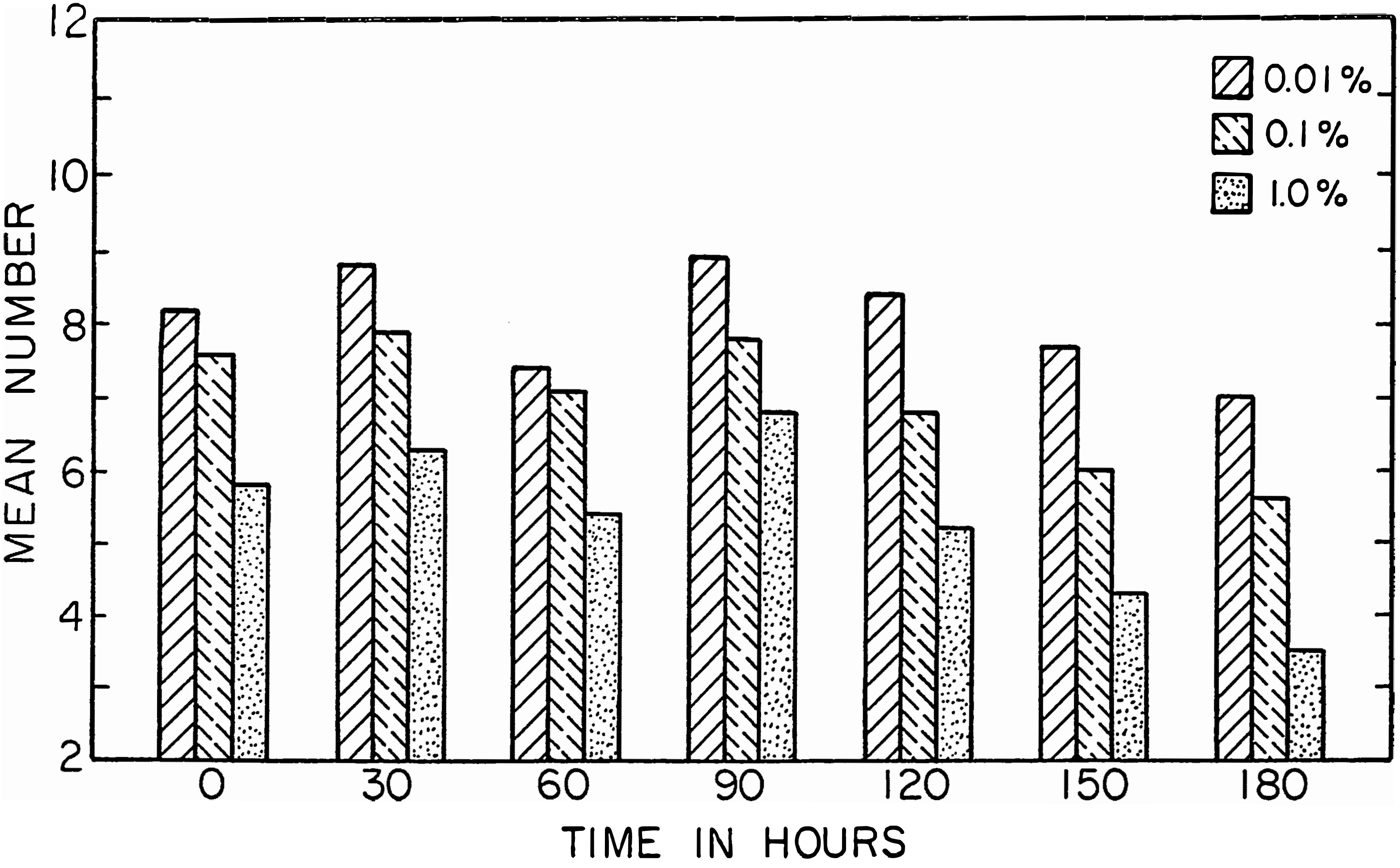

Figure 11. Mean number of B. *germanica* found on plywood panels treated with three concentrations of Triton X-155

