SUBLETHAL VAPOR-INDUCED RESPONSES OF THE GERMAN COCKROACH TO COMMERCIAL PESTICIDE FORMULATIONS

by

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Dissertation submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY
in
Entomology

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August, 1988
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(ABSTRACT)

The overall purpose of this study was to examine the effects of an insecticide vapor pulse on the dispersal response of the German cockroach, Blattella germanica (L). An apparatus was designed to expose test cockroaches to vapors from commercial pesticide formulations. Insecticide vapor-induced dispersal responses were recorded during a two hour period from different strains that had been allowed to acclimate to a harborage.

The dispersal response of large nymphs from a pesticide susceptible laboratory strain (VPI) was compared to two propoxur resistant field strains (Carver, Kenly) after exposure to four propoxur formulations and their blanks. Vapors from the petroleum-based oil and aerosol formulations induced significantly more dispersal than vapors from water-based emulsifiable concentrate

and wettable powder formulations. Vapors from formulations containing the toxicant generally induced a significantly faster dispersal response than did their blanks. Exposure of cockroaches to the vapors of diazinon, malathion, and cyfluthrin indicated that the class of pesticide can also influence the dispersal response. Strain differences were found in experiments with the propoxur formulations, their blanks, malathion and cyfluthrin. Slow dispersal precluded demonstration of significant strain differences in experiments with diazinon.

Exposure of mixed age groups of four strains to vapors from 1% propoxur-in-oil, an aerosol and their blanks indicated that dispersal patterns were similar to the single age class experiments. Inter- and intra-strain differences were found. Dispersal of the field strains was distinguished from that of the laboratory strain by more variable responses and differences among age classes. The strongest inter- and intra-strain differences were in response to the solvents rather than the complete formulation.

The air concentration of propoxur from a 1% oil formulation was estimated at 146 pg/ml. Condensation of the toxicant onto the apparatus was also observed. Vapor pulse characteristics for the four propoxur formulations indicated that the equilibrium vaporization rate in the test apparatus was approximately 0.5 mg/min for the oil and aerosol formulations and 1.0 mg/min for the WP and EC formulations.

Results suggest that effective control strategies must be tailored for each target population.

ACKNOWLEDGMENTS

I wish to express my abundant and sincere thanks to Dr. Mary Ross for her encouragement and direction throughout the course of my doctoral program. Her enthusiasm and love of research was contagious. I wish also to thank the members of my graduate committee: Dr. Mullins, Dr. Fell, Dr. Turner, and Dr. Hansen for their guidance and material support of my research.

Thanks must also go out to and of the "roach room" for not only their technical assistance but their friendship. In addition, I would like to acknowledge of the Statistics Department. Also I would like to thank for his advice and information on the test strains

The congenial work environment in the Entomology Department fostered by people like , and many others has made my stay at VPI & SU both pleasant and productive. In addition, I wish to give special thanks to who will always be a good friend.

I would also like to acknowledge of the Navy Health Science Education and Training Command and the National Pest Control Association for their financial support.

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INTRODUCTION AND LITERATURE REVIEW

The effects of insecticides on the German cockroach, Blattella germanica (L.) are complex. Although much work has been done on the physiological and toxicological aspects of these effects (Barson and Renn 1983; Scott and Matsumura 1981, 1983), comparatively little effort has been made to describe the effects of insecticides on behavior. The present study is concerned with behavior induced by insecticide vapors. To begin to understand this type of behavior, it is essential to review several basic concepts including: vapor formation, vapor perception by cockroaches, methodology, terminology, and the implication of these vapor-induced responses on the development of resistance.

Pesticide Vapor Formation.

It is crucial to have a basic understanding of the physical chemistry of vapor formation and the factors that influence them. The majority of studies on cockroach/pesticide interactions deal with the toxicological or behavioral reactions of the cockroach in terms of the toxicant without examining the nature of the formulations being evaluated (Ebeling et al. 1966, 1967;

Rust and Reierson 1977; Flynn and Schoof 1966). Responses induced by the vapors of a pesticide formulation should be considered as a function of the whole formulation and not exclusively as a reaction to the toxicant. In addition, most experiments have not been designed to investigate the initial impact of pesticide vapors on the target insect since the pesticide formulations being evaluated were allowed to dry thoroughly before being tested (Schneider and Bennett 1985; Cochran 1982, 1987; Ebeling et al. 1966, 1967, 1968). The implications of this are important because a cockroach's first contact with a pesticide, other than direct contact, is frequently in the form of vapors. Toxic vapor formation may be one of the major determinants of toxicant exposure of cockroach populations and may contribute to the development of both physiological and behavioral resistance. Lack of information on this period of insecticide vaporization seriously limits our understanding of the first crucial effects of a pesticide on cockroach populations.

The process of vaporization and volatilization is complex, depending on many factors, including the interaction of the chemical potentials of each component of a pesticide formulation (Atkins 1978). Dose transfer, as defined by Hall (1987), is the delivery of a toxicant to a point where there is contact with an organism sufficient to result in a measurable, predictable, biological response. This concept was developed to help tie together the physical chemistry aspects of a pesticide formulation and the behavioral or toxicological effects due to pesticide droplet impingement.

Pesticide volatilization is often thought to be dependent solely on the vapor pressure of the toxicant under study. Vapor pressures for most of the common pesticides are well documented and, except for the fumigants, are quite low (Graydon and Fosbraey 1982). However, what is not well understood is the carrier effects of highly volatile solvents in a pesticide formulation and their contribution to the vaporization of the actual toxicant. The amount of toxicant in the air from a drying pesticide formulation is determined by its distribution between the liquid phase and the air as a function of its solubility, molecular weight, vapor pressure, temperature, air flow (Burkhard and Guth 1981), partition characteristics and stability (Hall 1986) in relation to the formulation as a whole.

Pesticides applied at different concentrations may have a similar rate of volatilization and give a similar area of deposit (Hall 1987). Hall (1986) demonstrated that contact with permethrin as an emulsifiable concentrate for mite control did not induce the same behavioral responses as those induced by a wettable powder or oil base formulations.

Commercial pesticide formulations are often composed of many ingredients that have a wide range of volatilities. Therefore, during evaporation from a surface, the percent composition of a formulation will change from one moment to the next, leaving the less volatile components behind (Hall 1986, 1987; Sundaram 1986). This will cause a progressive decrease in evaporation and pesticide vaporization over time, resulting in an "exponential" decay of the mixture from the evaporating surface and a similar rapid decay in the air concentrations of those chemicals involved (Sundaram 1986). The drying of a pesticide formulation would result in a large "vapor pulse" followed by a rapid decline in air concentration as the formulation's vapors, including the toxicant, sought equilibrium by diffusion or condensation. The period of time when a pesticide formulation approaches "dryness" is crucial to the study of behavioral responses because chemokinses and chemotaxes of insects function best when close to a distinct gradient such as exists near a vapor pulse (Kennedy 1976).

Perception

The means by which the German cockroach perceives vapors from a pesticide formulation is also consequential to the understanding of behaviors caused by them. There are two types of vapor receptor cells: "specialist" and "generalist", the first of which has an extremely high specificity for odors and the latter which has a broad overlapping odor spectra (Morita and Shiraishi 1985). It has been shown by electroantennogram (EAG) (Bret 1985) and other studies (Reddy 1970) that the antennae of a German cockroach will respond to vapors of pesticides. However, information on threshold air concentrations is lacking. Furthermore, the correlation between a spike potential from an EAG and actual behavior has not been established. The morphology (Zacharuk 1985; Ishii 1971) and electrophysiology (Morita and Shiraishi 1985) of vapor sensitive sensilla, however, are well documented.

In the German cockroach, the antennae are the primary organs of olfactory perception (Reddy 1970). Vapor sensitive sensillae are found on other parts of the cockroach body, such as the tarsi, maxillary palps, labial palps, and the cerci, but they are thought to be of minor significance (Reddy 1970). Contact chemoreceptors are mainly located on the legs (Reddy 1970). The differentiation between direct tarsal contact and vapor detection by sensillae is critical to the conceptual bases of behavioral resistance (Lockwood et al. 1984).

Methodology

Many techniques have been used to evaluate insect/insecticide relationships. Much of the work on repellent compounds was done with mosquitoes (Khan et al. 1972, 1975) and houseflies (Virgona et al. 1983). Schreck (1977) reviewed early work in this area, including studies by Bar-Zeev and Schmidt (1959) who used radiolabelled compounds to repel mosquitoes from membrane feeders.

Methods for evaluating "repellent" compounds against cockroaches have been around for many years (Goodhue and Tissol 1952). Ebeling et al. (1966, 1967, 1968) used a choice box system that would present the cockroach with a selection between entering or avoiding an area that had been treated with a residual insecticide. Rust and Reierson (1977) placed test cockroaches in a central hub and allowed them the choice between several areas. Goodhue and Tissol (1952) used the choice of two shelters made from one pint ice cream cartons. Goodhue (1960) used the number of cockroaches found resting on treated index cards as an indication of the repellent nature of a chemical. Schneider and Bennett (1985) did a comparative study of the above methods and pointed out the advantages and disadvantages of each. Randall and Brower (1986) used glass plates separated by steel spacers to develop an index to evaluate various chemicals for repellent, neutral or aggregative properties. The above studies did not deal with the effects of chemical vapors, as each compound tested was allowed to dry before being used and the test cockroaches were allowed direct access to the toxic treated surfaces. Also, cockroaches were stressed by depriving them of food and water for up to 24 hours prior to the experiments to help induce movement.

Elbert and Behrenz (1986) developed a method for the determination of flushing effects using harborages made of paint coated aluminum plates. They developed a flushing time index for 50% of the population (FT_{50}). Aerosols were sprayed directly into these harborages and the number of cockroaches dispersing was recorded. Unfortunately, the effect of the vapor cannot be distinguished from direct contact with the aerosol spray. Furthermore, test cockroaches closest to the spray nozzle shielded those that were farther away. Other methods for evaluating aerosol spray against cockroaches include those of the American Society for Testing and Materials (ASTM 1980, 1984).

Methods for evaluating the German cockroaches' response to both aggregation pheromone (Ishii and Kuwahara 1967) and dispersant pheromone (Suto and Kumada 1981; Ross and Tignor 1985) are similar to the repellency studies of Goodhue (1960). The test cockroaches were allowed to contact either the pheromone treated dry filter paper or pesticide treated index cards. In this type of experiment, no distinction could be made between contact or olfactory detection of the chemicals by the test cockroaches.

Relatively few studies have dealt specifically with the responses of German cockroaches to pesticide vapors. Reddy (1970) used a simple binary choice chamber to test the effects of both the liquid and vapor phase of a repellent compound, MGK R-874. Bret and Ross (1985a, b) used a system of two interconnecting aquaria to study dispersal induced by propoxur vapors.

Since this is an area significant to cockroach control, but one in which few techniques have been employed, the development of a test apparatus suitable for such studies would be a useful contribution.

Terminology.

As interest in the responses of insects to insecticides developed, a variety of terms have come into use to describe various types of insect reactions. However, some confusion on the meaning and use of these terms has arisen, depending on the emphasis of the work involved.

Dethier (1947) realized that the term "repellency" was used in different ways by various investigators. He broadened the definition of repellent to include both a vapor repellent and a contact repellent. He further distinguished between two different kinds of behavioral repellency, of which one form was an immediate directional avoidance reaction or taxis and another form was an irritating effect which caused an increase in activity. A further effort to clarify terms resulted in the following definition (Dethier et al. 1960):

> Repellent- a chemical that causes insects to make oriented movements away from its source.

Muirhead-Thomson (1960) meanwhile utilized the terms "repellency" and "irritability" to describe induced behavior in mosquitoes. Lockwood et al.(1984) used these terms to describe responses involved in different types of behavioral resistance:

> Repellency- occurs when an insect is stimulated to leave the immediate toxic treated environment <u>before</u> contact with a treated surface.
>
> Irritability- occurs when an insect is stimulated to leave the immediate toxic environment <u>upon</u> contact with a treated surface.

Confusion in the use of these terms originated mainly from various other author's failure to define their terms when discussing their own work or comparing it to that of others. An example of how a problem could develop is given as follows: the work of Ebeling et al. (1966, 1967, 1968) used repellent compounds and described "repellency" in experiments where the response was apparently due to contact. According to Lockwood et al. (1984) this would be "irritability". Both terms are compatible if looked at in detail such that a vapor repellent (Dethier 1947) causes repellency (Lockwood et al.1984) and a contact repellent (Dethier 1947) causes irritability (Lockwood et al. 1984). The other major problem with terminology has been the use of the term "irritant" or "irritant effect" which should be distinguished from irritability. Dethier (1956) used the following to describe one of two forms of repellency:

> "....and the other as some sort of irritating effect- causes insects to increase their activity (orthokinesis) with the result there would be a decreased number on the surface causing excitation. "

To clarify the confusion he proposed that a chemical that induced this type of response be designated as a locomotor stimulant (Dethier et al. 1960).

Garson and Winnike (1968) discussed the relationship between volatility and repellency. They defined "olfactory repellents" as those materials which are sufficiently volatile to keep insects at a distance. "Gustatory repellents", on the other hand, were defined as those chemicals which are "so slightly volatile" that the insect must approach and touch the treated surface before being repelled. They also described two types of repellent action, one that is time dependent (effective repellency) and one that is time independent (intrinsic repellency). "Intrinsic repellency" is exhibited when a known amount of material causes some degree of repellency independent of time. Effective repellency is a repellent response measured as a function of time.

"Dispersal" and "dispersants" are also terms that might lead to confusion. Matthews and Matthews (1978) described one type of dispersal as a large scale spatial adjustment in terms of populations or species, including migration initiated by a depletion in local resources. This term might be applied to the movement of cockroaches throughout an apartment complex to search for food. Movement that is induced by dispersants such as disperant pheromones (Suto and Kumada 1981) or insecticide vapors (Bret and Ross 1985b) has a different connotation in that it involves short distance displacement from a harborage. Evans (1984) defined "dispersal as a general term for all forms of displacement to new habitats". This includes responses to crowding, food stress and other stimuli, as well as large-scale movements, such as migration. Vapor-induced dispersal as the subject of this study is defined as a short distance displacement response from a harborage caused by chemical vapors.

The term "flushing" (Elbert and Behrenz 1986; Ebeling and Reierson 1973) is another term which might be confused with vapor-induced dispersal. Flushing can be defined as the utilization of an aerosol spray formulation to purposefully initiate cockroach displacement for survey or control purposes. In theory the flushing agent would cause the insect to vacate its harborage and run across a residual toxic application (Ebeling and Reierson 1973). The difference between flushing and vapor-induced dispersal is best explained in terms of particle size. A flushing application includes both aerosol droplets (15-20 microns) and vapors (<15 microns). On the other hand, vapor-induced dispersal is only a function of a formulation's vapors. Particle size is the criticial factor as to the amount of pesticide impinging on an insect. If a particle is too small, it will be carried about by convection currents; if it is too large, it will fall-out quickly without impinging. The differences in the size of the particles and their corresponding differences in volumes can play a role as to whether behavioral or toxicological responses are observed. Flushing probably induces both behavioral responses, such as dispersal and toxicological responses, such as knockdown.

There are many additional terms that occur in the literature that have not gained wide acceptance including: "expellency" (Whicham 1988), defined as repellency of an insect after it enters a domicile; and "cidophobia" (ASTM 1981), defined as the sensitivity of target insects that increases their aversion to high mortality within a population.

Vapors and Resistance.

The interrelationship between physiological and behavioral responses in the development of resistance is not clear (Pluthero and Singh 1984). Changes in vapor-induced responses to insecticides may have evolved in a manner similar to the adaptation of insects to toxic secondary plant compounds (Pluthero and Singh 1984). If this response was modified, it could be expected to impact on the efficacy of control measures. Nevertheless, behavioral modification of an insect population in response to pesticide exposure was thought to be of minor importance in the overall resistance picture (Georghiou 1972). Ebeling et al. (1968) realized the importance of chemical induced responses to cockroach control and stated that repellency (irritability) was "the most important factor affecting the insecticidal efficiency of blatticides", although he did not consider this in relation to behavioral modifications.

Scott and Matsumura (1981, 1983) stated that the main type of pesticide resistance is due to "target insensitivity". Rashatwar and Matsumura (1985) found that the biochemical mechanism for pyrethroids was not due to either metabolic or penetration differences but was related to the reduction in calcium sensitivity in the nerve Na^+/Ca^{2+} exchange system.

The combined action of both physiological and behavioral adaptations could be very beneficial to cockroach survival following a pesticide treatment. Physiological resistance could protect the cockroach against lethal effects from unavoidable contact with the insecticide while the behavioral mechanism could limit contact with the insecticide (Pluthero and Singh 1984). The basic assumption behind the relationship involves both the development of an increased ability to detoxify a particular pesticide, as well as a modification in behavior (Pluthero and Singh 1984).

As physiological resistance is selected for in a cockroach population, one of three behavioral responses in terms of dispersal might also develop: (1) reduced sensitivity as mediated by an increased ability to detoxify, resulting in a decreased dispersal response; (2) increased sensitivity and increased dispersal; (3) no relationship between dispersal behavior and physiological resistance. A cockroach with a type 1 response might not sense the toxicant effect as readily as a susceptible cockroach and thus show less dispersal than a sensitive strain (Pluthero and Singh 1984; Bret and Ross 1986).

The advantage of a reduced dispersal response to cockroaches might be in the avoidance of a toxic treated surface or the continued occupation of a "superior" harborage providing exposure did not have lethal effects. The vapor pulse of most pesticides is of short duration so the ability of a resistant strain to remain in or near a favorable harborage until the vapor dissipated might be an adaptive advantage. On the other hand, increased sensitivity to pesticide vapors might provide the cockroach with the ability to vacate a harborage before the situation deteriorates to a lethal point. Examples of each of the above relationships are found in the literature for a variety of insects and are described in conjunction with their theoretical implication by the reviews of Lockwood et al. (1984), Pluthero and Singh (1984), and Georghiou (1972).

A behavioral response to a stimulant is probably under the control of many genes (Pluthero et al. 1982) that might work independently from those that control physiological resistance (Pluthero and Singh 1984). This situation could lead to contradictory results when examining different resistant strains of the same organism

(Lockwood et al. 1984). Many factors can come into play to determine what type of resistance develops. They include the type of selective pressure as a function of pesticide treatment history, including formulation types, and environmental factors and genetic variation within the pest population (Lockwood et al. 1984).

Because of the multiplicity of factors involved, it is conceivable that the development of behavioral resistance to pesticide vapors could take on any one of the three forms mentioned above. As mentioned by Lockwood et al.(1985), there are many examples of a given species even within a single study demonstrating different physiological and behavioral resistance relationships.

PART I. VAPOR-INDUCED DISPERSAL TEST APPARATUS: DESIGN RATIONALE AND SPECIFICATIONS

INTRODUCTION

Three patterns of German cockroach movement have been described: evasive, exploratory or aggregative (Sommers 1975, 1978; Ballard et al. 1984). There are many factors that could induce a cockroach to move besides pesticide vapors. The design of any apparatus to study dispersal should take into account the causative factors from each of the above patterns. The overall apparatus design in a vapor-induced dispersal study would be to duplicate the conditions of a "superior harborage". Test cockroaches should be placed in a set of conditions that promote aggregation, and at the same time reduce the chance of dispersal by causes other than that of chemical vapors.

The following paragraphs illustrate factors that might influence cockroach aggregation, movement and dispersion patterns. They are followed by a description of an apparatus for testing vapor-induced dispersal and a set of conditions that would limit any movement of the test cockroaches except those caused by the introduced chemical vapor.

FACTORS PROMOTING AGGREGATION

The German cockroach spends at least 75% of its time at rest (Cornwell 1968), usually in aggregations of conspecifics (Ishii and Kuwahara 1967). They frequently aggregate in preferred micro-environmental locations known as "harborages" (Cornwell 1968) or refuges (Denzer et al. 1985). The main factors promoting the formation of aggregations within harborages are pheromonal and thigmotactic stimuli (Berthold and Wilson 1967). Cockroaches exhibit a positive thigmotactic response (Bell and Kramer 1979; Berthold and Wilson 1967). Adult cockroaches seem to show no orientation preference in a harborage (Cornwell 1968). However, the nymphs of some species show a preference for resting on vertical surfaces (Bell et al. 1972). Ishii and Kuwahara (1967) described an aggregation pheromone for the German cockroach. Wileyto et al. (1984) reported that the pheromone served to transfer information to conspecifics on habitat selection and aggregation make-up.

FACTORS PROMOTING DISPERSAL

Movement by Blattella germanica usually consists of short runs at a speed of about 0.25ms^{-1} and halts (Chadwick 1985). Factors that might cause a cockroach to disperse from a harborage are: excessive temperature, vibrations (Ballard et al. 1984), low humidity (Guthrie and Tindall 1968), circadian rhythm (Baker et al. 1980; Dreisig and Nielsen 1971), lack of food or water, (Berthold and Wilson 1967), a dispersant pheromone (Suto and Kumada 1981; Ross and Tignor 1985, 1986), noise (Cornwell 1968), chemical contamination (Ebeling et al. 1967, 1968; Reddy 1970), air movement (Bell and Kramer 1979; Camhi and Tom 1978), and light (Guthrie and Tindall 1968). It is difficult to discuss these factors meaningfully by themselves or in absolutes since they may all work in concert with each other to produce a local environmental state.

Cornwell (1968) and Sommers (1978) stated that the German cockroach is not photonegative. Cornwell (1968) attributed the cockroaches nocturnal habits not so much to the presence or absence of light, but to the cockroach seeking refuge from the lower relative humidity during the day. Nocturnal activities by the German cockroach are also strongly influenced by circadian rhythm. Cornwell (1968) indicated that the circadian rhythm activity period of the German cockroach begins as early as 6:00 P.M. in the winter and as late as 3:00 AM in the summer. Dreisig and Nielsen (1971) stated that there is a delay in the activity period of the circadian rhythm for the German cockroach when exposed to a constant source of light. However, nocturnal activity is released not by presence or absence of light itself, but by gradual reduction in illumination. They also found that an increase in temperature would delay the onset of circadian activity.