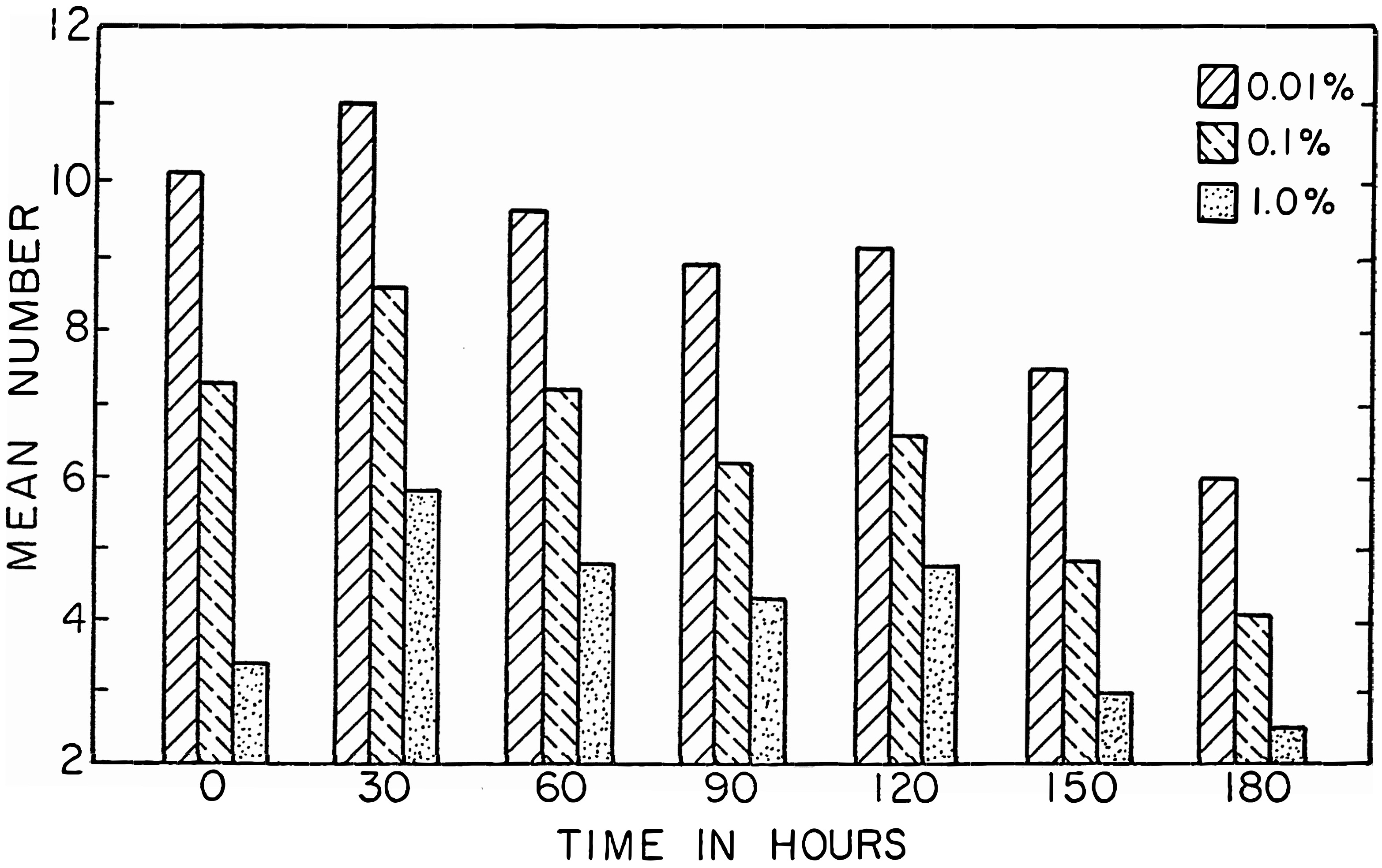

Figure 12. Mean number of B. germanica found on plywood panels treated with three concentrations of Volpa-3

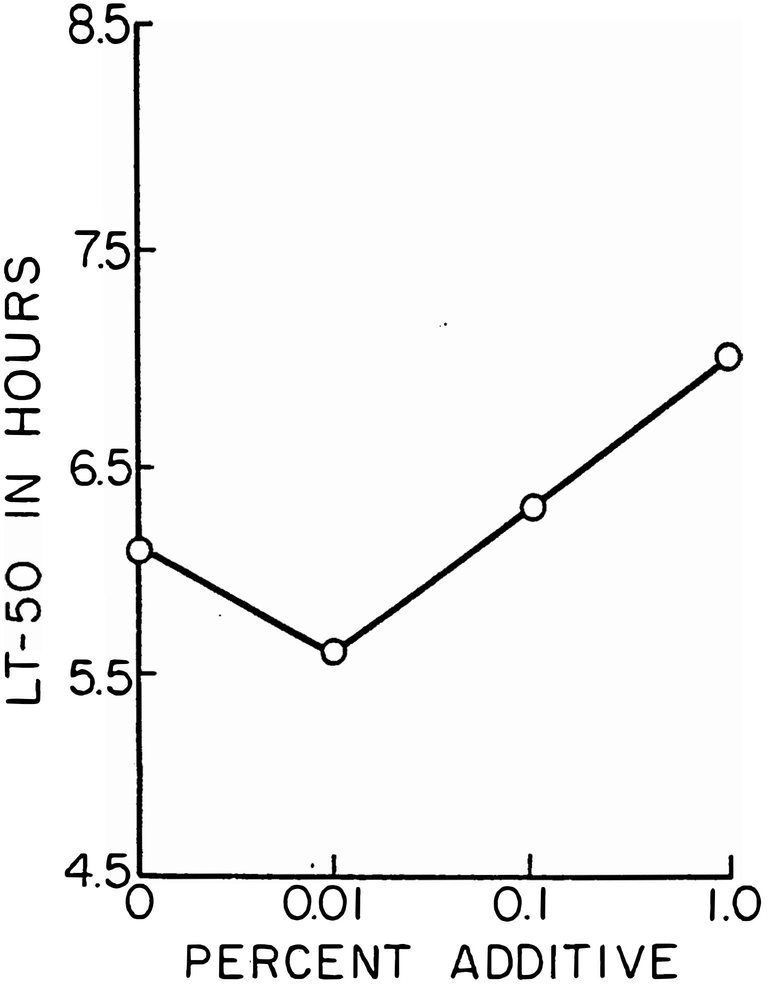

Figure 13. Toxicity of residues of 1.0% diazinon plus three levels of piperonyl butoxide to P. americana

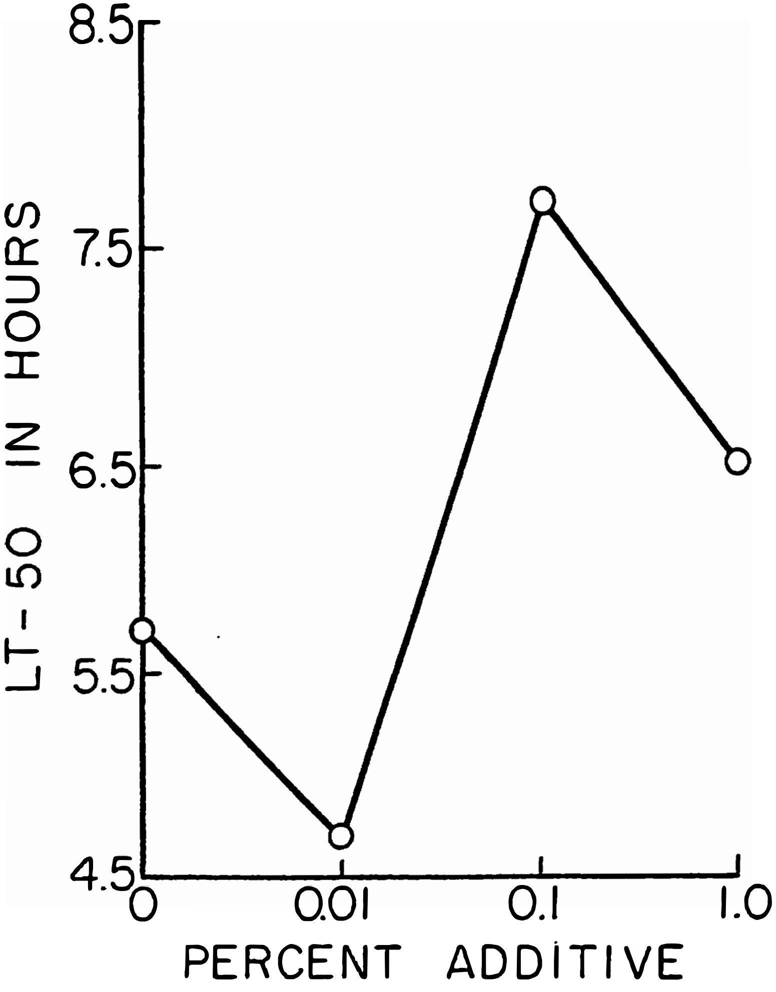

Figure 14. Toxicity of residues of 1.0% diazinon plus three levels of R-11 to P. americana