Normally, undesiccated cockroaches are not influenced by humidity gradients, but those that are desiccated respond readily to high relative humidity (Cornwell 1968). The German cockroaches preferred temperature range, if it has food and water, is 24-33°C (Cornwell 1968). Berthold and Wilson (1967) indicated that food deprivation would induce exploratory activity. Reynierse et al. (1972), however, reported that for the cockroach _Nauphoeta_ _cinerea_ food and water deprivation reduces activity.

Air movement, vibrations, and noise might also disrupt an experimental harborage. Bell and Kramer (1979) indicated that the German cockroach perceives the direction of air current through its antennae and cerci, and that the threshold of perception was between 0.015-0.03 m/sec.

Ballard et al. (1984) found that exploratory behavior in the German cockroach increased with increased temperature, environmental familiarity and food deprivation. It decreased with increased population density and fecal contamination (Ebeling and Reierson 1970).

APPARATUS SPECIFICATIONS

Each test apparatus (Fig. 1) was made of two 750 ml Pyrex crystallizing dishes connected by a short tube. The first dish (aggregation dish) was modified by making a 10 mm diameter exit hole in the side of the dish, flush with the dish floor. At this exit hole a tube flange of Pyrex glass (20 mm long; 10 mm i.d.) was attached. The tube flange was constricted upwards at the point of attachment to the floor of the first dish to form a glass dam 7 mm high at the exit hole. The second dish (dispersal dish) was modified similarly, but without the glass dam. Both dishes were joined by a connecting tube fitting over the tube flanges of each dish. Gasket seals between the connecting tube and flanges were made of Parafilm wax. The connecting tube was made from a glass cylinder (57 mm long; 17mm i.d.). The glass cylinder was modified by removing a central length (30 mm long) of roughly half the circumference of the cylinder side, and covering it with a piece of 30 mesh brass screen. This open mesh window was always placed face down when connecting the two dishes.

The sides of the interior of both dishes were covered with a thin film of petroleum jelly to keep the test cockroaches on the floor of the dishes and to prevent their escape. Two mesh screens strips (40 mm by 10 mm) that had been exposed to cockroach aggregations were folded into the shape of a "W" and placed into both dishes. The exit hole from the first dish was sealed with a Parafilm strip. A Petri dish (1.8 cm high; 14.5 cm diam.) was used as a cover for the first dish. Test cockroaches were placed into the first dish for at least three hours to allow them to acclimate before the introduction of vapors.

Pesticide vapors were introduced into the apparatus by treating a Whatman no. 1 filter paper strip (430 mm by 10 mm) with 300 ul of each test formulation. The treated collar was then placed on the inner upper lip of the first dish approximately 5 cm above the floor. After the vapor source had been placed inside the first dish, the wax barrier was removed and the number of test cockroaches moving out of the first dish recorded at 10 minute intervals for two hours.

The apparatus was designed to allow chemical vapors to build up in the first dish and to diffuse from the second dish as demonstrated in Part IV. Cockroaches were prevented from making contact with the filter paper collar by the petroleum jelly.

DISCUSSION

Those factors that promote aggregation and that might cause movement other than that caused by the test vapors were taken into account in the design of the apparatus. The screens provided both thigmotactic and aggregation stimuli. To reduce dispersal by circadian rhythm all tests were run from 10:00 - 17:00 hours (10:00-14:00 acclimatization; 14:00 - 17:00 to determine dispersal). To reduce exploratory activity, the cockroaches had access to water and food *ad lib.* until testing. Overhead lighting was kept constant throughout the experiment. All tests were run at times when there was minimal air movement, noise, or vibrations. Experiments were conducted at 22 ±°C and a 55±10% RH. Untreated filter paper collars were used in the control experiments. The latter were placed prior to that of the treated collars to prevent accidental introduction of chemical vapors.

The formation, shape, and intensity of a vapor pulse are dependent on the surface area and surface texture of the vapor source. Differences in vapor pulse intensity and shape might affect the dispersal response of insects to pesticides. Location of the vapor source in relation to the location of the test insects is also important. Care must be taken that each cockroach has an equal chance of exposure to pesticide vapors and that vapor free pockets or shielding effects are reduced. The filter paper collar releases vapors from all sides of the test apparatus and not from just one point source. This allows for an even release and probably for minimal variation in the accumulation of vapor in the test apparatus (See Part IV).

This apparatus offers the advantage of small size and glass construction. Small size allows for the efficient utilization of space, as in a small environmental chamber. Small size and the glass construction also facilitate cleaning. Transparent construction permits the use of video-recording techniques.

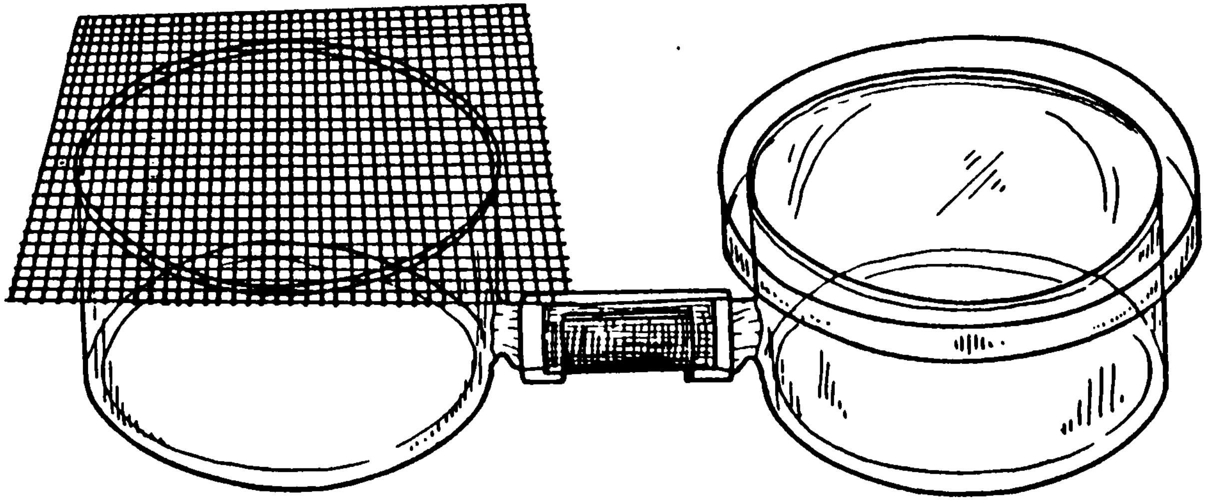

Figure 1. Apparatus used to test vapor-induced dispersal in the German cockroach. Vapors are introduced into the dish at right (Dish-1, aggegation dish) by mean of a filter paper collar (Drawing provided by J. Mullins).

PART II. THE IMPACT OF FORMULATION TYPE AND INERT INGREDIENTS ON PESTICIDE VAPOR INDUCED RESPONSES OF THE GERMAN COCKROACH

INTRODUCTION

The components of every commercial pesticide formulation are listed on its label as either "active" or "inert ingredients". The term "inert" has a benign connotation and suggests that any impact on insects to be minimal. Recently, however, the EPA has identified vapors from at least 50 of these "inert compounds", including benzene, as being toxic to man (Thomsen 1987). Roy (1954) used EAGs to demonstrate that air concentrations of benzene and toluene caused antennal spiking in American cockroaches. He also stated that at "exactly the same concentration" of the antennal spikes, the cockroaches made "violent efforts" to avoid the vapors. Vapors from these "inert compounds" might also impact on other target species, such as the German cockroach.

There have been studies to examine the effects of inert ingredients on the German cockroach. Bennett and Wright (1971) applied a number of spray constituents to wood panels, allowed them to dry for 24 hours, and tested them for repellency (irritability). They tested synergists (MGK-264), repellents (MGK Repellent 326) and surfactants (ie. Altox, Triton X-100), and found that most were repellent. Sterling and Howell (1972) added different compounds to a diazinon formulation and tested it for repellency (irritability) against the German cockroach at 1, 7, 21, and 42 days. They also used a variety of synergists, repellents and surfactants and found that many of these pesticide additives were repellent. Both of the above studies evaluated the additives but not their vapors.

Reddy (1970), on the other hand, evaluated a known repellent, MGK R-874, in both the vapor and liquid phase using acetone as a solvent. A German cockroach was dropped into a binary choice chamber and a reading was taken at three minutes. The cockroach was then removed and the process repeated ten times. Bret and Ross (1985a) examined vapor induced dispersal from aquaria of the German cockroach with a propoxur-in-oil formulation. The above studies dealt with vapors but they did not examine the effects of the inert ingredients in the formulations tested.

The development and variation of resistance patterns in insects is well documented (Gould 1984). Georghiou and Taylor (1977) examined some operational factors and concluded that dosage, treatment threshold, frequency of application, and maintenance of refugia would impact on the development of resistance. Mani and Wood (1984) discussed the evolution of physiological resistance in terms of the proportion of insects that escaped an "initial pesticide dose". They stated that space sprays would lead to less resistance than would short lived deposits or persistent insecticides. They also concluded that "escape" exerted a strong influence on the evolution of resistance. If vapor-induced dispersal contributed to this "escape phenomena", it too might be a significant factor in the development of resistance. However, behavioral resistance may only be manifested in the environment in which it evolved (Lockwood et al. 1984). The use of commercial pesticide formulations might therefore be critical in any bioassay which hopes to demonstrate a "field" behavioral response.

The purpose of the following study was: (1) to determine if vapors from commercial formulations without the active ingredient (blanks) could induce dispersal of the German cockroach; (2) to compare dispersal by vapors of the same pesticide in different formulations; and (3) to determine if there were strain differences within German cockroaches exposed to the blanks, different formulations or different classes of pesticides.

MATERIALS AND METHODS

Strains: A pesticide susceptible strain and two resistant strains of the German cockroach were selected for testing. The VPI strain is a pesticide susceptible strain that has been reared in the laboratory for over forty years. It was originally collected from a dairy barn in Blacksburg, Va. The Kenly strain is moderately resistant to propoxur compared to the VPI strain (6.1X), highly resistant to malathion (>50X) and highly resistant to pyrethrin (>240X) (Cochran pers. comm.). It has been maintained in colony since 1984 and was originally collected from a house in Kenly, N. C. The Carver strain is highly resistant to propoxur (17.4X), highly resistant to malathion (>60X), and highly resistant to pyrethrin (>80X)(Cochran pers. comm.). It was originally collected from an apartment in Gainesville, Fla., during 1983. The above resistance ratios were calculated by dividing the LT_{50} of the VPI strain into the LT_{50} of the resistant strain for each of the above pesticides (Cochran 1987).

Pesticides: The following commercial pesticides formulations or their blanks were used: 1% propoxur in oil (Octogon Roach Spray, Octogon Process, Edgewater, N. J.); 1% propoxur aerosol (Raid Ant and Roach Spray, S. C.

Johnson & Son, Racine Wis.); 70% propoxur wettable powder (S-Baygon W. P., Mobay Chemical Corp., Kansas City, Mo.); 13.9% propoxur emulsifiable concentrate (S-Baygon 1.5 EC., Mobay Chemical Corp., Kansas City, Mo.). Diazinon 4E (AG 500 Ciba-Geigy Corp.); 25% cyfluthrin flowable concentrate (S-Bay FCR 1272, Mobay Chemical Corp.) and a 98% technical grade malathion formulation (American Cyanamid Co.) were also used. All formulations were applied to a strip of filter paper at the rate of 300 ul of 1% solution or equivalent volume of blank. The emulsifiable concentrate, wettable powder, and flowable were formulated with water. The aerosol formulations were first sprayed into a small beaker until an adequate volume of material could be obtained. The technical grade malathion was mixed with a 50:50 mixture of Risella Oil (Shell Chemical Co.) and mineral spirits to make a 1% formulation.

Experimental Procedure: The test apparatus was set up as described in Part I. Ten large nymphs (5th and 6th instars) were obtained from one of the three.strains and placed into the apparatus to acclimate. Test formulations were introduced by application onto a paper collar attached to the top of the first dish (Dish-1). The number of cockroaches dispersing into the second dish was recorded manually at 10 min. intervals for 2 hours. Each test was replicated 6 times. Controls were run with each replicate.

Statistical Analysis: The results of each replicate were adjusted by Abbott's (1925) formula for dispersal in the controls. Control dispersal seldom exceeded 15%. In a few instances, where there was a high dispersal rate in a control due to inadvertent disturbance, the replicate was redone. The mean time/dispersal responses from all replicates were pooled and analyzed by probit analysis (SAS Institute 1982). Dispersal time for 50% (DT_{50}) of each test population was estimated for each strain against each test formulation. Values were considered to be significantly different if the 95% Fiducial limits did not overlap. Plots of the Probit analysis were done for comparisons during the 120 minute test period.

RESULTS

When exposure to vapors of the test materials resulted in significant dispersal, dispersal occurred within the experimental time period. In some cases most of the dispersal occurred within the first hour, with little if any dispersal occurring after the first 60 minutes. There was no mortality among the test cockroaches during the experimental periods.

Comparison of dispersal induced by several commercial formulations of propoxur and their blanks

The results of the dispersal experiments with the commercial propoxur formulations and their blanks are shown in Table 1, Table 2, and Figs. 2-4. The results are presented below according to strain. However, certain general patterns emerge that are common to all three strains. Figs. 2-4 show that the order from most rapid to least dispersal was the oil formulation, followed by the aerosol, aerosol blank, and last by the oil blank, regardless of the strain. Dispersal by the emulsifiable concentrate (EC) was, at the most, very slow, and that induced by the wettable powder (WP) even slower or undetectable (Table 2). Also, no response was found in any of the experiments with the blank formulations of the EC or WP.

VPI strain: Estimates of the times required for 50% of the VPI nymphs to disperse (DT_{50}s) showed that significant levels of dispersal occurred in the experiment with propoxur-in-oil, the oil blank, the aerosol, and the aerosol blank (Table 1). However, dispersal was significantly slower following exposure to vapors of the oil blank than in the experiment with the oil formulation, the aerosol, or the aerosol blank. The DT_{50}s in the latter three experiments did not differ significantly from one another. The nymphs did not show a dispersal response in the experiment with the wettable powder (WP), its blank, or the blank of the emulsifiable concentrate (EC) (Table 2). However, a very slow dispersal occurred during exposure to vapors of the EC (projected DT_{50} = 142 min).

Fig. 2 shows dispersal of the VPI strain nymphs during the 120 min exposure to the propoxur formulations and their blanks. The dispersal in the experiment with the oil blank was markedly lower than in the other three experiments. As expected from the DT_{50}s, dispersal in the latter experiments was similar. However, the data show a tendency towards more rapid dispersal in the experiment with propoxur-in-oil than in that with the aerosol formulations. At the end of the 120 minute interval in the experiment with propoxur-in-oil, approximately 95% of the test population had dispersed. In the experiments with the aerosol and its blank, there was 92% and 85% dispersal, respectively, after 120 minutes.

<u>Kenly Strain</u>: Significant dispersal of the Kenly strain was observed following exposure to both the oil and aerosol formulations and their blanks (Table 1). Dispersal in the experiment with the oil formulation was significantly faster than in the experiments with oil blank, aerosol, or the aerosol blank. The DT_{50} in the aerosol experiment indicated dispersal was also

significantly faster than from its blank but slower than in the oil formulation.

Dispersal of the Kenly strain throughout the experiment with the propoxur formulations and their blanks is shown in Fig. 3. Dispersal was markedly faster in the propoxur-in-oil experiment compared to the other three experiments. The experiments with the oil and aerosol blanks showed that in addition to the lack of significance between DT_{50}s the slopes of their lines were similar. The percent dispersal at the end of the experiment with the propoxur-in-oil was 99% compared to the aerosol and its blank at 89% and 70% respectively.

<u>Carver</u> <u>Strain</u>: DT_{50}s in the experiments with nymphs of the Carver strain indicated significant dispersal occurred following exposure to the oil formulation, the aerosol, and the aerosol blank (Table 1). There was, however, a very slow dispersal response in the experiment with the oil blank. The dispersal response in the experiments with the oil and the aerosol were similar, but were significantly different from dispersal induced by the aerosol blank. The experiment with the propoxur-in-oil induced the fastest dispersal, followed by the aerosol and the aerosol blank.

The dispersal of the nymphs of the Carver strain exposed to the propoxur formulations is shown in Fig 4. The dispersal response in the experiments with the active ingredient and the aerosol blank were markedly faster than that of the oil blank formulation, as indicated by the slopes of the lines. At the end of the each experiment (120 min), percent dispersal induced by the oil formulation was 92%, the aerosol formulation 84%, the aerosol blank 72%, and the oil blank 31%.

Comparison of dispersal responses to the propoxur formulations and their blanks according to strain

As noted above, certain patterns of dispersal were common to all three strains. Nevertheless, strain differences occurred in all experiments where there was significant dispersal (Table 1).

VPI Strain: Dispersal in the VPI strain was more similar to that in the Kenly than to the Carver strain. The experiments in which the DT_{50}s did not differ significantly occured with the oil blank and the aerosol (Table 1). One difference from the Kenly strain was a slower response to the propoxur-in-oil formulation. There was also a stronger dispersal response to the aerosol and aerosol blank in the VPI strain compared to the Kenly strain.

Kenly Strain: The Kenly strain exhibited an extremely rapid response to propoxur in oil compared to both the VPI and the Carver strains (Table 1). Surprisingly, the response to the aerosol blank formulation was significantly slower than with the VPI strain. On the other hand, the oil blank induced a similar slow rate of dispersal in both strains.

Carver Strain: Dispersal patterns of the Carver strain differed markedly from the Kenly and VPI strains. The Carver strain exhibited the most differences when compared to the other two strains (Table 1). In the experiment with propoxur-in-oil, the DT_{50} was longer than in the Kenly strain. Unlike the VPI and Kenly strains, Carver nymphs dispersed very slowly in the experiment with the oil blank, requiring at least 10 times longer for 50% of the nymphs to disperse. The response to the aerosol blank was similar to the result obtained with the Kenly strain, but was significantly slower than in the VPI strain.

The Effects of Diazinon, Malathion, and Cyfluthrin on Dispersal

The results of experiments with diazinon, malathion and cyfluthrin are shown in Table 3 and Figs 5-7. The eventual dispersal of 50% could be projected in all experiments except with the Kenly strain exposed to diazinon. Estimates of DT_{50}s suggested strain differences. However, the results were highly variable, statistical significance was found in the malathion experiment where the VPI nymphs dispersed more rapidly than those of the Kenly and Carver strains. In the diazinon experiment the projected DT_{50}s (Table 3) indicated that the VPI and Carver strain nymphs also dispersed faster than the Kenly strain nymphs. Fig. 5, however, shows that the initial dispersal of the VPI nymphs by diazinon was less than that of the Kenly and Carver strains, although at the end of the experiments a similar percentage from each strain had dispersed. This implies that although the projected DT_{50} were significantly different there were probably no real strain differences induced by this diazinon EC formulation. A different pattern of dispersal was induced by malathion (Fig. 6). The VPI strain dispersed faster than either the Kenly or Carver strains after exposure to malathion, but the percentage dispersed after 120 minutes was 60% compared to 50% and 41%, respectively, for the Kenly and Carver strains.

In the experiment with cyfluthrin, the VPI strain dispersed very slowly. On the other hand the Carver and Kenly strains dispersed very rapidly compared to the VPI strain, but their DT_{50}s were not significantly different from each other. However, at the end of the experiment, the percent dispersal of the Kenly strain exceeded that of the other two strains (Fig. 7).

DISCUSSION

The basis for the present comparisons of vapor-induced dispersal is that each experiment used the same amount (300 ul) of a 1% test formulation or an equivalent volume of its blank. The propoxur formulations and their blanks, with the exception of the wettable powder, were applied at label recommended rates for cockroach control. Vapors from commercial formulations were used, with the exception of the malathion formulation. It should also be kept in mind that the percent toxicant in a commercial pesticide is not always what is stated on the label. Differences from the label can be attributed to lack of quality control or an extended storage period (D. Mullins pers. comm.). Results have some relevance to field situations because the vapors are more representative of what cockroach populations actually encounter in the field as opposed to an arbitrary laboratory mixture.

The use of similar concentrations of the toxicant, however, does not mean that equal amounts of the toxicant are vaporized into the air. Conversely, the amount of toxicant found in the air is a function of the formulation. In addition, independent formulation effects are also seen depending on the "noxious" nature of its ingredients. The dispersal responses discussed below cannot be viewed as strictly a function of the toxicant, but must be viewed as a reaction to that particular formulation and the interrelationship of the toxicant and the "inert ingredients" applied under a given set of environmental conditions.

The results of the experiments may be viewed from several perspectives: (1) comparisons of the vapor-induced response of a pesticide in different formulations; (2) variation in the response to vapors of different pesticides; (3) strain differences. These aspects are discussed separately below, but it should be kept in mind that they are all interrelated within the general context of an overall dispersal response.

Dispersal induced by formulations and their solvent systems

It is not surprising that vapors from the propoxur-in-oil and propoxur aerosol blanks induced dispersal. Burden and Smittle (1960) found that many inert compounds are repellent to the German cockroach, including kerosenes. The solvent system of the propoxur-in-oil is probably a petroleum distillate; that of the aerosol almost certainly contains the most volatile compounds (propellants) of any of the four propoxur formulations. The longer-lasting dispersal and overall higher percentage of insects that dispersed in the oil-based formulations (oil and aerosol) compared to that of the water-based formulations (EC and WP) can be attributed to the differences in the degree that the toxicant is volatilized by a formulation's solvent system. Vapors of the complete formulations generally induced a more rapid dispersal than that of their blanks. This indicates that the insects responded to constituents of both the solvent system and the active ingredients. It must, however, be emphasized that vaporization of the toxicant is a function of the total formulation and any response to it cannot be attributed just to the active ingredient.