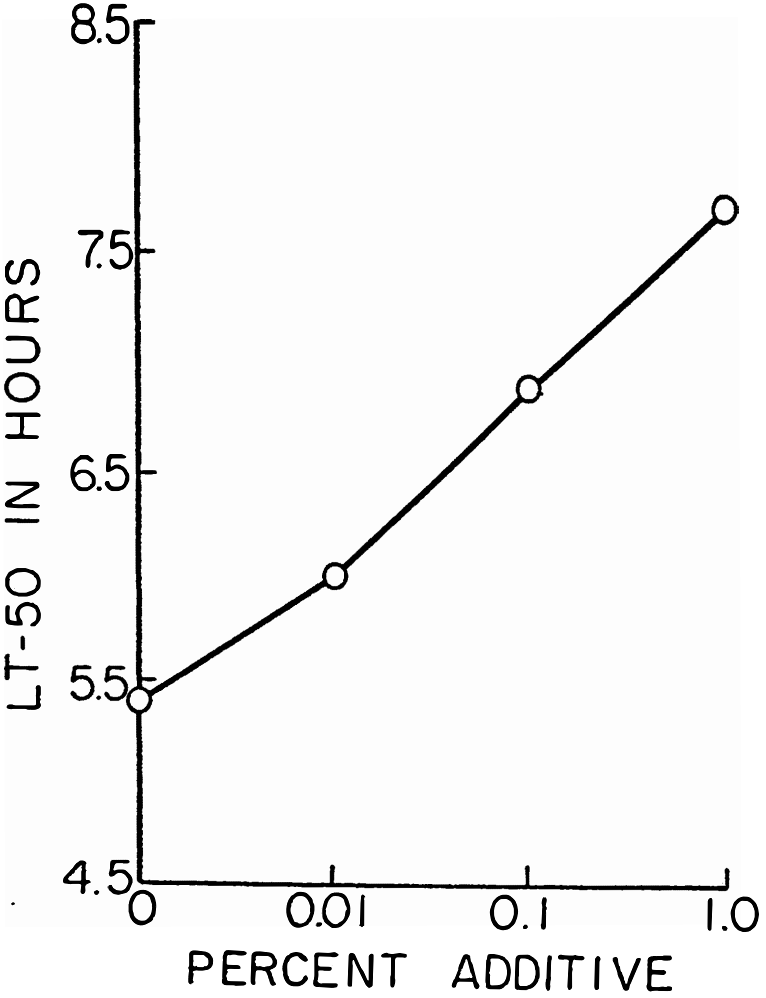

Figure 15. Toxicity of residues of 1.0% diazinon plus three levels of Triton X-155 to P. americana

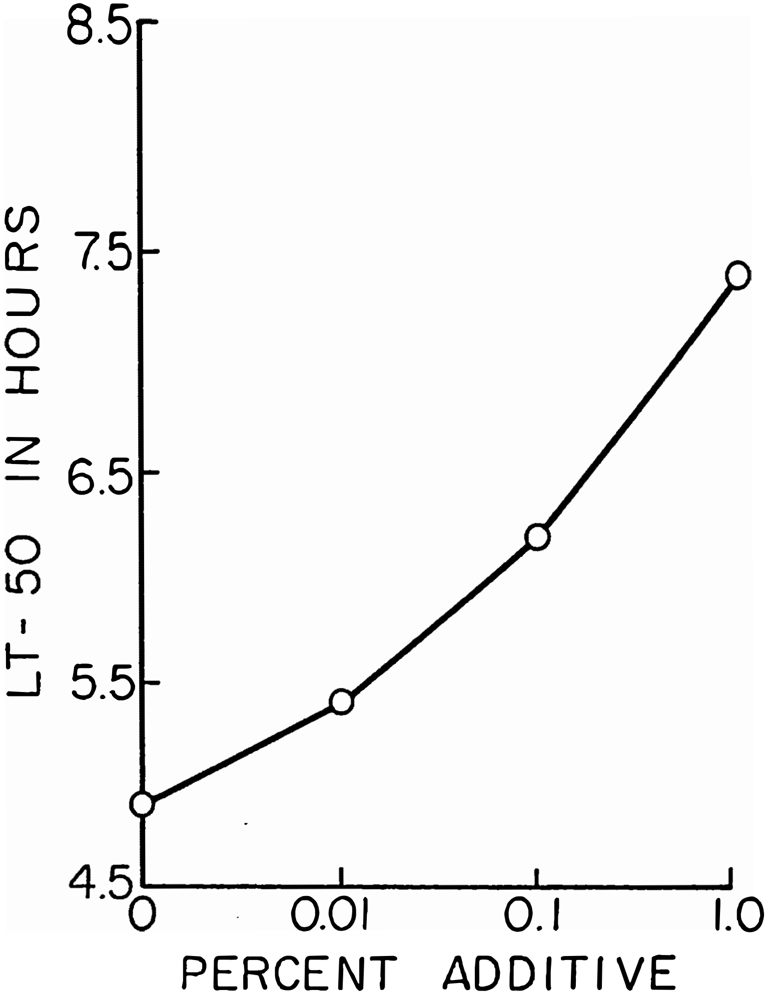

Figure 16. Toxicity of residues of 1.0% diazinon plus three levels of Volpa-3 to P. americana

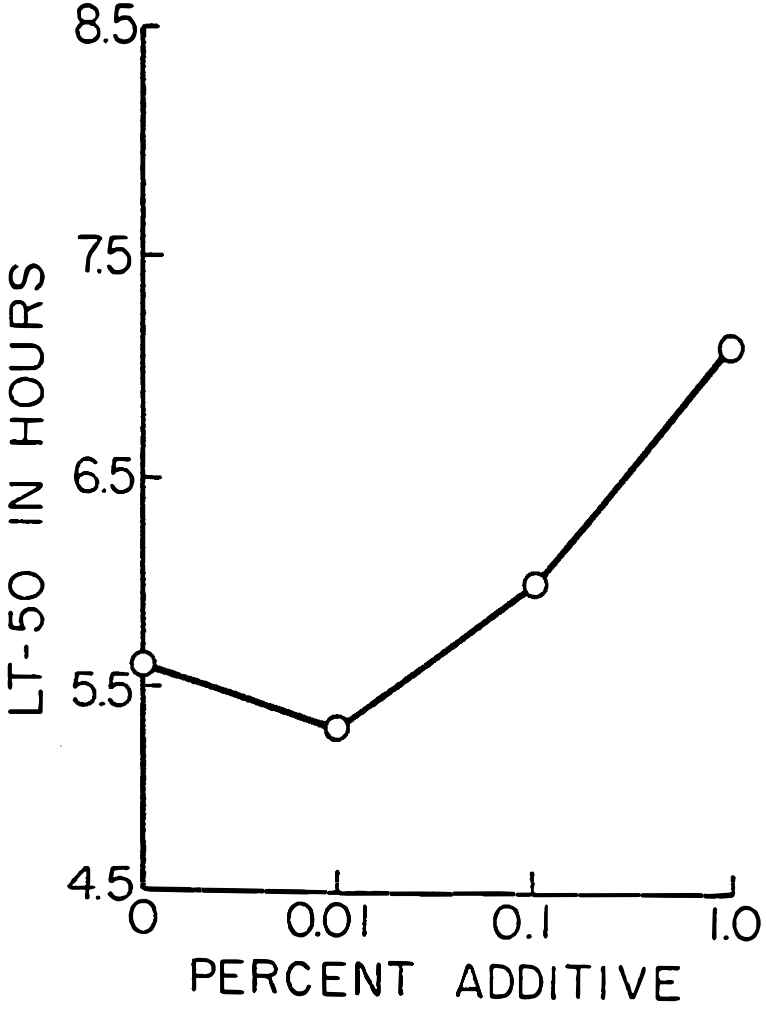

Figure 17. Toxicity of residues of 1.0% diazinon plus three levels of piperonyl butoxide to B. germanica