The occurrence of a slight, yet detectable response of the VPI and Carver strain nymphs to the propoxur EC, but not its blank, indicated that some vaporization of propoxur occurred. The only response to the WP was in the Carver strain, but it was slower than that induced in the experiment with the EC. These differences, including the very slow response compared to that from the aerosol and oil formulations, are not surprising in view of the solvent system (water base) used in the EC and WP formulations (Meister 1984). Solvents of an EC are generally a combination of non-volatile solvents and a volatile aromatic solvent (xylene). On the other hand, the WP formulations have few, if any volatile components. The aromatic solvent in the EC probably resulted in a slightly higher vaporization of propoxur than from the WP. Neither the WP nor EC formulations approached the amount of dispersal induced by that of the oil and aerosol formulations. The vapors from the EC or WP would be unlikely to cause rapid dispersal although, depending on the strain, a limited dispersal response might occur. The response induced by the EC and WP formulations would not induce an insect to move rapidly away from a treated area. Thus, it is unlikely that these materials would have a significant impact on the modification of behavioral avoidance in field strains.

Dispersal as related to different pesticides

Dispersal in the experiments with the organophosphates, malathion and diazinon, was considerably slower than that from the propoxur oil and aerosol formulations. As in the comparisons between the several propoxur formulations, this is accounted for by the low vaporization of the toxicant by the formulations tested (Hall 1986, 1987). The diazinon was a commercial EC formulation and the malathion formulation was made by mixing technical grade malathion with a viscous mineral oil and mineral spirits. The experiments with the Carver and VPI strains exposed to diazinon EC had comparable DT_{50}s to those seen in the experiments with the propoxur EC.

The results of the experiments with formulations that have high vaporization rates of the toxicant (oil and aerosol) showed a basic difference from those with low vaporization of the toxicant (EC and WP) in that the EC and WP generally had a slower dispersal rate and larger fiducial limits. These findings make us question whether the response was a "true repellency"', distinguished by an avoidance response, or an interference with a normal behavior pattern (Reddy 1970). According to Haynes (1988), the latter response is slow, compared to that generally found in cases of sensory perception. The class of pesticide, as reflected in its mode of action and symptoms of intoxication, might be a factor in the interpretation of any response induced by vapors. Propoxur is a carbamate; malathion and diazinon are organophosphates. Both of these classes of pesticides have similar modes of action. Their symptoms of intoxication in insects include: restlessness, hyperactivity, convulsions and paralysis (Matsumura 1985). Cyfluthrin is an alpha-cyano class (Type II) synthetic pyrethroid. Type II pyrethroids cause cockroaches to become ataxic and uncoordinated with periods of convulsions and hyperactivity (Gammon et al. 1981).

The concept of thresholds, sublethal dose, intoxication and behavioral response due to the action of a toxicant is complex. A crucial point is that levels of exposure do exist below which no effect occurs. However, a level of exposure below a toxic threshold does not preclude the initiation of behavioral responses. Cox (1987) proposed a spectrum of decreasing thresholds of successively milder effects as decreasing amounts of toxicant are encountered. Death is at one end of the spectrum followed by intoxication, then behavioral effects until, at the biochemical level, some type of molecular reaction always occurs where there is no threshold at all. A confounding problem in the interpretation of the vapor-induced dispersal results is whether or not the behavioral responses are independent from or are occurring simultaneously with behaviors induced by intoxication (Haynes 1988). If, at the sublethal dosages used here, locomotor activity was increased, it would heighten the probability that some individuals would move out of the vapor filled dish. This would be a result of intoxication and not a perception avoidance response to the insecticide vapor. This neurotoxic response to the insecticide might mask or alter an avoidance dispersal behavior. Partial expression of the latter could account for a tendency towards strain differences. The latter would develop from selection for differences in sensory perception (Haynes

1988).

The "inert" ingredients must also be viewed from a behavior/intoxication standpoint. The petroleum solvents in the oil and aerosol formulations might possibly act as indifferent narcotics. As with the neurotoxic active ingredients, an indifferent narcotic also reaches a threshold concentration in the lipid "biophase" of the nervous system and induces narcosis (O'Brien 1962). He stated that the molecular structure of the compound was unimportant for narcosis, but the main criterion for toxicity was liposolubility. The symptoms of intoxication of insects by "inert ingredients" are unknown.

The results of the experiment with cyfluthrin showed that this formulation caused a very rapid dispersal. Even though one strain (VPI) showed relatively little dispersal, that which did occur was over within 20 minutes. The role played by ingredients of the solvent system in the dispersal response, either through promoting volatilization of the toxicant or because of their own repellent properties, is unknown. As shown here for propoxur formulations, repellency of pyrethroids is also known to vary with different formulations (Gould 1984).

Strain differences in dispersal

It can be assumed that the development of propoxur, malathion, and pyrethrin resistance in the Kenly and Carver strains reflected a history of exposure to either these materials or those imparting cross resistance to them. Undoubtedly modifications of their behavior were associated with the use of insecticides, but precisely how these changes came about is unknown. The existence of strain differences in response to solvent systems suggests that solvent systems have played a more important role in behavioral modification of field strains than is generally realized. The genetic make-up of the population and methods, types, and frequencies of insecticide applications are other factors that may have influenced selection for changes in insecticide-induced dispersal.

The largest strain differences in dispersal occurred with materials that had the strongest effects on dispersal. They revealed differences that were either not apparent or difficult to detect in experiments with materials to which the cockroaches were less sensitive, such as the EC and WP.

The response modifications reported here are complex, even though the study was limited to comparisons between two field strains and our standard susceptible laboratory strain. However, in the case of propoxur, a trend may be evident. The highly-resistant Carver strain, like the Bowling Green strain (Bret and Ross 1985b), generally showed a lesser response to propoxur than the susceptible strain. DT_{50} for the Carver strain was also larger than in the moderately propoxur-resistant Kenly strain. Perhaps the mechanism responsible for high propoxur resistance imparts a loss of sensitivity. Alternatively, once a high level of physiological resistance is achieved, it may be advantageous for the cockroaches to remain within or near a favorable harborage. Possibly behavioral resistance in a population with high physiological resistance involves a decreased dispersal response, whereas, in other populations, a rapid dispersal might be advantageous.

A lower DT_{50} for the VPI nymphs exposed to the propoxur-in-oil formulation than to the oil blank indicated a response to the active ingredient. Therefore, the similarity of DT_{50}s in the experiment with the propoxur aerosol to its blank seemed contradictory. However, examining the percent dispersal at the end of the experiment indicates that the stronger overall response was still to the formulation with the toxicant.

The Kenly strain was remarkable for its rapid response to propoxur-in-oil. This may be an example of behavioral resistance where selection was for individuals with particularly high sensitivity to propoxur. Sensitivity to the active ingredient is suggested since response to the oil blank was no higher than that of the susceptible strain. Kenly nymphs did not disperse as rapidly as those of the susceptible strain on exposure to the aerosol formulation, but this may have reflected its slower response to the aerosol blank.

The development of behavioral resistance could account for the extremely rapid dispersal of the two field strains as compared to the lack of dispersal in the susceptible strain when exposed to vapors of cyfluthrin. If so, physiological resistance of the field strains to pyrethrins suggest the co-evolution of physiological and behavioral resistance, a possibility discussed by Lockwood et al. (1984). A physiological response that reduced locomotion in the susceptible strain may have played a role in the selection process. It would clearly be a disadvantage as it would enhance exposure of the susceptible insects to an insecticide. Selection for individuals with enhanced sensory perception and consequently a sufficiently rapid dispersal to avoid deleterious toxicological effects may have accompanied selection for genes imparting resistance.

As suggested earlier, the occurrence of few strain differences in the experiments with the water based formulations and malathion may have been due to the lack of exposure to significant levels of the toxicant or exposure to noxious ingredients that might induce dispersal. This does not rule out the possibility that clearer strain differences might be found if different formulations of malathion or diazinon were used. It is noteworthy that dispersal of the VPI nymphs in the experiment with malathion was rapid in comparison to that of the other two strains. This supports the hypothesis proposed earlier that neurotoxicity stimulated locomotion. One would expect the susceptible strain to be the most markedly affected, resulting in a more rapid movement and higher probability of movement out of the original container than seen in the Carver and Kenly strains, both of which are resistant to malathion, as well as propoxur. Neither was resistant to diazinon, but whether this made a difference in the results is questionable since little dispersal occurred in any of the strains.

Table 1. Comparisons of the time required for dispersal of 50% (DT_{50}) of the test population for three strains of the German cockroach exposed to two oil based formulations of propoxur or their blanks.

Strain	Formulation*	DT_{50} (min)	95% (FL)**
VPI	Oil	26.0	(22.7-29.2)
	Oil Blank	65.3	(55.4-78.3)
	Aerosol	21.6	(17.3-25.7)
	Aerosol Blank	25.3	(18.9-31)
Kenly	Oil	8.4	(6.9-9.7)
	Oil Blank	50.3	(41.9-59.7)
	Aerosol	26.3	(21.3-30.9)
	Aerosol Blank	52.7	(44-62)
Carver	Oil	29.5	(25.2-33.6)
	Oil Blank	819.8	(304-2257)
	Aerosol	31.6	(26.2-36.8)
	Aerosol Blank	50.1	(45.2-54.95)

* Dose of 300 ul of a 1% formulation or equivalent volume of blank.

** Significant differences are indicated by the nonoverlap of the 95% FL.

Table 2. Comparison of the projected DT_{50}'s for two strains of the German cockroach exposed to two water based formulations of propoxur or its blanks.

Strain	Formulation*	DT_{50} 95% (FL)**
VPI	Emulsifiable Concentrate (EC)	142 (102-264)
	EC Blank	-----@------
	Wettable Powder (WP)	-----@-----
	WP Blank	-----@-----
Carver	Emulsifiable Concentrate	248 (135-1504)
	EC Blank	-----@-----
	Wettable Powder	289 (169-1008)
	WP Blank	-----@------

* Dose of 300 ul of a 1% formulation or equivalent volume of blank.

** Significant differences are indicated by nonoverlap of the 95% FL.

@ Indicates the slope of the line did not differ significantly from zero at the 1.0 level.

Table 3. Comparisons of the DT_{50}'s for three strains of the German cockroach exposed to a 1% formulation of diazinon, malathion or cyfluthrin.

Pesticide*	Strain	DT_{50} (min)	95% (FL)**
Diazinon	VPI	208.3	(156.1-341.8)
	Kenly	ND	-----------
	Carver	451.5	(247-2000)
Malathion	VPI	44.8	(22.8-72.2)
	Kenly	106.1	(73.0-235.8)
	Carver	238.8	(151.4-636.8)
Cyfluthrin	VPI	226.2	(108.6-)
	Kenly	4.7	(1.2-9.1)
	Carver	18.6	(0.6-47.9)

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* Dose of 300 ul of 1% formulation applied to filter paper strip.

** Significant differences are indicated by nonoverlap of the 95% FL.

ND No dispersal, number greater than 24 hours (1440 min).

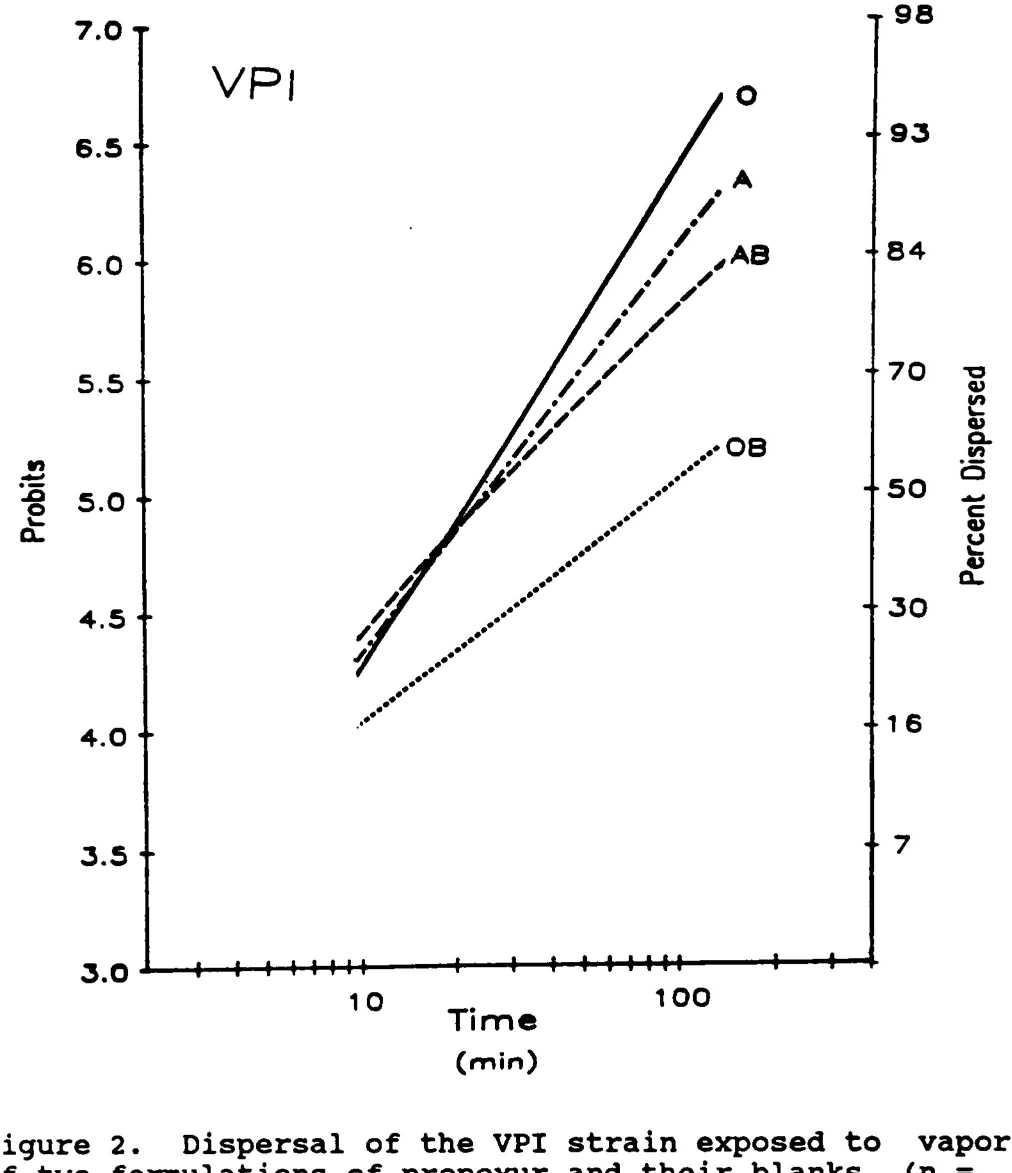

Figure 2. Dispersal of the VPI strain exposed to vapors of two formulations of propoxur and their blanks (n = 60). O-oil; OB -oil blank; A-aerosol; AB-aerosol blank.

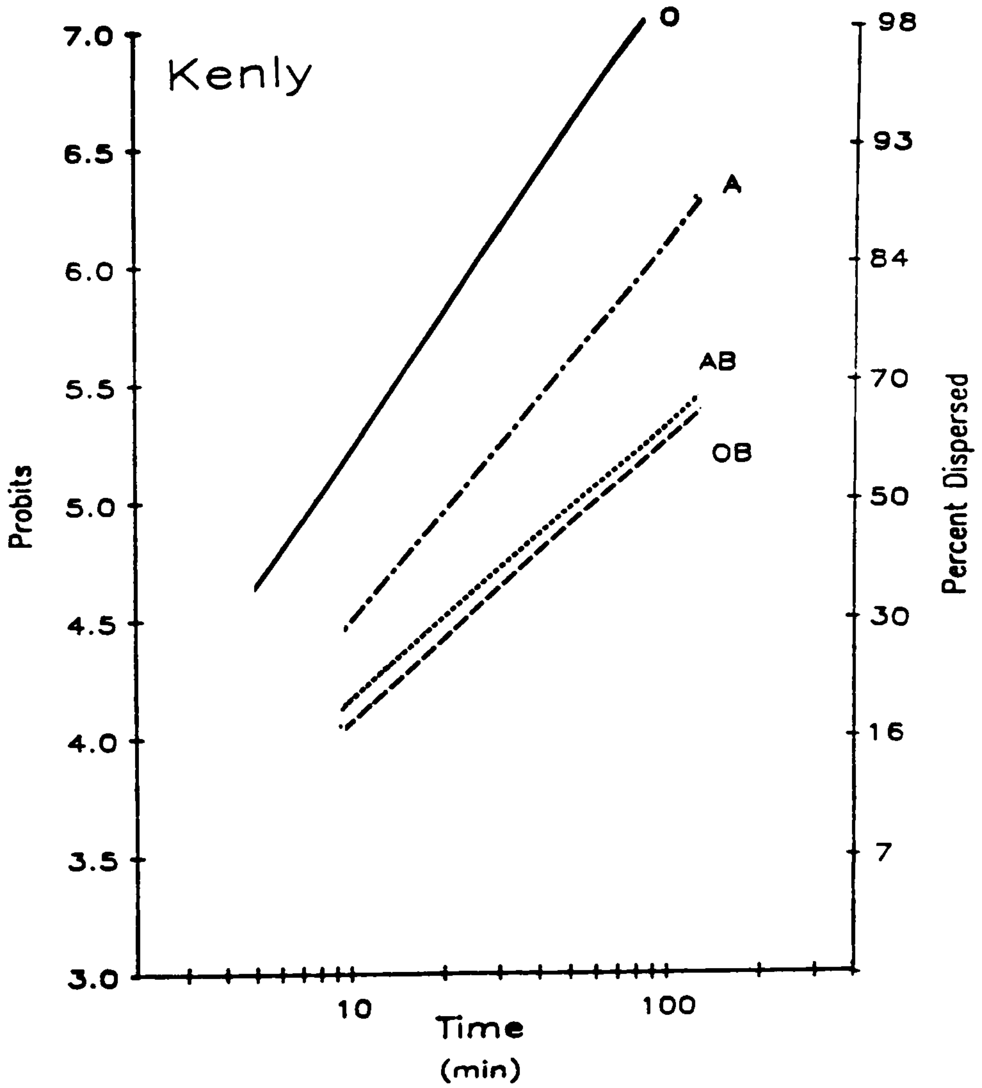

Figure 3. Dispersal of the Kenly strain exposed to vapors of two formulations of propoxur and their blanks (n = 60). O-oil; OB-oil blank; A-aerosol; AB-aerosol blank.

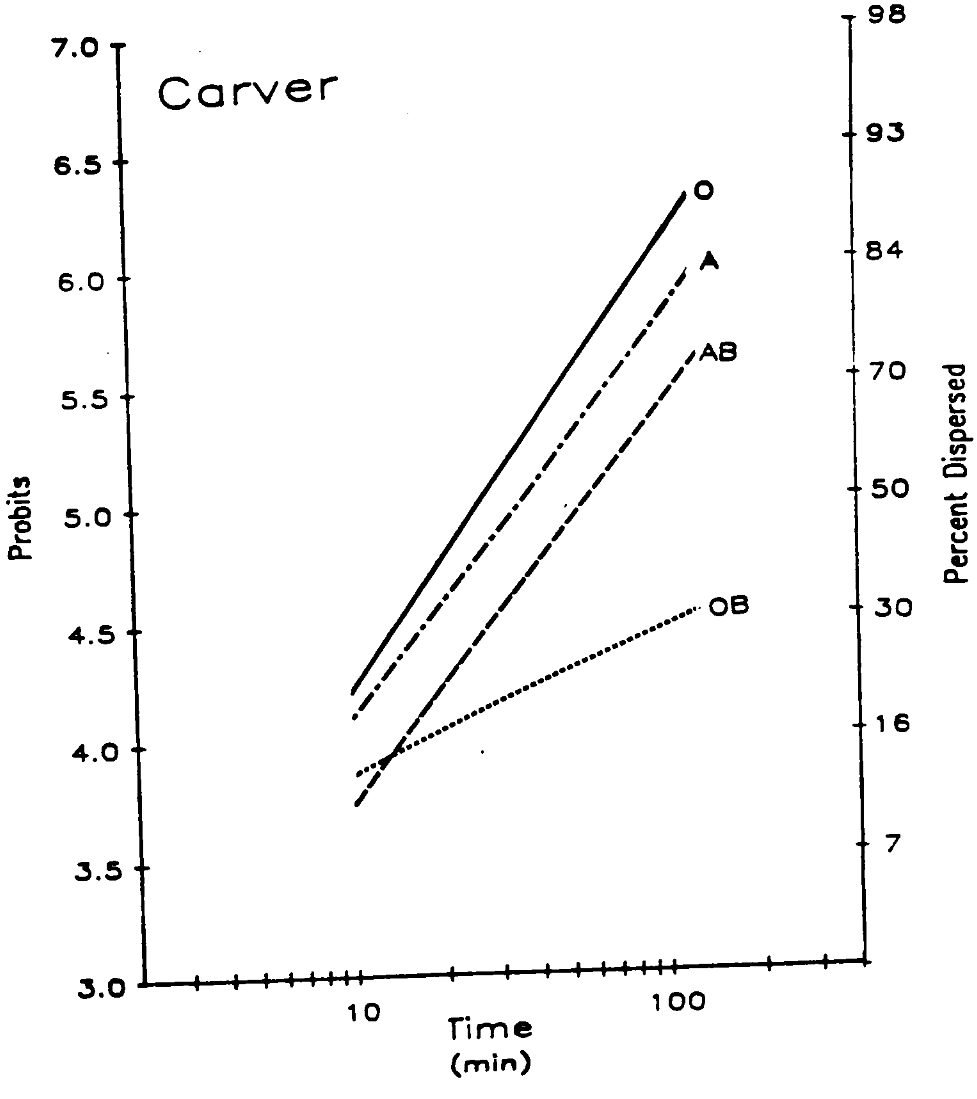

Figure 4. Dispersal of the Carver strain exposed to vapors of two formulations of propoxur and their blanks (n = 60). O-oil blank; OB-oil blank; A-aerosol; AB-aerosol blank.

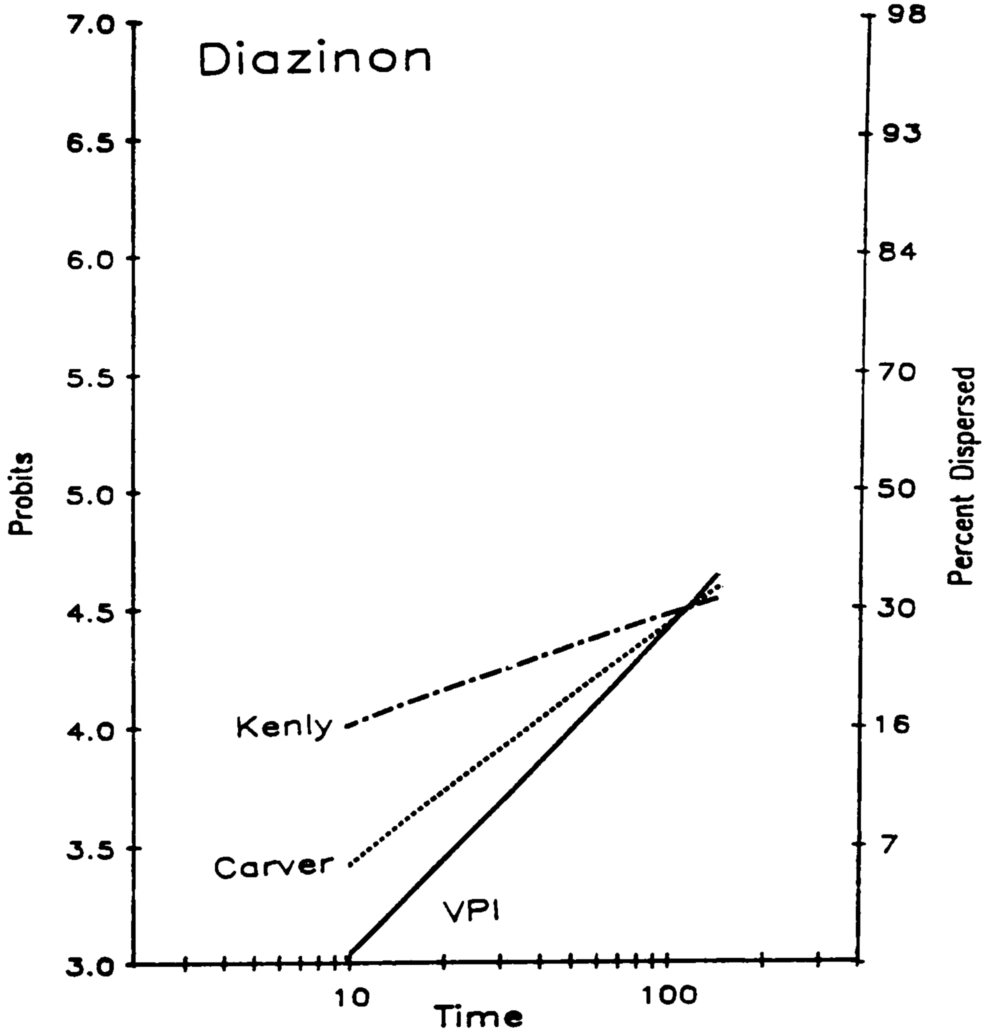

Figure 5. Dispersal of three strains of the German cockroach exposed to vapors of a 1% formulation of diazinon (n = 60).

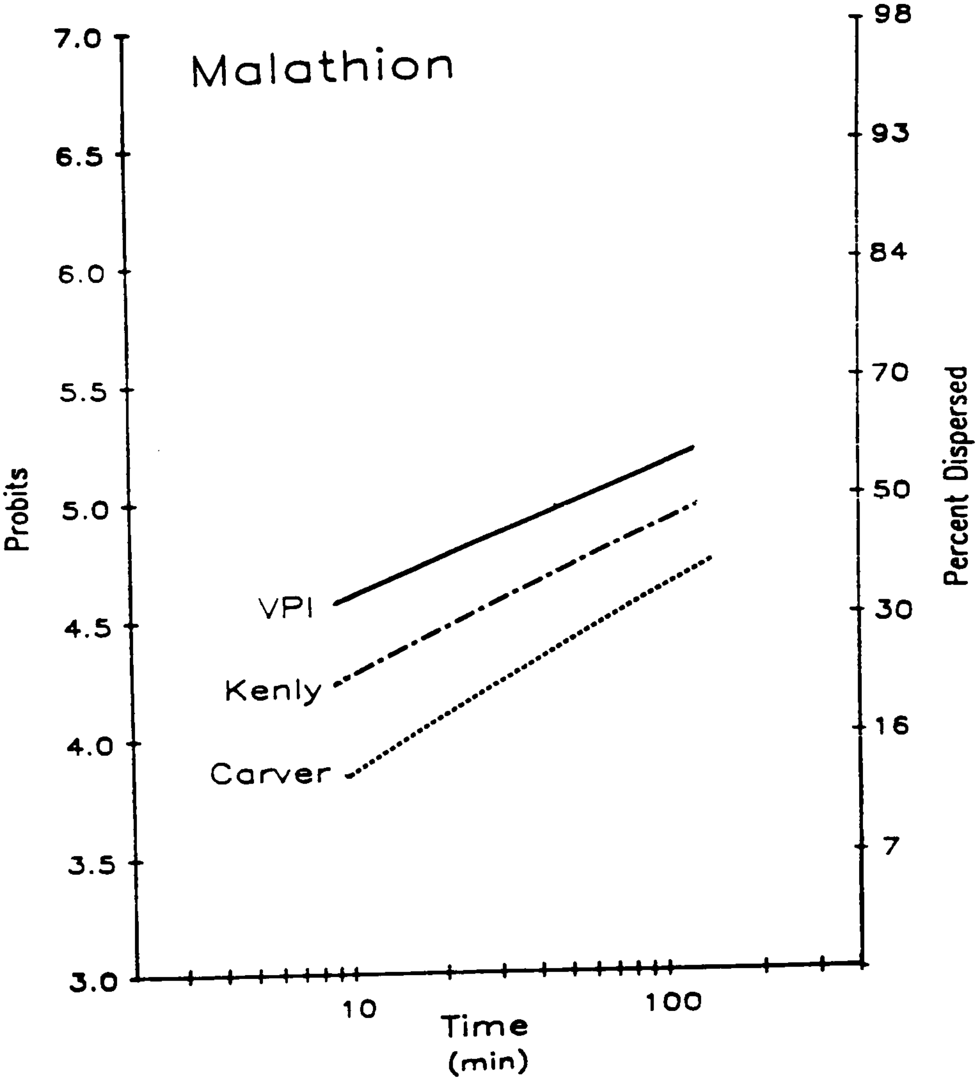

Figure 6. Dispersal of three strains of the German cockroach exposed to vapors of a 1% formulation of malathion (n = 60).

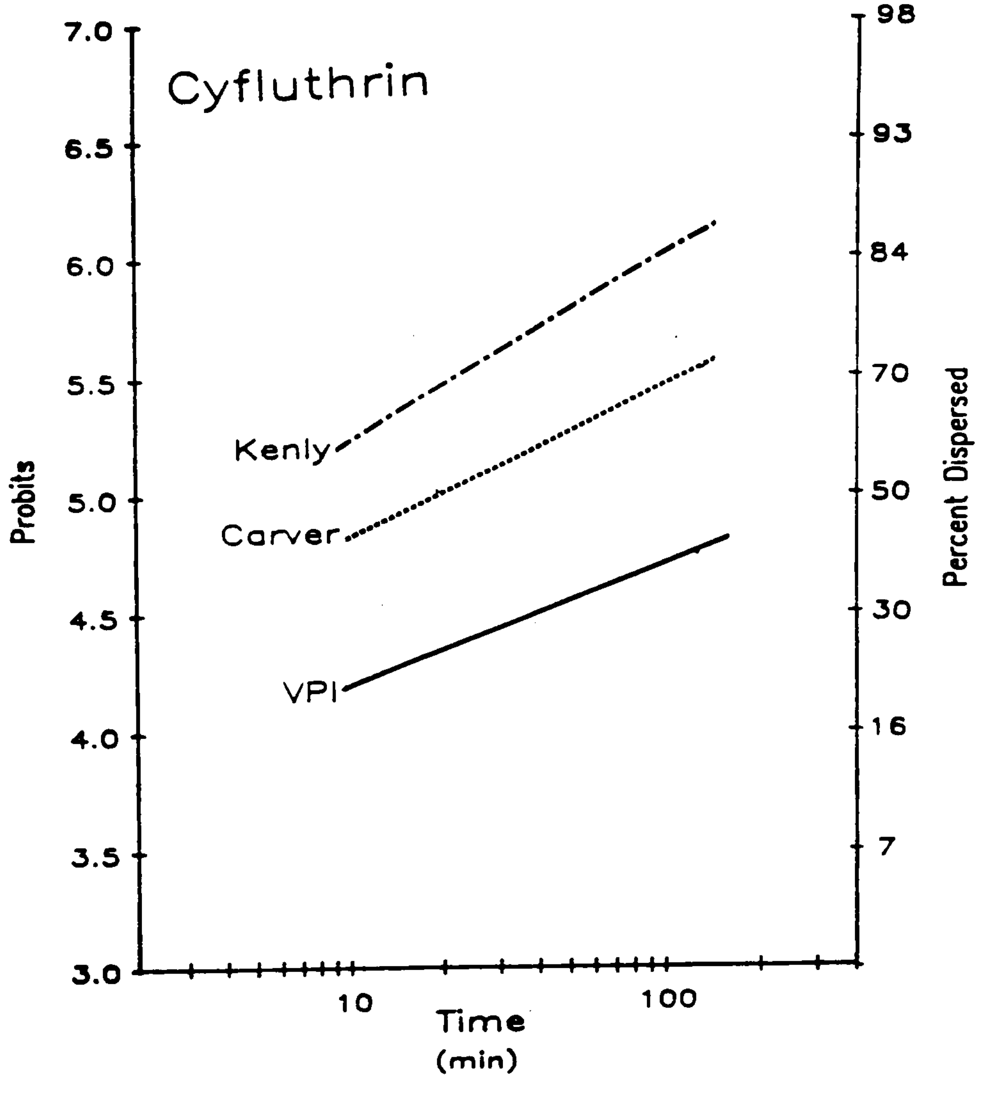

Figure 7. Dispersal of three strains of the German cockroach exposed to vapor of a 1% formulation of cyfluthrin (n = 60).

PART III. MIXED-AGE CLASS DISPERSAL OF THE GERMAN COCKROACH WHEN EXPOSED TO VAPORS FROM COMMERCIAL PESTICIDE FORMULATIONS

INTRODUCTION

To better approximate what takes place in the field it was necessary to expose cockroaches to formulations that are actually used in control operations. Accordingly, since a harborage in the field is usually occupied by all age classes, it is important to evaluate vapor-induced dispersal in mixed age groups. One age class might respond differently than another to any given pesticide formulation. The presence of one age group might modify the dispersal response of another. The response of an age-class in one strain might be modified differently from that of another strain by selection pressures on each strain. Presumably a major selection pressure on a cockroach population comes from the use of insecticides.

The response of any age class to pesticide vapors or the interpretation of dispersal results might be influenced by inherent differences in the level of activity among age classes. The general order of circadian activity for the German cockroach was reported to be highest in males, followed by nongravid females, gravid females, older nymphs and younger nymphs (Sommers 1975). Silverman (1986) concurred and found that nongravid females and males moved out of a harborage to seek water more than gravid females. Bret and Ross (1985a) found that the middle instars move the most, followed by adults and early instars, which showed the least amount of movement, but in those experiments emigration movements to a new harborage were studied rather than a search for food and water.

The age class composition in a harborage might also influence the stability of that aggregation following exposure to pesticide vapors. Mizuno and Tsuji (1974) examined harboring behavior of the German cockroach and found that adults and late instars were found in separate shelters, while the first instars tended to coexist in one shelter. Evan and Breed (1984) found that the tendency for sibling Leucophaea maderae to associate was strongest in first-instar nymphs and is absent in second instar nymphs.

Harborage overcrowding might also influence the dispersal response. Breed et al. (1975) described agonistic behavior in the German cockroach. They found that gravid females are more aggressive than non-gravid females and that the amount of aggression increased with population density. Overcrowding might also cause the release of dispersant chemicals as described by Ross and Tignor (1985) and Suto and Kumada (1981).

Physiological responses to pesticides might also be age-class dependent. El-Aziz et al. (1969) found that the last nymphal instar of the German cockroach had over five times as much catecholase activity as mixed sex adults. High levels of this and other enzymes was reported to be one of the main factors for carbamate resistance.

All age-classes of the German cockroach secrete aggregation pheromone, but the responsiveness to this pheromone varies with age (Ross and Tignor 1986). High concentrations of aggregation pheromone might inhibit locomotion (Burk and Bell 1973). How the presence of aggregation pheromone might mediate vapor-induced dispersal among the different age classes is not known.

The purpose of this section was to examine: (1) the differences in age class dispersal response when exposed to different formulations of propoxur and their blanks; (2) to determine if strain differences exist between different age-classes.

MATERIALS AND METHODS

Strains: Three resistant and a pesticide susceptible strain of the German cockroach were selected for mixed-age class testing. The VPI strain is a pesticide susceptible strain that was originally collected from a dairy barn in Blacksburg, Va. It has been maintained in the laboratory for over forty years. The Mandarin strain is a pesticide resistant strain that was collected from a restaurant in San Diego, Calf. Compared to the VPI strain it is moderately resistant to propoxur (4.2X), highly resistant to malathion (>60X), and slightly resistant to pyrethrin (1.9X) (Cochran pers. comm.). The Carver strain was originally collected from an apartment in Gainesville, Fla. during 1983. It is highly resistant to propoxur (17.4X), highly resistant to malathion (>60X), and highly resistant to pyrethrin (>80X) (Cochran pers. comm.). The Kenly strain was collected from a house in Kenly, N.C. in 1984. It is moderately resistant to propoxur (6.1X), highly resistant to malathion (>50X), and highly resistant to pyrethrin (>240X) (Cochran pers. comm.). The above resistance ratios were calculated by dividing the LT_{50} of the VPI strain into the LT_{50} of the resistant strain for each of the above pesticides (Cochran 1987).

<u>Pesticides</u>: The following commercial pesticide formulations or their blanks were used: 1% propoxur in oil (Octogon Roach Spray, Octogon Process, Edgewater, N. J.); 1% propoxur aerosol (Raid Ant and Roach Spray. S. C. Johnson & Son, Racine Wis.);

<u>Experimental Procedure</u>: The dispersal test apparatus consisting of two 600 ml crystallizing dishes connected by

a glass and wire mesh tube, was set up as described in Part I. Five males, 5 gravid females, 10 large nymphs (5th and 6th instars), and 10 small nymphs (2nd and 3rd instars) were drawn from one of the four strains and held in the first dish of the test apparatus to acclimate. Test formulations were introduced by application of a 300 ul dose of a 1% solution of the test formulation or equivalent volume of blank onto a filter paper collar. The collar was placed onto the upper lip of the first dish. The number of cockroaches dispersing into the second dish were recorded manually every 10 minutes for 2 hours. Each test was replicated 6 times. Controls were run with each replicate.

Statistical Analysis: The results of each replicate were adjusted by Abbott's (1925) formula for dispersal in the controls. The mean time/dispersal response for each ten minute interval was converted to a proportion of 10 (n = 10) (Birch pers. comm.) and analyzed by probit analysis (SAS Institute 1982). DT_{50}s are not reported unless they occurred within the 120 minute experimental period.

RESULTS

The results of the dispersal experiments with mixed-age classes of the four strains exposed to commercial formulations of propoxur and their blanks are shown in Tables 4-8 and Figs. 8-23. The results below are presented by: (1) within strain comparisons; (2) strain differences.

Within Strain Comparisons

VPI Strain: The experiments with the VPI strain (Table 4 and Figs. 8-11) were characterized by a close similarity in the dispersal response shown by each age class to each formulation tested. Dispersal response induced by the oil blank was significantly slower than in the experiments with the propoxur-in-oil, aerosol, and aerosol blank (Table 4). In fact, during the first 10 minute interval the oil blank induced only a few of the nymphs and adult males to disperse (Figs. 8-11). However, the females of the VPI strain initially dispersed faster after exposure to the oil blank than the aerosol and its blank, but by the end of the experiment the overall percent dispersal was lower (Fig. 11). Exposure to the propoxur-in-oil induced significantly faster dispersal compared to the aerosol and the aerosol blank for all age classes except the males (Table 4). Even though the fiducial limits (FL) overlapped for the VPI males exposed to vapors from the oil and aerosol formulation, their general dispersal response was similar to the other age classes (Figs. 8-11). There were no significant differences in the dispersal response induced by the aerosol compared with its blanks in any of the age groups (Table 4, Figs. 8-11).

All age classes of the VPI strain showed at least some response to the four test formulations. Estimates of the DT_{50}s fell within the experimental period for all tests except that of the small nymphs in the experiment with the oil blank. Although dispersal of small nymphs fell slightly short of the 50% mark, it was evident that they did respond to the oil blank (Fig. 8).

While significant differences between age classes in response to the aerosol and aerosol blank were not observed, general trends show a slower response by the adult females. Also, the response of the adults to the oil blank tended to be stronger than that of the nymphs.

<u>Carver</u> <u>Strain</u>: The results of the experiments with mixed age groups of the Carver strain exposed to the vapors of two propoxur formulations and their blanks are shown in Table 5 and Figs. 12-15. Significant differences can be seen by formulation type and by age class. The most striking characteristic response was that no single age class exposed to the oil blank reached the 50% dispersal level after 120 minutes (Table 5). In addition, broad fiducial limits in the dispersal response to the aerosol blank indicated a high variability in all age

classes.

Although the most rapid response of all Carver age classes was to the oil formulation, small nymphs did not respond as rapidly as the large nymphs or the adults (Table 5). As a result, the total percent dispersal of the small nymphs by the oil formulation at the end of the experiment was less than in the other age classes (Fig. 12 vs Fig. 13-15).

Both the large nymphs and the small nymphs responded more slowly than the males after exposure to the aerosol blank. The nymphs showed markedly less dispersal within the first 10 minutes than the adults (Figs. 12 & 13 vs. Figs. 14 & 15). Large nymphs demonstrated a significantly faster response to the aerosol than to the aerosol blank (Table 5). Small nymphs showed a tendency in this direction but the results did not differ significantly.

Adult responses differed from those of the nymphs in that the DT_{50}s in the experiments with the aerosol were closer to DT_{50}s those of the aerosol blank (Table 5). In addition, the adults not only responded rapidly to the oil, but narrow fiducial limits indicated very little variability in their response.

<u>Kenly Strain</u>: The results of the experiments with the Kenly strain mixed age groups were notable for their extremely broad fiducial limits, especially in the experiments with small nymphs (Table 6). The dispersal

response of the nymphs differed from the adults in the experiments with the oil blank. Only adults showed a DT_{50} response within the experimental period. The lack of significant differences within each age class between the response to the oil and to the aerosol blank is apparently due to the high variability in response to the aerosol blank.

As a result of the wide fiducial limits in the experiment with small nymphs, there were no significant differences in the DT_{50}s in the experiment with the oil, aerosol, or aerosol blank (Table 6). Nevertheless, the DT_{50}s in the oil experiment and probit plot indicate that the response was similar to the other age classes in that it caused the most rapid dispersal of any of the test materials.

The response of the adults to the oil blank differed from that of the nymphs. The DT_{50}s in the experiment with the oil blank fell within the 120 minute exposure period (Table 6). On the other hand, the total percent dispersal for the nymphs barely reached 30%, with the small nymphs showing very little response within the first 30 minutes (Figs. 16-19).

No significant differences occurred between the response to the aerosol and its blank within any age class (Table 6). Surprisingly, the DT_{50}s in the experiments with the aerosol in all age groups suggested a slower dispersal than in the experiments with the aerosol blank. However, the percent dispersal at the end of the 120 minute period was higher in the aerosol experiment than with the aerosol blank (Figs. 16-19). Small nymphs tended to disperse more slowly than other age classes in the experiments with the aerosol and aerosol blank (Table 6). The response of the small nymphs to the aerosol was significantly slower only in comparison to that of the males.

Mandarin Strain: Perhaps the most characteristic response of this strain was its high variability in all experiments, but especially in those with the formulation blanks (Table 7). However, at least 50% of all age classes dispersed in the experiments with the oil blank. Nymphal dispersal in the experiments with the aerosol was similar to that of the aerosol blank, whereas the adult response to the aerosol was either significantly faster (females) or showed a strong tendency towards a more rapid dispersal (males) than in the experiments with the aerosol blank.

Estimated DT_{50}s of the small nymphs not only had large fiducial limits but suggest dispersal was slower than in other age classes (Table 7). The response of small nymphs was significantly slower than the large nymphs in experiments with the aerosol formulations. The small nymphs dispersed significantly faster in the experiments with the oil formulation as compared to the oil blank (Table 7). However, there were no other significant differences in responses of the small nymphs to the other test formulations. The patterns of dispersal were like those seen throughout the other dispersal studies but the total percent dispersal at the end of the experiments was notably less than in the other age classes (Fig. 20 vs. Figs. 21-23).