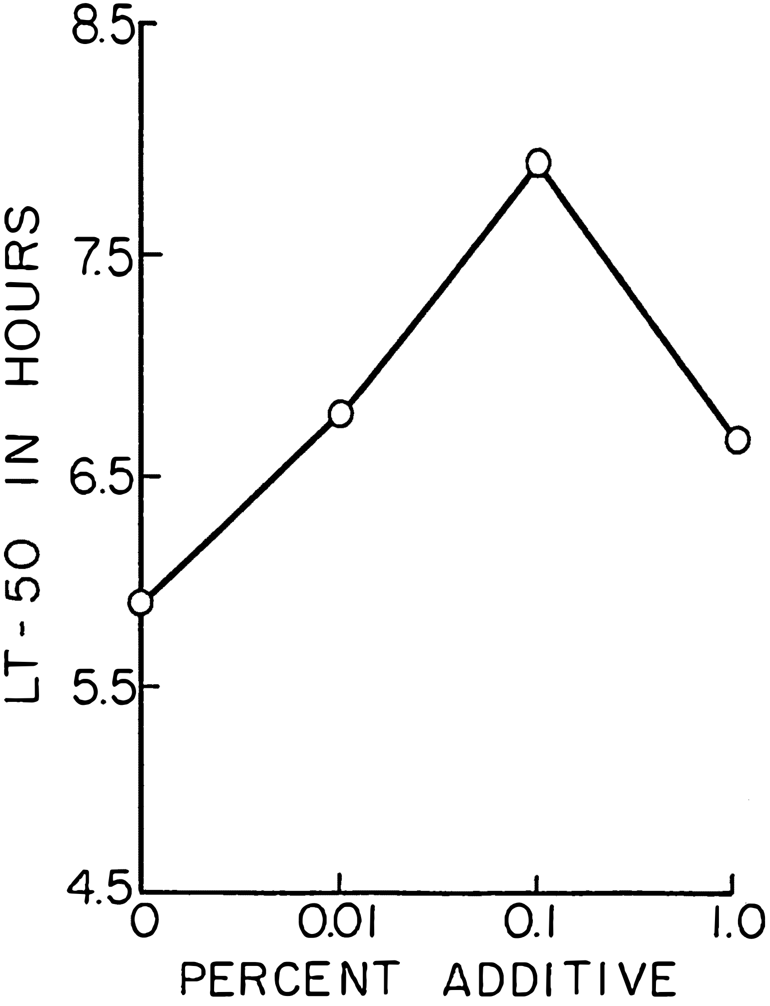

Figure 18. Toxicity of residues of 1.0% diazinon plus three levels of R-11 to B. germanica