Dispersal of large nymphs was notable for the significantly rapid response induced by the aerosol blank compared to the adults (Table 7). Total percent dispersal for the large nymphs exposed to the aerosol blank was greater than in the other age classes (Fig. 21). Fig. 21 also shows that the percent dispersal at the end of the experiment with the aerosol blank was less than that from the aerosol, even though the DT_{50} estimates, like those of the small nymphs, did not differ significantly (Table 7). Overall, dispersal throughout the experiment with the large nymphs was similar to that of the adults (Figs. 21-23).

Response of adult males was similar to that of the females, with the adult response, as noted above, distinguished from that of the nymphal classes by greater differences between response to the aerosol and its blank. However, Figs. 22-23 show a divergence from the pattern common to these and earlier experiments (Part II) in that the percent dispersal at the conclusion of the experiments was higher in that with the oil blank than the aerosol blank.

Comparisons Between Strains

General: The VPI strain was characterized by a similar response across age classes in experiments with each test material. Also, fiducial limits were narrower than in the other strains. Each of the field strains were characterized by intra-strain differences by individual age classes. The latter varied from strain to strain. For example, Carver was the only strain in which the total percent dispersal of all age classes in the experiment with the oil blank was less than 50%. Also, the Carver strain was the only strain where age classes differed significantly in their response to the oil formulation. The Kenly strain was notable for high variability (broad FL) in the dispersal of small nymphs and the occurrence of significant differences between the response of nymphs and adults (oil blank). The Mandarin strain was the only strain where at least 50% of each age class dispersed during the 120 minutes of the experimental period in experiments with the oil blank.

Small nymphs: Experiments with the small nymphs indicated that there were no statistical strain differences in dispersal after exposure to the oil, oil blank, or the aerosol formulations. The VPI small nymphs responded significantly faster to the aerosol blank compared to the Carver but neither was significantly different from small nymphs of the Kenly or Mandarin strains. Variability was generally greater (broader FL) in the field strains with moderate resistance to propoxur (Kenly and Mandarin) than in the highly resistant Carver strain.

Large nymphs: The DT_{50}s for the large nymphs did not show any significant strain differences when exposed to the oil, oil blank, and aerosol. However, the response of the Carver strain nymphs was significantly slower to the aerosol blank than that of the other three strains. The results with materials causing the most rapid dispersal (oil, aerosol and aerosol blank) had narrower fiducial limits than those of the small nymphs.

Adult males: The response of males to the oil and aerosol formulation was not significantly different across strains except that the Carver and Mandarin males responded significantly faster to the oil than the VPI males. Carver males were the only males where dispersal was less than 50% in the experiment with the oil blank. Otherwise, the only notable difference in the DT_{50}s was a significantly faster response of the Kenly males compared to the Mandarin males in experiments with the aerosol blank.

Adult females: The response of the females across strains to the oil, aerosol, and oil blank was similar. The only significant differences were in response to the aerosol blank. Here the response of the Mandarin females was significantly slower than that of the VPI and Kenly females.

DISCUSSION

The exposure of mixed age classes to commercial pesticide formulations best approximates in the laboratory what happens when cockroach populations are actually exposed to pesticide vapors in the field. The discrimination of behaviors caused by perception/avoidance (P/A) from intoxication/hyperactivity (I/H) is no clearer in the mixed age classes than in the single age class experiments. It must be kept in mind that, while in a temporal sense the P/A probably occurs before the I/H, the difference in time could be very minimal. In the experiments seen above P/A and I/H probably occur together over most of the vapor exposure period. This, however, might be a moot point as both factors are expressed in varying degrees depending on the strain, the pesticide, and formulation. These factors may in fact be inseparable. However, they do interact to produce the overall response seen in the above experiments. Recognition that the dispersal response has these two components is important, but it is the overall dispersal effect that is the focus of this study. Work on the discrimination of P/A from I/H and the difference in degree they impact on the overall dispersal response will be left for future research.

The dispersal response in the mixed age groups will be discussed from three aspects: (1) differences in response according to formulation; (2) the response of mixed age groups; and (3) strain specific characteristics.

Formulation differences

When the response to the total formulations is compared to that of the blanks, no consistent relationship was seen in the sense that a strong response to the solvent system increased the response to the total formulation. Most data from the experiments with the oil did indicate response to the active ingredient in that the response to the complete formulation was stronger than the response to its blank. This, however, was not true for the aerosol and the aerosol blank. The situation varied markedly with the strain, but frequently there was little difference between the response to the total aerosol formulation and its blank.

Mixed age groups

Studies on mixed age groups added a new facet to the study of strain differences as compared to the study of large nymphs. These studies show that strain differences include intra-strain variation in response to insecticide vapors from one strain to another. Bret and Ross (1985b) saw strain differences between a susceptible and field strain in dispersal of adults but not nymphs when exposed to propoxur vapors. The present studies demonstrate this type of difference also exists between field strains.

Variation in the response of different age classes of the German cockroach to insecticides was documented by Woodbury (1938) and to pheromones by Ross and Tignor (1986). It is not surprising that such differences exist in the experiments exposing mixed age groups to pesticide vapors. This indicated that the effects of selective pressure on cockroach populations have resulted in intra-strain variation among age class components of a population. Both intra and inter-strain differences that have developed almost certainly reflect different histories of insecticide application.

The primary and over-riding pressure on German cockroach populations is probably from the use of pesticides. Since the major differences within and between strains were in response to a particular formulation rather than just the toxicant, it appears that the composition of the formulation has been a major factor in selecting for different responses among age/sex classes.

At present knowledge of the inter- and intra-strain differences is too sparse to attempt to relate them to either the level or type of physiological resistance to insecticides. However, the Carver strain had the highest resistance to propoxur. A history of more intense selection pressure might have contributed to lesser variability than in the other field strains. Certain general similarities among age classes were shown from the mixed age group experiments. Adult males and females show a generally similar response to all test formulations. The small nymphs frequently showed the highest variability and the slowest response to the test vapors. Perhaps small nymphs retain comparatively high genetic variability. Adults and large nymphs likely received similar exposures in the course of an insecticide treatment.

Strain-specific characteristics

Each strain had its own particular characteristics and, in the field strains, it is evident that testing for dispersal of one age class would not necessarily be indicative of the dispersal response of the other age classes.

VPI strain: The VPI pesticide susceptible strain differed from the field strains in several aspects: (1) less variability, which probably reflects the homogeneity of selection by long-term laboratory rearing; (2) similarity in response by different age classes vs. variability in field strains. This indicates that selection pressures on field strains act differently on different age classes. It is known that the lethal effects of insecticides (Woodbury 1938) and response to pheromones (Ross and Tignor 1986) vary with age class. In view of such variations, it is not surprising that age classes respond differently to insecticide vapors or that differences in selection pressures on field strains have modified age-class responses in different ways.

Kenly Strain In experiments with the large nymphs, exposure to vapors of the oil formulation induced a more rapid dispersal than in the VPI and Carver strains, although significant differences were not seen in the mixed age groups. The DT_{50}s are suggestive of this difference since they were higher than those of the VPI and Carver strains for each age class, even though they did not differ significantly.

The most outstanding characteristic of dispersal in the Kenly was the difference between adults and nymphs in the experiments with the oil blank, where the DT_{50}

response within the test period was limited to the adults. Without information on whether there is a general lack of response to the oil blank among susceptible field strains, no sound hypothesis can be advanced about a decreased selection response of the Kenly nymphs to the oil blank. Comparison to the response of the VPI nymphs to the oil blank suggests that this may have occurred. Another tendency notable among Kenly mixed age groups was a rapid response of the adults and large nymphs to the aerosol blank, although differences were statistically significant only in comparison to adults of the Mandarin strain and large nymphs of the Carver strain.

Mandarin strain: Dispersal in the Mandarin strain resembled that in the Kenly in that the results for small nymphs had extremely large fiducial limits. Whether this indicates less selection pressure and consequent maintenance of higher genetic variability in this age group is unknown. Propoxur resistance in the Kenly and Mandarin strains, although present, is lower than in the Carver strain. The Mandarin is distinguished from the other field strains by its overall (all age classes) higher response to the oil blank, but whether this is due to a heightened perception and avoidance response is unknown.

Carver strain The most outstanding characteristic of dispersal in this strain was a very low response to the oil blank. Possibly there is a relationship between the development of propoxur resistance and a loss of sensitivity, as suggested by Ross and Bret (1985a). Otherwise, the most obvious characteristics were lower variability of the small nymphs compared to the other field strains and, in comparison to the VPI small nymphs, a slower dispersal response. These characteristics also may be related to selection sufficient to cause a relatively high resistance to propoxur.

Table 4. Dispersal of mixed age groups of the VPI strain exposed to two formulations of propoxur and their blanks.

Age Class	Formulation	DT_{50} (min)	95% (FL)*
Small Nymphs	Oil	12.3	(5.2-18.6)
	Oil Blank	128.3	(97.2-264.4)
	Aerosol	33.5	(23.4-42.5)
	Aerosol Blank	37.4	(27.8-46.2)
Large Nymphs	Oil	10.9	(4.3-16.8)
	Oil Blank	96.8	(75.2-157.2)
	Aerosol	29.7	(18.5-39.6)
	Aerosol Blank	28.6	(18.7-37.4)
Males	Oil	15.7	(10.3-21.3)
	Oil Blank	91.3	(67.3-164.5)
	Aerosol	28.1	(19.1-36.2)
	Aerosol Blank	35.6	(23.5-46.4)
Females	Oil	12.5	(6.3-17.8)
	Oil Blank	81.2	(64.4-113.6)
	Aerosol	43.5	(32.6-54.2)
	Aerosol Blank	48.3	(33.8-64.2)

* Significant differences indicated by nonoverlap of the 95% FL.

Table 5. Dispersal of mixed age groups of the Carver strain exposed to two formulations of propoxur and their blanks.

Age Class	Formulation	DT_{50} (min)	95% (FL)*
Small Nymphs	Oil	24.7	(15.6-32.7)
	Oil Blank	ND**	----------
	Aerosol	54.2	(39.9-72.1)
	Aerosol Blank	85.6	(58.6-188.6)
Large Nymphs	Oil	9.4	(2.9-15.4)
	Oil Blank	ND**	----------
	Aerosol	32.8	(22.5-42.3)
	Aerosol Blank	66.1	(49.2-93.1)
Males	Oil	6.3	(3.3-8.7)
	Oil Blank	ND**	----------
	Aerosol	37.2	(24.2-49.5)
	Aerosol Blank	34.2	(20.7-46.26)
Females	Oil	8.5	(4.9-8.7)
	Oil Blank	ND**	----------
	Aerosol	38.6	(27.5-49.5)
	Aerosol Blank	56.8	(12.2-521.6)

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* Significant differences indicated by nonoverlap of the 95% FL.

** No DT_{50} estimate. Dispersal ceased within the 120 min experimental period without reaching 50%.

Table 6. Dispersal of mixed age groups of the Kenly strain exposed to two formulations of propoxur and their blanks.

Age Class	Formulation	DT_{50} (min)	(95% FL)*
Small Nymphs	Oil	8.1	(0.1-220)
	Oil Blank	ND**	---------
	Aerosol	71.3	(43.3-182.7)
	Aerosol Blank	44.4	(14.4-83.3)
Large Nymphs	Oil	5.5	(1.3-9.5)
	Oil Blank	ND**	-----------
	Aerosol	40.4	(29.9-50.6)
	Aerosol Blank	23.7	(4.4-39.4)
Males	Oil	5.8	(1-10.6)
	Oil Blank	80.1	(57.2-142.5)
	Aerosol	28.1	(20.1-35.3)
	Aerosol Blank	20.4	(2.0-35.8)
Females	Oil	2.2	(1-11.1)
	Oil Blank	78.9	(54.7-150.3)
	Aerosol	42.5	(27.4-58.5)
	Aerosol Blank	26.2	(6.0-42.6)

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* Significant differences indicated by nonoverlap of the 95% FL.

** No DT_{50} estimate. Dispersal ceased within the 120 min experimental period without reaching 50%.

Table 7. Dispersal of mixed age groups of the Mandarin strain exposed to two formulations of propoxur and their blanks.

Age Class	Formulation	DT_{50} (min)	(95% FL)*
Small Nymphs	Oil	28.2	(6.9-45.5)
	Oil Blank	103.2	(77.4-189.5)
	Aerosol	67.1	(38.3-178.6)
	Aerosol Blank	57.1	(29.7-118.2)
Large Nymphs	Oil	6.1	(.5-13.2)
	Oil Blank	64.5	(36.2-161.9)
	Aerosol	22.1	(11.5-31.1)
	Aerosol Blank	13.4	(0-41.1)
Males	Oil	6.1	(0-10.2)
	Oil Blank	45.3	(31.9-59.7)
	Aerosol	34.7	(20.1-48.3)
	Aerosol Blank	65.6	(44.6-109.2)
Females	Oil	7.1	(1.5-14.9)
	Oil Blank	70.6	(46.7-135)
	Aerosol	29.3	(18.0-39.4)
	Aerosol Blank	114.9	(67.6-1493.2)

* Significant differences indicated by the nonoverlap of the 95% FL.

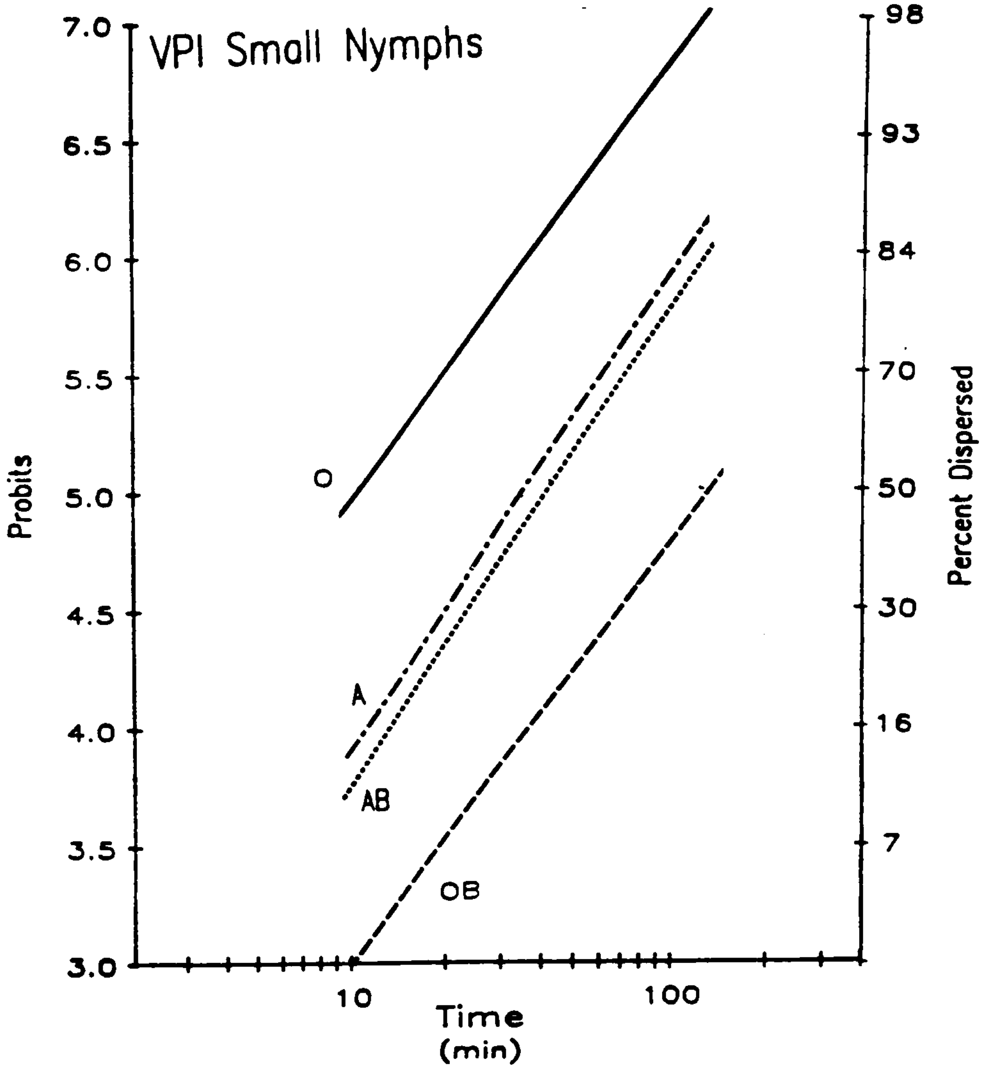

Figure 8. Dispersal of small nymphs of the VPI strain exposed to the vapors of two formulations of propoxur and their blanks (n = 60). O-oil; OB-oil blank; A-aerosol; AB-aerosol blank.

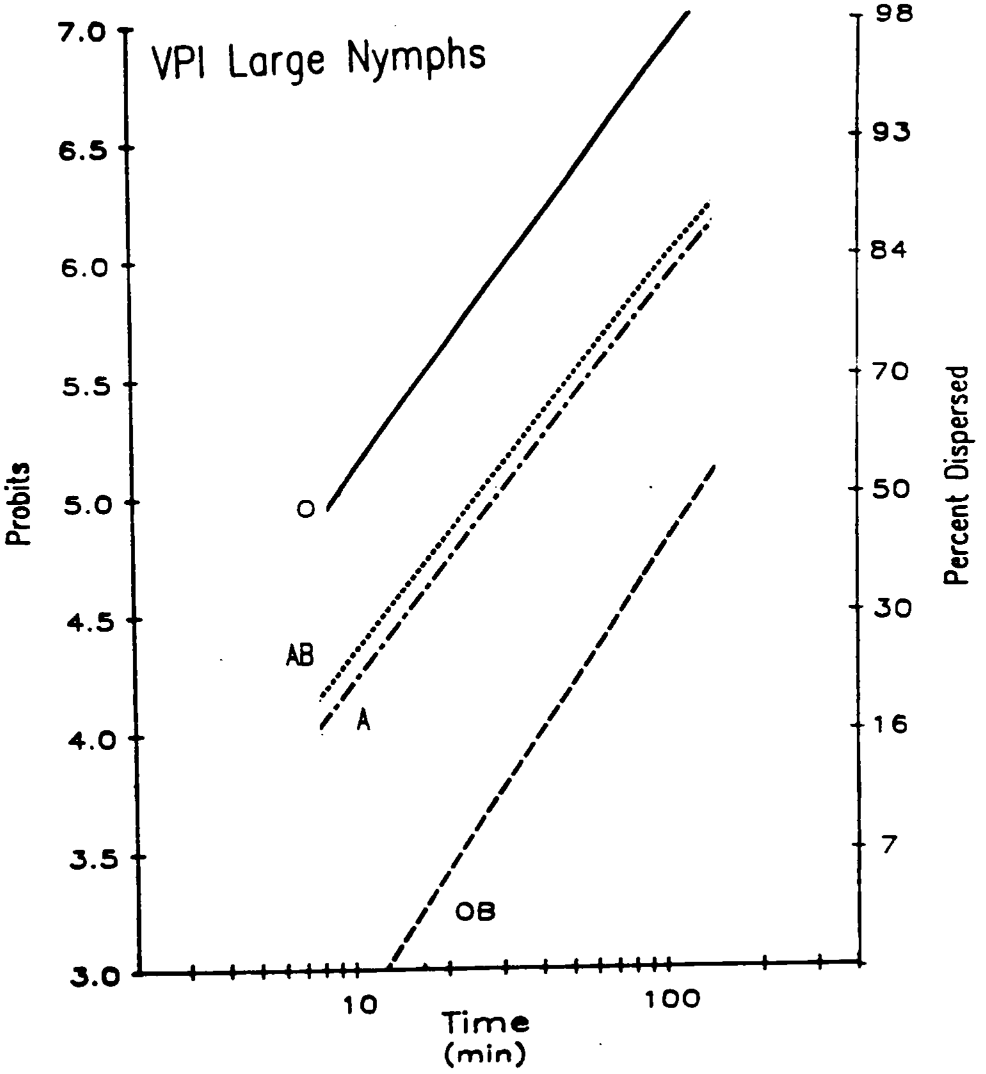

Figure 9. Dispersal of large nymphs of the VPI strain exposed to the vapors of two formulations of propoxur and their blanks. O-oil; OB-oil blank; A-aerosol; AB-aerosol blank.

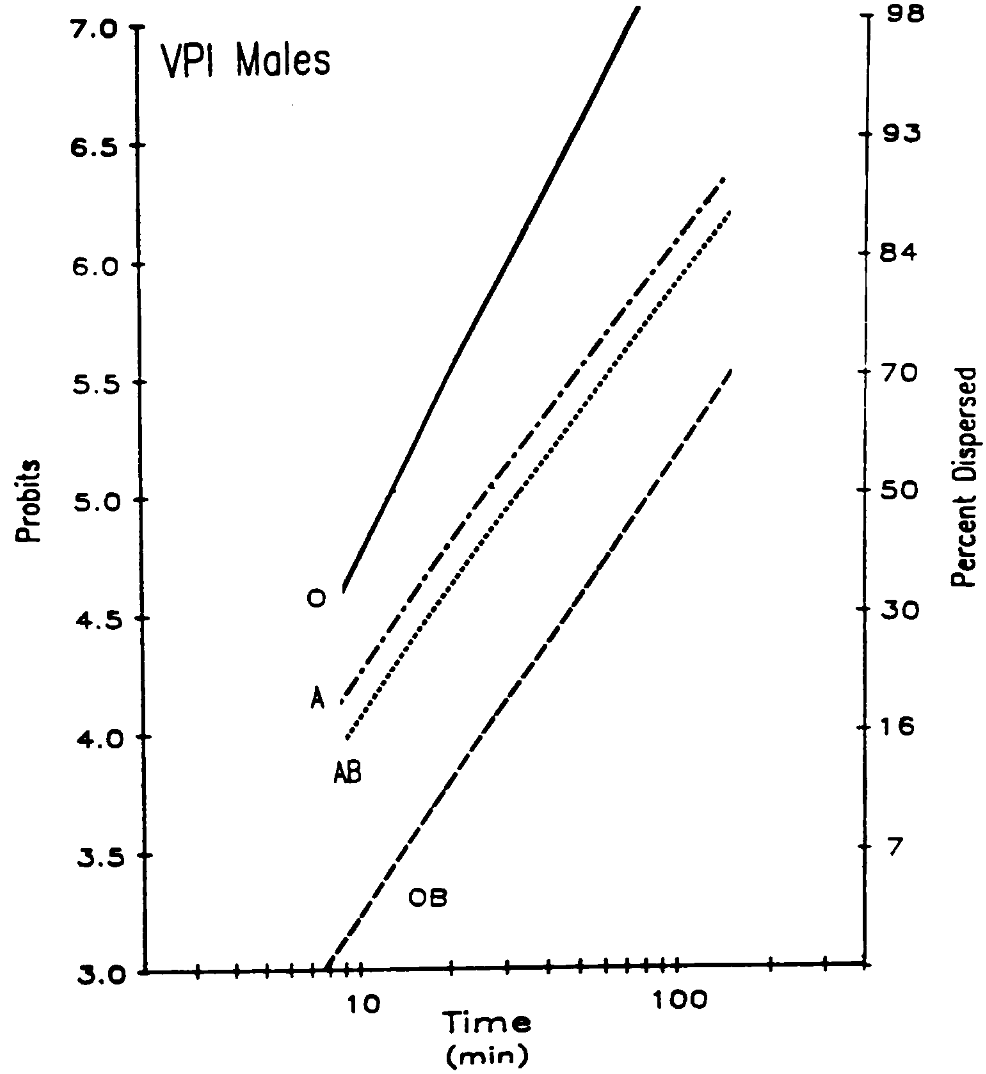

Figure 10. Dispersal of males of the VPI strain exposed to the vapors of two formulations of propoxur and its blanks. O-oil; OB-oil blank; A-aerosol; Ab-aerosol blank.

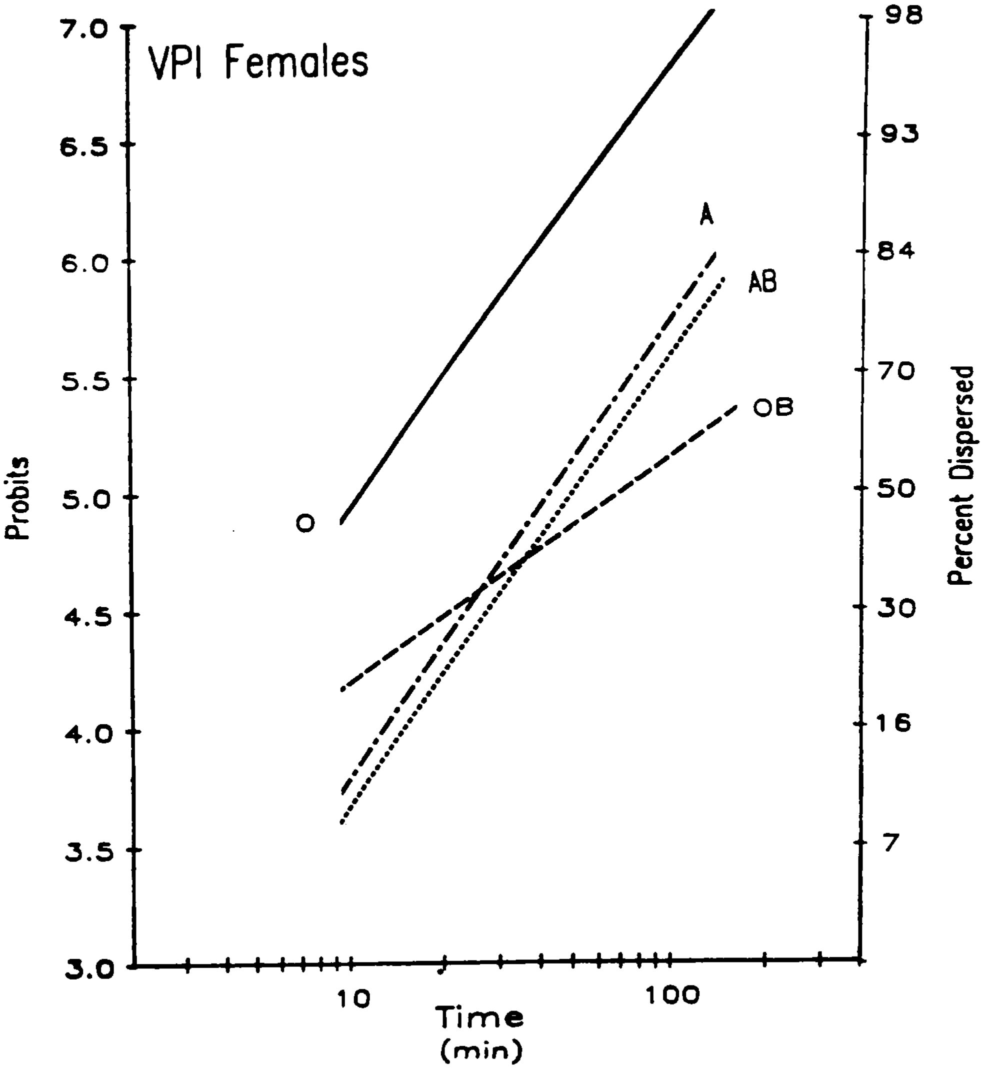

Figure 11. Dispersal of females of the VPI strain exposed to the vapors of two formulations of propoxur and its blanks. O-oil; OB-oil blank; A-aerosol; AB-aerosol blank.

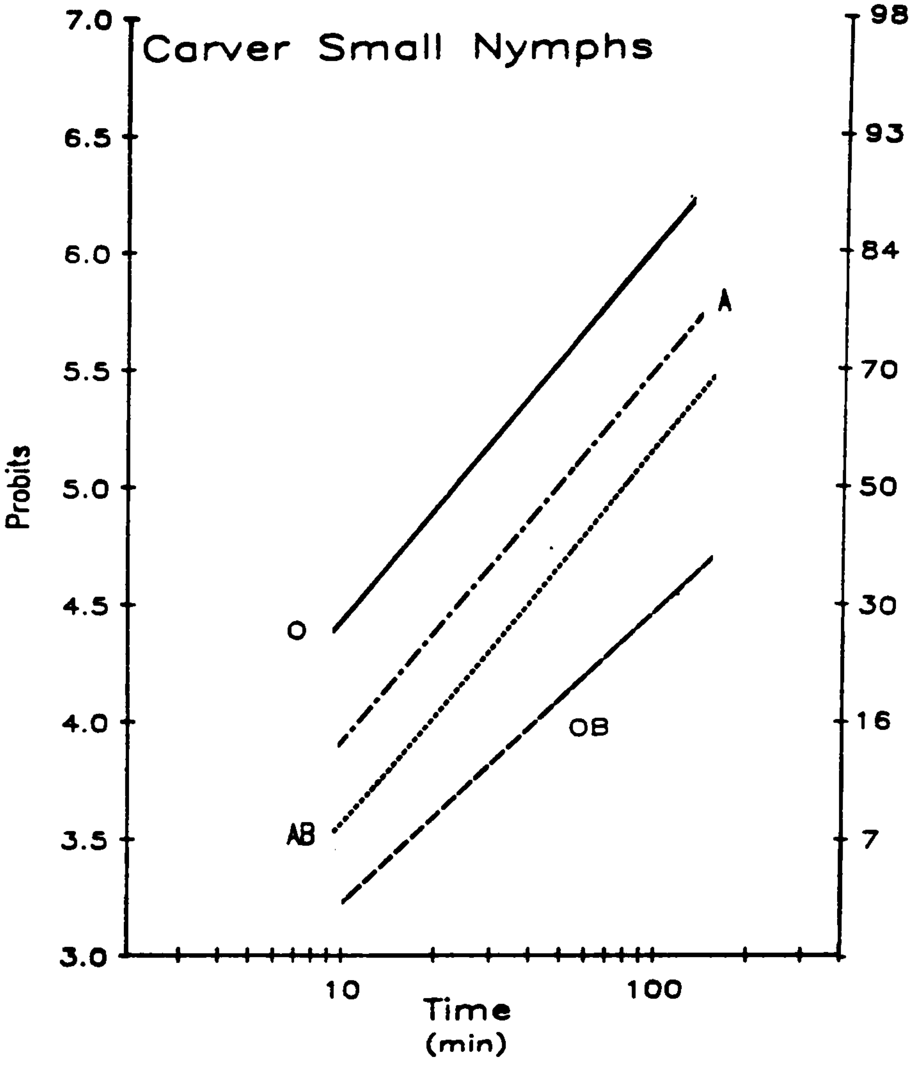

Figure 12. Dispersal of small nymphs of the Carver strain exposed to the vapors of two formulations of propoxur and its blanks. O-oil; OB-oil blank; A-aerosol; AB-aerosol blank.

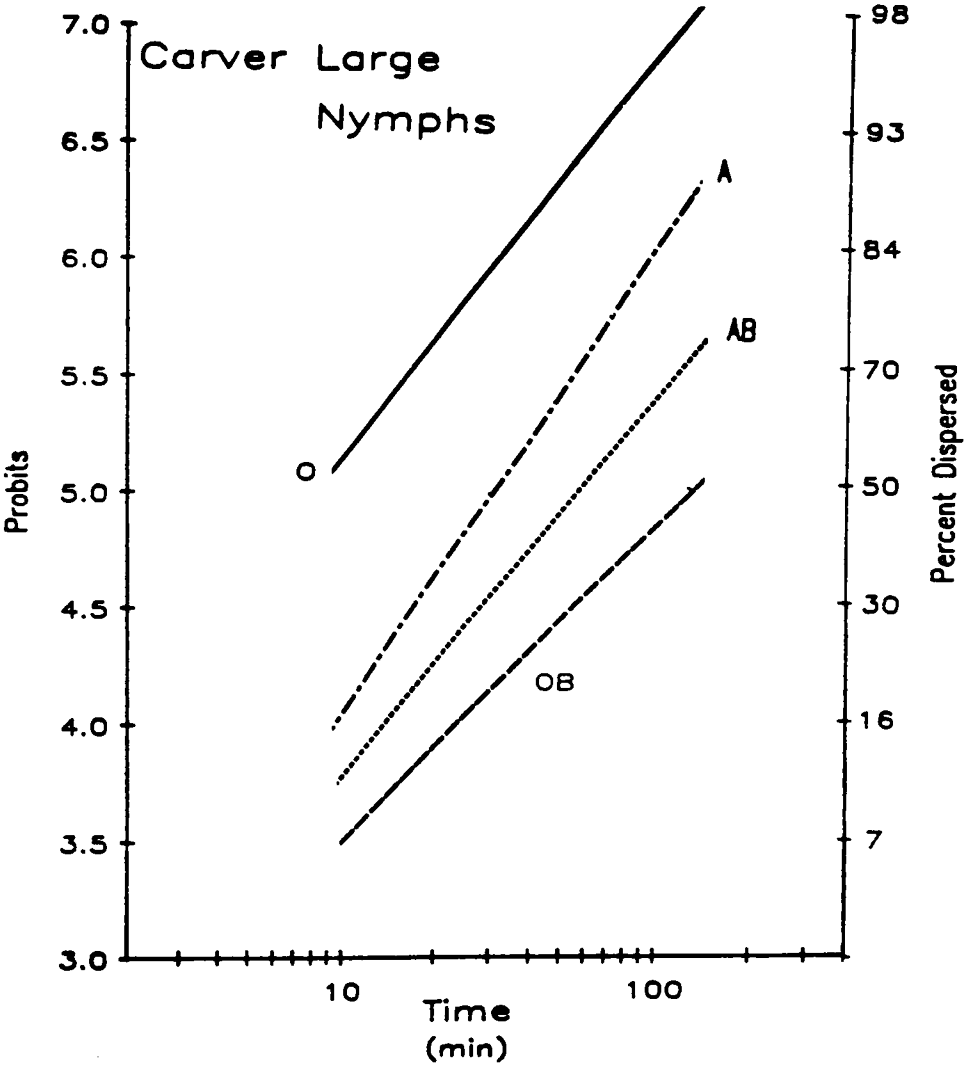

Figure 13. Dispersal of large nymphs of the Carver strain exposed to the vapors of two formulations of propoxur and its blanks. O-oil; OB-oil blank; A-aerosol; AB-aerosol blank.

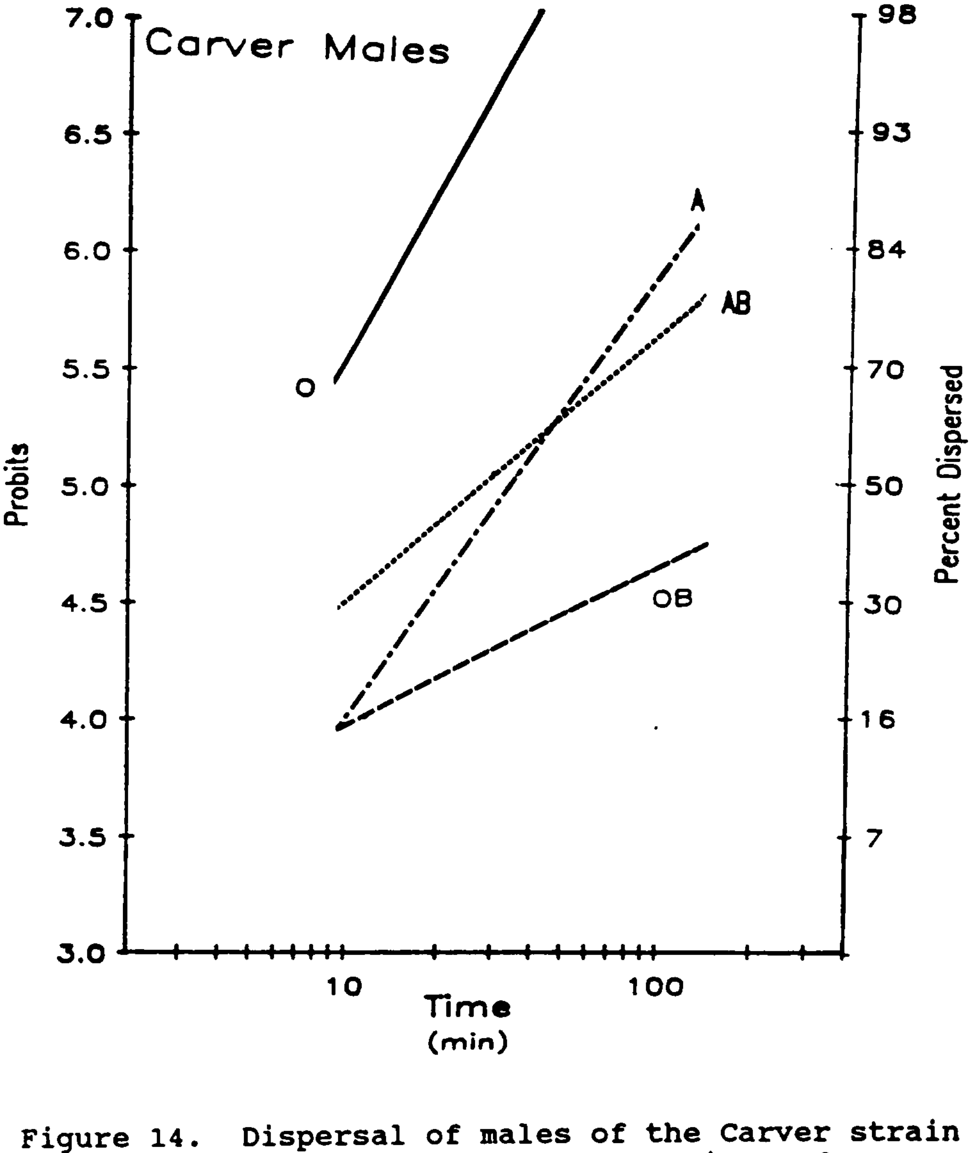

Figure 14. Dispersal of males of the Carver strain exposed to the vapors of two formulations of propoxur and its blanks. O-oil; OB-oil blank; A-aerosol; AB-aerosol blank.

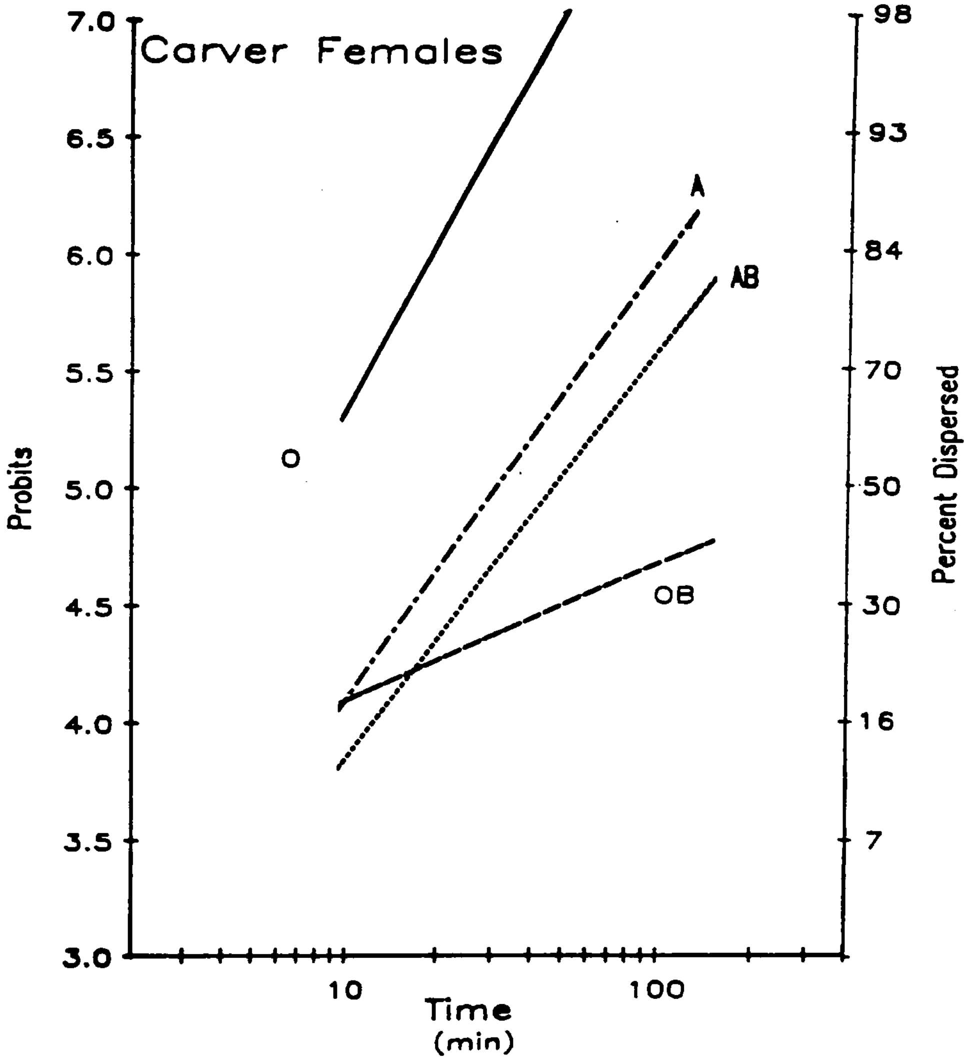

Figure 15. Dispersal of females of the Carver strain exposed to the vapors of two formulations of propoxur and its blanks. O-oil; OB-oil blank; A-aerosol; AB-aerosol blank.

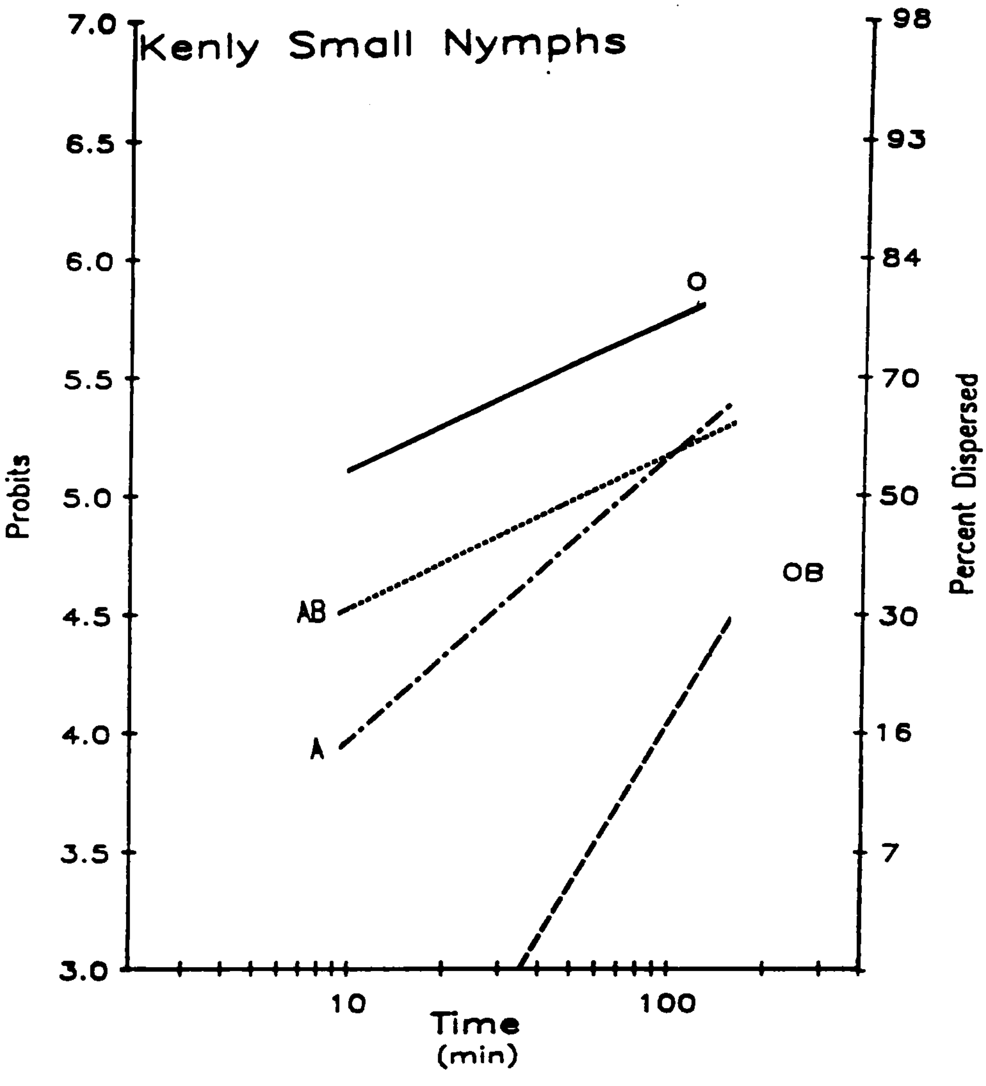

Figure 16. Dispersal of small nymphs of the Kenly strain exposed to the vapors of two formulations of propoxur and its blanks. O-oil; OB-oil blank; A-aerosol; AB-aerosol blank.