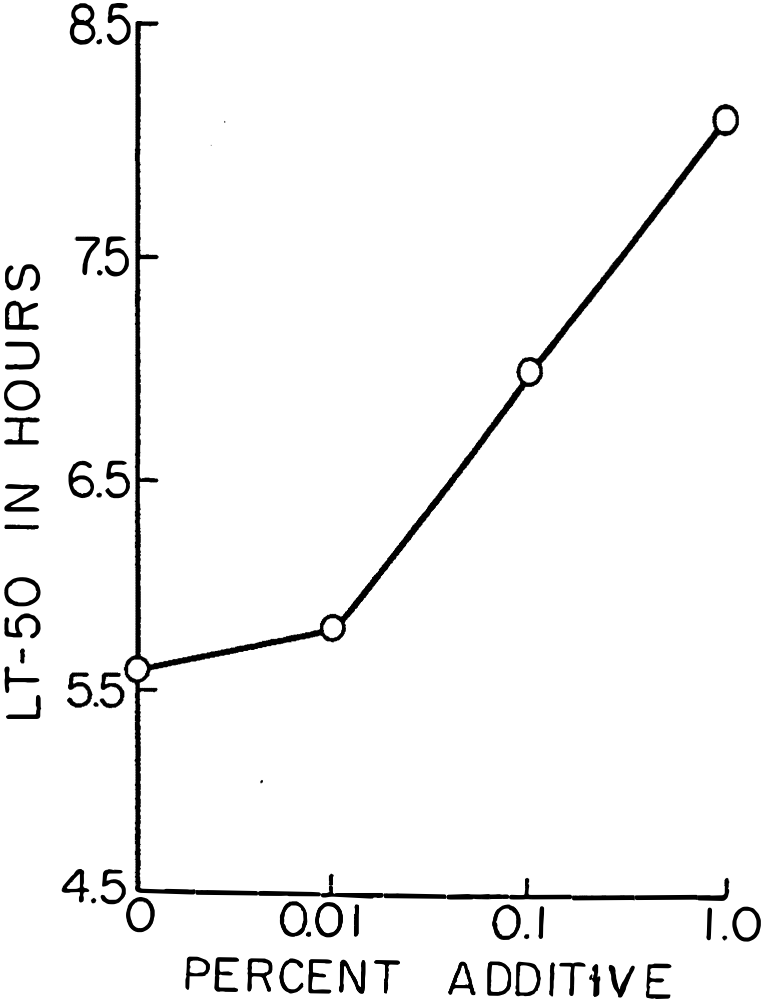

Figure 19. Toxicity of residues of 1.0% diazinon plus three levels of Triton X-155 to _B. germanica_

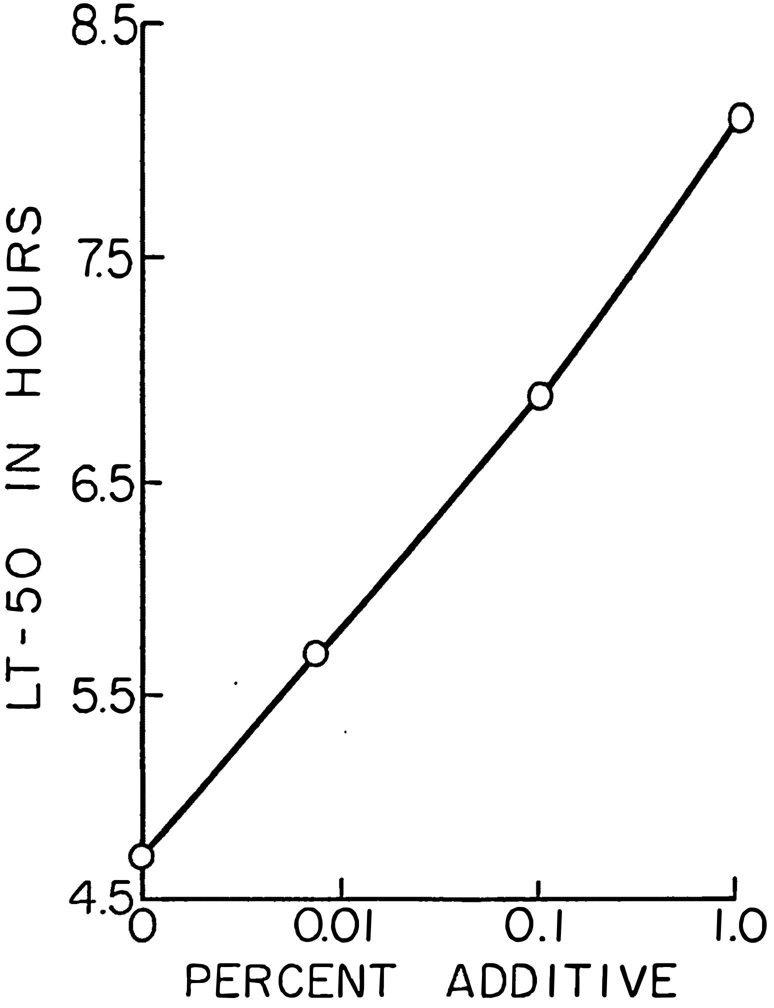

Figure 20. Toxicity of residues of 1.0% diazinon plus three levels of Volpa-3 to _B. germanica_

VITA

Laurence Johnston

Candidate for the Degree of

Doctor of Philosophy

Thesis: THE BIOLOGICAL EVALUATION OF FOUR SPRAY ADDITIVES WITH *BLATTELLA GERMANICA* (LINNAEUS) AND *PERIPLANETA AMERICANA* (LINNAEUS)

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