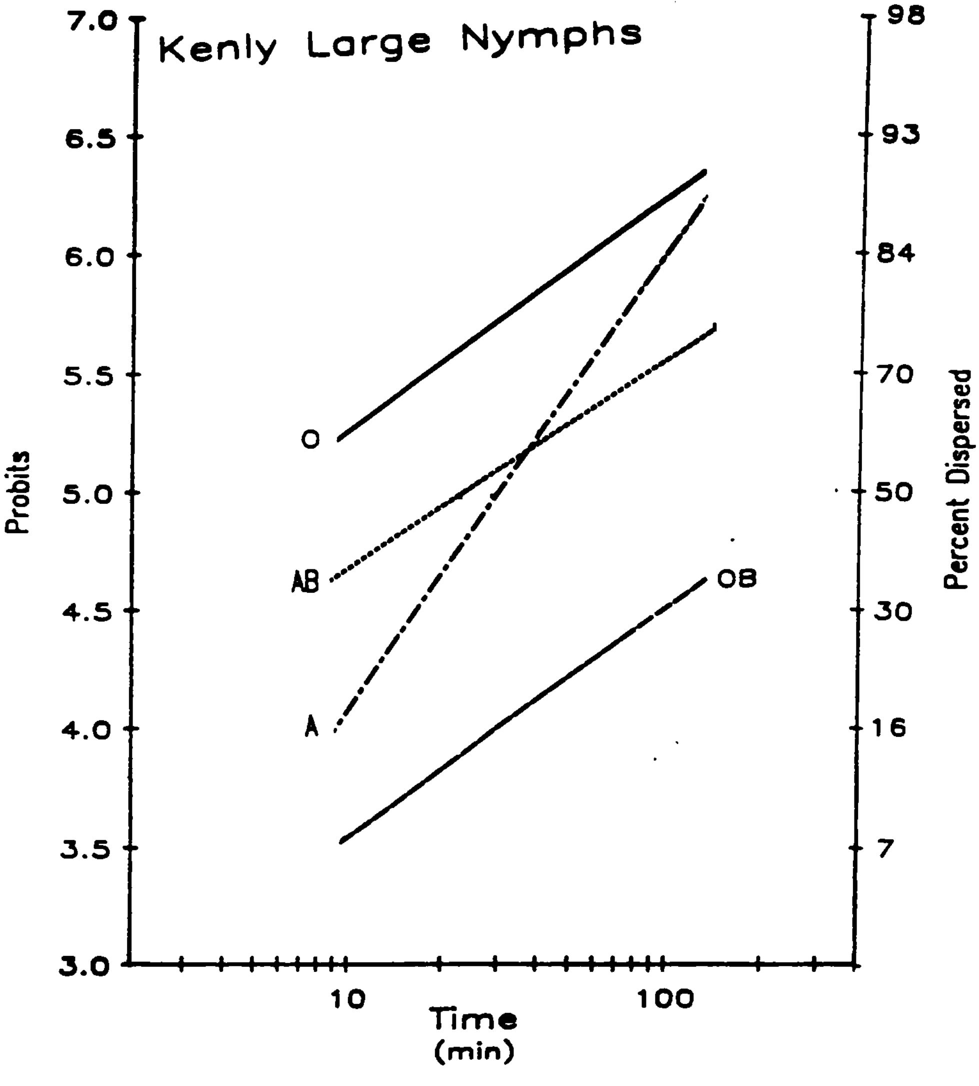

Figure 17. Dispersal of large nymphs of the Kenly strain exposed to the vapors of two formulations of propoxur and its blanks. O-oil; OB-oil blank; A-aerosol; AB-aerosol blank.

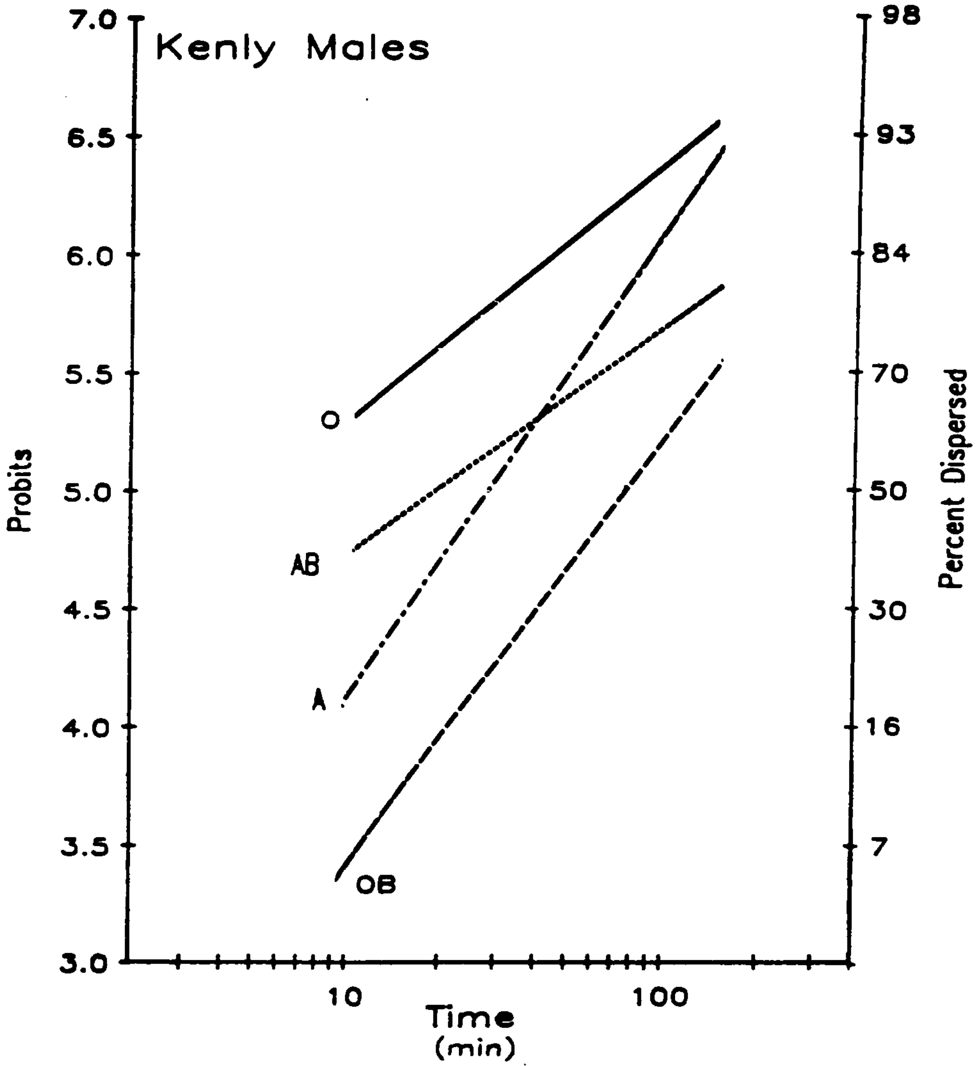

Figure 18. Dispersal of males of the Kenly strain exposed to the vapors of two formulations of propoxur and its blanks. O-oil; OB-oil blank; A-aerosol; AB-aerosol blank.

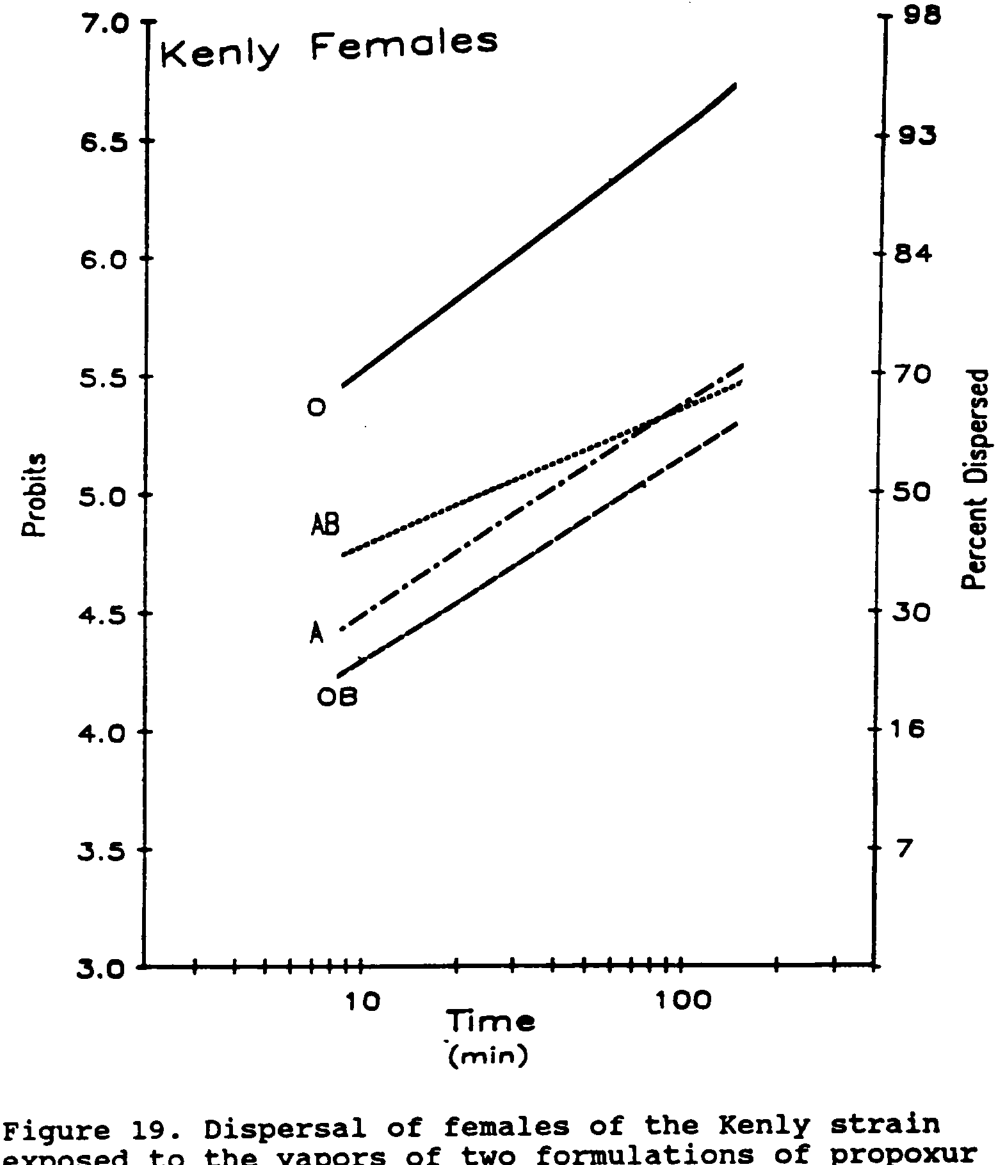

Figure 19. Dispersal of females of the Kenly strain exposed to the vapors of two formulations of propoxur and its blanks. O-oil; OB-oil blank; A-aerosol; AB-aerosol blank.

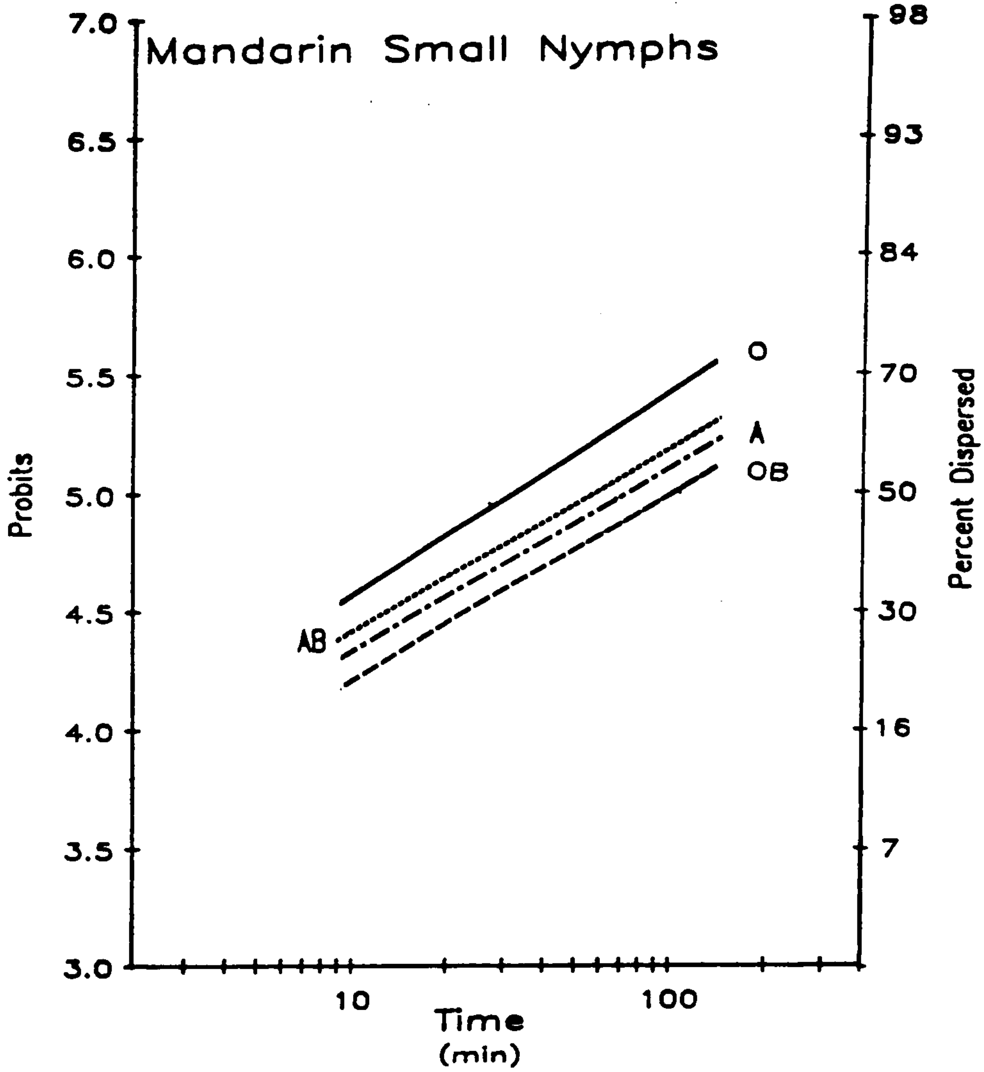

Figure 20. Dispersal of small nymphs of the Mandarin strain exposed to the vapors of two formulations of propoxur and its blanks. O-oil; OB-oil blank; A-aerosol; AB-aerosol blank.

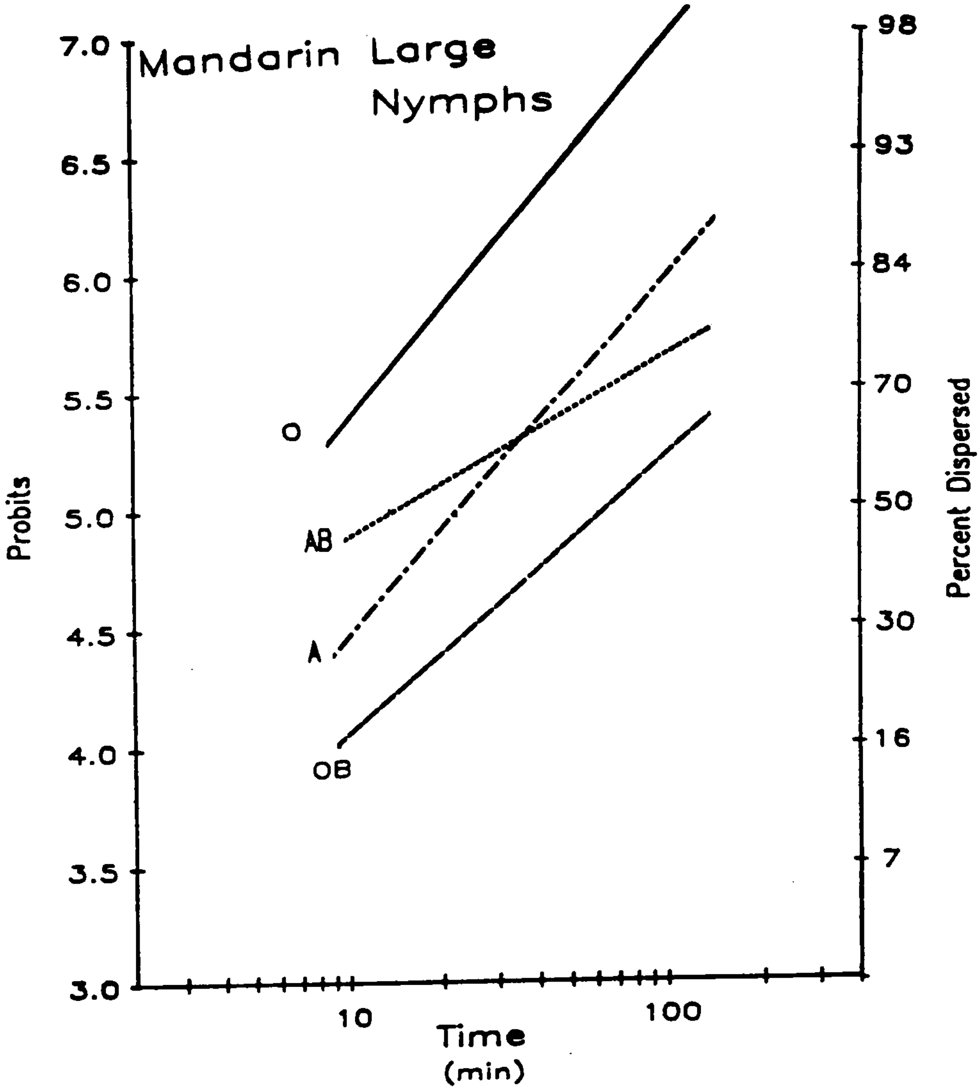

Figure 21. Dispersal of large nymphs of the Mandarin strain exposed to the vapors of two formulations of propoxur and its blanks. O-oil; OB-oil blank; A-aerosol; AB-aerosol blank.

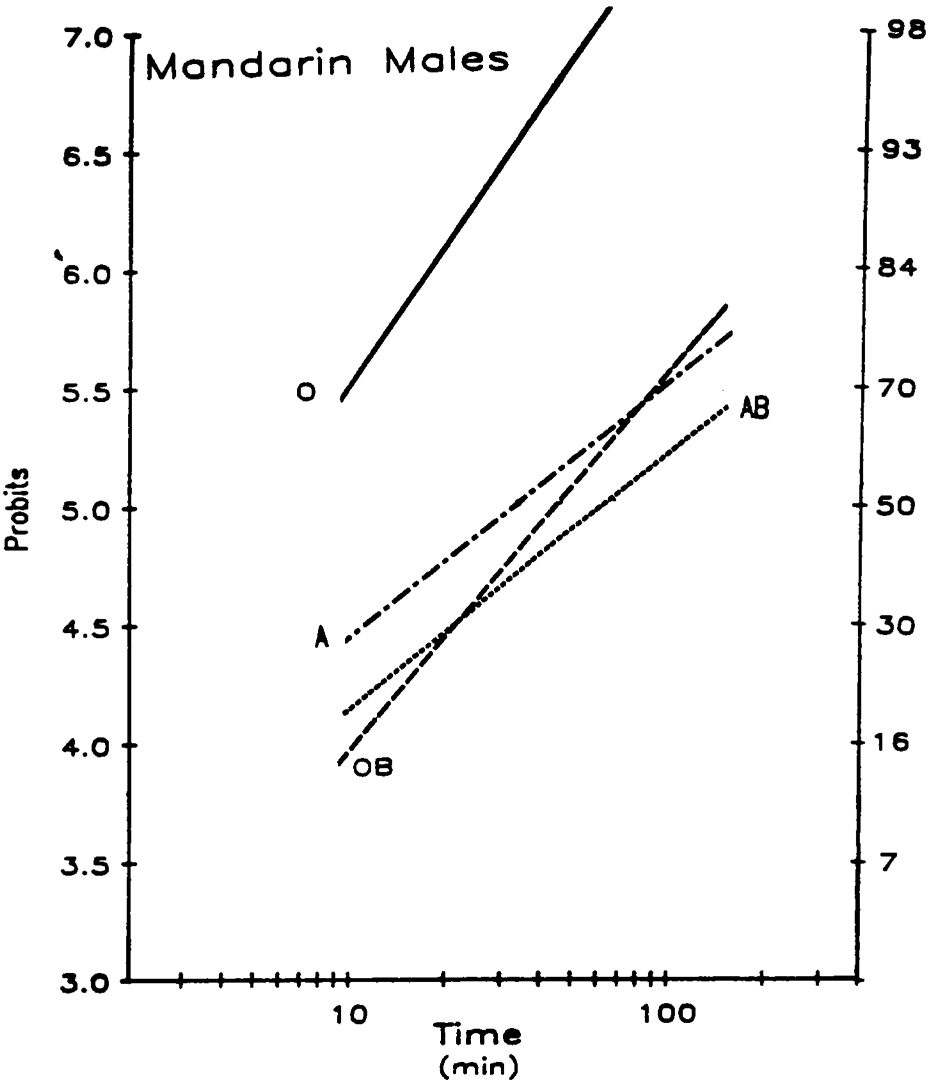

Figure 22. Dispersal of the males of the Mandarin strain exposed to the vapors of two formulations of propoxur and its blanks. O-oil; OB-oil blank; A-aerosol; AB-aerosol blank.

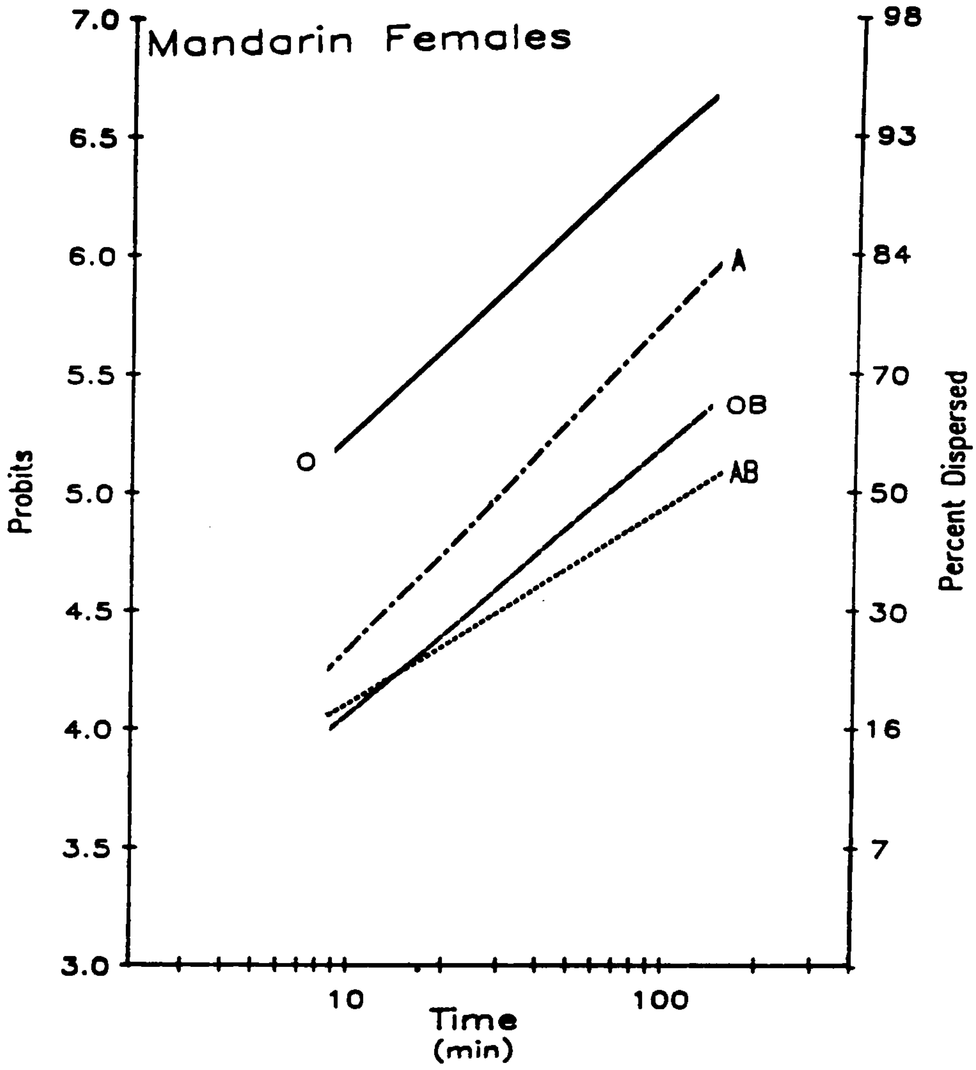

Figure 23. Dispersal of the females of the Mandarin strain exposed to the vapors of two formulations of propoxur and its blanks. O-oil; OB-oil blank; A-aerosol; AB-aerosol blank.

PART IV. VAPOR PULSE CHARACTERISTICS OF COMMERCIAL PESTICIDE FORMULATIONS USED AGAINST THE GERMAN COCKROACH

INTRODUCTION

The physics of pesticide application is a dynamic and complex process that is poorly understood. This process, sometimes known as dose transfer, involves many components, including: atomization, transport to target, impaction, deposition, movement and redistribution (Hall 1987; Hislop 1987). Other factors, such as the environment, operating conditions, target properties, the pesticide formulation and the active ingredient also affect dose transfer and thus play an important role in pesticide efficacy (Young 1986). A contributing factor to the rapid development of insecticide resistance has been improper dose transfer application technology and inappropriate pesticide placement (Hall 1986).

In conjunction with the concept of dose transfer, Hislop (1987) defined optimum pesticide deposition as the application of a biologically effective dose on a target with maximum safety and economy. The majority of the work on pesticide transfer and performance has been done in the area of agricultural chemicals. There has been little effort devoted to dose transfer problems for pesticides used in cockroach control programs.

The problem of effective dose transfer is complicated by redistribution of the pesticide after application. Redistribution from a surface or a target can occur as either vapor or particulate matter (Ebeling and Reierson 1963). Indeed, as a focus of this study, formulation material may first become redistributed as a vapor pulse which may then condense onto another surface some distance from the original site of application. While leading to an undesirable loss of the toxicant, redistribution can also lead to a sublethal impact on a target insect. Exposure to vapors or deposits of small concentrations could influence the behavioral or intoxication response of a target insect and may promote the development of resistance. The idea that a toxicant in the form of vapors may have effects on an insect in addition to, or independently of those from the treated surface is seldom taken into account in cockroach control programs.

There is a lack of information on air concentrations of pesticide formulations with regard to their threshold levels for detection, intoxication, or behavioral responses by the target insect. Air concentrations for the highly volatile fumigants are well documented. In general, these compounds are dispersed in large concentrations into large volumes of air (methyl bromide 5

- 7 kg/150 m^3) and for over 24 hours or more (Matthews 1979). They are generally used to saturate a restricted air space. Little is known about the sensory threshold of the target insect to these compounds. Dittrich (1966) determined air saturation point for the use of dichlorvos against mites at 145 mg/m^3.

Shinoda et al. (1983) determined that 50% of German cockroaches tested showed knockdown (KT50) after 30 minutes by an air concentration of permethrin of 40 ug/l (40 ng/ml) and that of phthalthrin of 35 ug/l (35 ng/ml). In addition, he noted that as much as 2% of the pyrethroids will condense on various surfaces regardless of surface texture.

Specific air concentration information is available for pesticides like propoxur, but it relates to human health exposure in the work place. While not having any direct relation to insect thresholds, these data do offer a base line figure for comparison. The threshold limit values for propoxur are divided into two categories. One is a Time Weighted Value (TWV). This is the concentration that a worker can be exposed to through an eight hour day without ill effect. The TWV for propoxur is 0.5 mg/m^3 (5 ng/ml). The other category is called a short term exposure limit (STEL) for a 15 minute interval. The STEL for propoxur is 2 mg/m^3 (20 ng/ml) (ACGIH 1983). The concept of short and long term threshold limits could also be employed when addressing the impact of pesticide vapor on insects.

Another area that might provide some insight into the responses of insect to airborne chemical concentration is the study of pheromones. While the detection of air-borne pheromones by insects has been thoroughly studied (Payne et al. 1986; Bell & Carde 1984), quantification of the concentration threshold that can be detected by insects is less well developed. Bell & Carde (1984) indicated that lepidopterous sex pheromones are emitted at the rate of 100 billionths of a gram per hour and that detection concentrations were in the order of 10^{-7} ug to 10^{-5} ug.

The most important impact of fume formation after pesticide application is manifested as the redistribution of the toxic material as vapors followed by the condensation of toxic material on exposed surfaces. The exposure of the target insect to these sublethal deposits of toxicant could result in the acceleration of both physiological and behavioral resistance. Sublethal responses may include the various dipersal responses described in the previous chapters. As more toxic compounds are used or more specifically targeted, it is increasingly important to know how this transfer process works.

In order to better understand the reaction of a cockroach when placed into a toxic chemical environment, it is necessary to characterize the vapor pulse emitted by various formulations and concentrations that cause dispersal. The objectives of this section are: (1) to estimate air concentrations of propoxur emitted from a oil formulation in the test apparatus used to test vapor-induced dispersal; (2) to determine if there is redistribution of propoxur and to estimate the amount of propoxur condensation in the test apparatus; (3) to examine the effects of modifying the test apparatus in regards to the phenomena of formulation volatilization and of toxicant vapor condensation; and (4) to estimate the vapor pulse originating from an oil, aerosol, emulsifiable concentrate (EC) and wettable powder (WP) formulations in the test apparatus and to compare them to the volatilization of known compounds.

MATERIALS AND METHODS

A. ESTIMATION OF TOXICANT AIR CONCENTRATION AND CONDENSATION

Air Scrub Studies: Polyurethane foam plugs have been used to capture insecticide volatiles by passing air continuously over samples in a closed system (Turner and

Glotfeity 1977, Petruska et al. 1985, D. E. Mullins pers. comm.). This technique combined with the use of a radiotracer produce a highly sensitive method for measuring volatilization of a propoxur formulation. This method eliminated the problem of derivitization that would be required with the use of HPLC, GLC and HPTLC.

An air scrubbing system (Fig. 24.) was designed to capture volatiles. The dispersal test apparatus was set up as described in the previous chapters. In brief, the apparatus is made of two glass crystallizing dishes connected by a wire and glass mesh tube. The inner sides of each dish were coated with a film of petroleum jelly. Screens folded into the shape of a "w" are placed into each dish as harborages. Cockroaches were acclimated for three hours prior to the placement of the treated filter paper strip. After the strip was placed on the upper inner lip of the first dish (dish-1) the exit from the first dish was opened. An 18 gauge (2 mm i.d.) needle was pushed through the screen of the connecting tube with the point lying on top of the vapor dam of the first dish. The needle was connected to a syringe tube (1 cc TB Plastipak Luer-Lok (B-D Rutherford, N. J.). The open end of the syringe tube was connected to a piece of Tygon tubing. The tubing led to another syringe tube which was connected to an 18 ga needle that had been pushed through a #5 neoprene stopper. The stopper-needle combination was then used to seal one end of a glass tube (10 cm long by 1.3 cm diam.). The glass tube contained three polyurethane plugs (3 cm long by 1.3 cm diam.). A vacuum air pump was connected to the other end of the glass tube with the stopper-needle-tubing combination. As a safety precaution, an additional glass tube containing three polyurethane plugs was placed between the first tube and the pump. Air flow from the needle tip at the vapor dam was measured by an air flow meter and adjusted by use of a hose clamp. The air scrub apparatus was placed in a fume hood. Exhaust from the pump was directed away from the chamber into the fume hood exhaust system with Tygon tubing (6.4 mm i.d.). A glass plate (20 cm by 20 cm) was placed underneath the connecting tube of the apparatus in an attempt to collect condensation emitting from the tube. Both dishes were covered with a Petri dish that had been modified by attaching a (3 cm long; 2 mm i.d.) glass tube in it center projecting upward. All tests were run at 22 ± 3°C and 70 ± 5%. RH.

Polyurethane plugs were prewashed before placing them into the glass tubes. They were allowed to soak at least 30 minutes in hexane (insecticide grade hexane was used throughout the experiment). The plugs were dried by pressing them into a small Buchner funnel atop a vacuum flask. This step was repeated three times. Plugs were allowed to air dry in a fume hood overnight.

Preliminary experiments were conducted to determine the optimal flow rate of air through the system, the amount of ^{14}C propoxur needed for recovery of detectable amounts, and the scrub time needed for the volatiles to be captured. A flow rate of 150 ml/min was selected. The turnover rate in the test chambers with this air flow was 6.6/30 min. A commercial 1% formulation of propoxur-in-oil (Octogon Process Inc. Lot C-4043) was spiked with Carbonyl-^{14}C propoxur (Mobay Chemical Corp., S.A. 17.98 mCi/mmole) in benzene at the rate of 5 uCi per 300 ul. A strip (1 cm x 130 cm) of Whatman no. 1 filter paper was treated with 300 ul of the radiolabelled spiked propoxur formulation and placed onto the upper lip of the first dish of the test apparatus. The glass cover of the first dish was replaced, and the pump turned on. Air was pulled through the 18 ga. needle over the vapor dam. Pesticide vapors were scrubbed from the air and trapped in the foam plugs. Scrubbing was done for 30 minute intervals for a 2 hours period. The pump was turned off at the end of each interval and a clean tube with three fresh plugs was placed in line.

Each plug was placed in a 450 ml French square bottle. The glass tube was rinsed with 5 ml of hexane into a French square bottle. The propoxur that was trapped in each plug was extracted by soaking the plug in 30 ml of hexane for 30 minutes. Each plug was removed and placed into a small Buchner funnel. The Buchner funnel was placed above the French square bottle and the plug squeezed repeatedly with the bottom of a 50 ml beaker until dry.

After the end of the 2 hour test period, the apparatus was disassembled and the separate parts were rinsed in 30 ml of hexane into individual French square bottles. The screen wire harborage, filter paper strips, and glass plates were placed individually into 30 ml of hexane and allowed to soak for a minimum of 30 minutes. The cockroaches from each test were placed into one bottle and allowed to soak for 30 minutes.

Each 30 ml sample from either the plugs or apparatus parts was removed, placed into a test tube, and concentrated down to 1 ml using a nitrogen flow evaporator at 40^{o}C. A 100 ul aliquot from each 1 ml concentrate was placed in 8 ml of scintillation fluid (Ecoscint) and counted. Counts were taken for 20 minutes or 0.7% counting error, whichever came first. The narrow ^{14}C-window of a Beckman (Model LS 3150T) scintillation counter was used.

Three replicates were completed. Counts were corrected for sample quenching. Counts per minute were converted to disintegrations per minute (DPM) using a Fortran program

"Isotope" (K. Tignor pers. comm.). Plug extraction efficiency was determined by spiking a foam plug with a known amount of ^{14}C-propoxur and extracting as above. Three 1 cm x 1 cm samples of each filter paper strip were placed in 8 ml of scintillation fluid and counted to determine any residual activity following hexane extraction of the filter paper. Air and surface concentration were estimated by using the ratio of recovered radiolabelled propoxur to that applied, multiplied by the number of pg in 300 ul of a 1% solution of propoxur.

Redistribution Studies: The apparatus was set up as above using ^{14}C propoxur, except that the air scrubbing system was not used. After 2 hours the apparatus was disassembled and the individual parts rinsed. The rinsate was concentrated and analyzed as described above. Four replicates were completed.

Closed System Study: The apparatus was set up as in the redistribution study except the glass and screen connecting tube was replaced with a 3 cm long solid Tygon tubing (1 cm id). After 2 hour the various parts of the apparatus were disassembled and individually rinsed, the rinsate concentrated and analyzed as above. Three replicates were completed.

B. FORMULATION VAPORIZATION RATES.

Vaporization Studies: Air concentration estimates of vapors from the four formulations of propoxur (oil, aerosol, EC, and WP) were obtained by calculating the differences in weight of the formulation applied to a filter paper strip as it evaporated over time into a known volume of air. The filter paper strip was folded flat into a "W" and placed on the weighing pan of a Mettler (Model 100) balance and its weight recorded. As in the dispersal studies (Parts II & III), 300 ul of the test formulation was applied to the strip. Its weight was recorded. Immediately after application of the test formulation the paper strip was enclosed by either inverting an apparatus test dish over the weighing pan (678 ml) or closing the doors on the balance box (6037 ml). The exit port of the inverted apparatus dish had been sealed with a piece of Parafilm M. Weights of the filter paper were then recorded every minute for 30 minutes. The weight differences between two time intervals divided by the volume of the container could be used to estimate the amount of volatilization per unit time and air concentration of the formulation at equilibrium. Three replicates of each formulation volume combination were performed.

Vaporization rates of solutions of known composition: A 300 ul sample of each of the following solutions were tested as above: water, mineral spirits (MS), acetone, trichloroethane (TCE), ethanol (ETOH), 50:50 acetone:mineral spirits; and 75:25 acetone:mineral spirits. This experiment was designed to determine the volatilization rates of known compounds and mixtures at various ratios in both the large and small volume. Three replicates were done for each of the above compound and volume combinations. Water and mineral spirits were chosen because they are representative of the base ingredient in the water and oil formulations respectively. Acetone, TCE, and ethanol were chosen because of their high volatility which might be comparable to the volatilization rates of certain ingredients found in the aerosol and EC formulations.

RESULTS

A. TOXICANT AIR CONCENTRATION AND CONDENSATION.

Air Scrub Study: Table 8 shows the amount of ^{14}C recovered from various surfaces of the test apparatus with an air scrub system. The majority of the propoxur recovered remained on the the filter paper strip (61.6%).

Mean recovery of radiolabelled materials from the total amount applied was 63%. Most of the propoxur condensed on the top, sides, and base of Dish-1. The highest projected amount of propoxur condensation was 152 ng/cm^2 from the sides of the apparatus. The estimated amount of propoxur for the base of Dish-1 was 33 ng/cm^2 followed by the top of Dish-1 with 10 ng/cm^2. Very little radiolabelled material was recovered from Dish-2 and the estimates of propoxur condensation are correspondingly low.

The estimated air concentrations in Dish-1 for each 30 minute scrub interval are shown in Table 9. Estimates of propoxur air concentrations were 146 pg/ml during the first 30 minute interval. The concentration then rapidly declined to 40 pg/ml during the next interval followed by 26 and 20 pg/ml for the remaining two intervals.

<u>Condensation</u> <u>Studies</u>: Redistribution of propoxur on the various parts of the test apparatus without air scrub is indicated in Table 10. The amount of material recovered was highest on the sides of Dish-1, followed by the base, top and screens. Overall recovery was slightly higher (67%) than in the air scrub experiment. Most of the pesticide remained on the filter paper strip as it did in the previous experiment. The projected amount of propoxur condensation was lower in Dish-1 than that in the air scrub system. However, the amount of propoxur condensation in Dish-2 was higher (0.50 ng/ml Table 10 vs.

0.13 ng/ml Table 8) than in the system with the air scrub.

The amount of propoxur condensation when the apparatus was used as a closed system (Table 11) was similar to the other tests. The major difference was the higher condensation levels on the top of Dish-2 compared to the previous experiments.

B. FORMULATION VAPORIZATION RATES.

Vaporization Studies: Vaporization rates for the four formulations of propoxur in a 6 liter volume are shown in Fig. 25. Both the oil and WP formulations reached equilibrium very rapidly, as did the water treatment. The EC formulation reached equilibrium at about 2 minutes after losing approximately 10 mg of material into the air. The aerosol formulation reached equilibrium at about four minutes after emitting over 20 mg of volatiles into the air. Equilibrium levels for the WP, EC and water treatment were approximately 6 mg/minute. The oil and aerosol equilibrium point was approximately 2 mg/minute. Equilibrium air concentration estimates were 993 pg/ml for the water base formulations and 331 pg/ml for the oil base formulations.

Fig. 26. shows the 30 minute vaporization rates of the oil and aerosol formulations compared to mineral spirits in the inverted test apparatus (678 ml). The

results are similar to the vapor profiles in the larger volume. Equilibrium rates were reached at approximately the same time as those in the larger volume but at lower levels of 0.5 mg/minute. The large weight loss in the first minute of this experiment can be attributed to the time lag and air currents created by the manipulation of the apparatus dish over the Mettler balance pan. The equilibrium air concentration estimate for the oil base formulation is 737 pg/ml/min.

The vaporization rate of the water based formulations are shown in Fig 27. They are also similar to the results obtained in the larger volume study. The equilibrium loss rate is approximately 1.0 mg/minute following an initial 3-5 minute pulse of vaporization. The equilibrium air concentration estimate for the water base formulation is 1.47 ug/ml/min.

<u>Vaporization of Known Volitiles</u>: The vaporization rates of the known materials in the 6 liter volume are indicated in Fig. 28. Acetone goes to complete dryness within five minutes of application, followed by TCE which goes to dryness at about 11 minutes. On the other hand, ETOH established an equilibrium rate of 2.6 mg/minute at 9 minutes.

Fig 29. shows the vaporization rate of a mixture of known materials in the 6 liter volume. All three mixtures containing acetone rapidly lost material by evaporation in

the first three minutes. The pure acetone went to dryness in 15 minutes. The mixtures that contained mineral spirits leveled off and reached equilibrium at the same time as the acetone.

DISCUSSION

Dispersal of the test cockroaches in previous sections must be viewed as a product of a vapor pulse originating from the total formulation applied to the filter paper strip. Upon attachment of the filter paper collar a vapor pulse is emitted as the formulation begins to dry. This pulse is composed primarily of "inert ingredients". As seen in the ^{14}C experiment, the total amount of toxicant in the vapor pulse of an oil formulation is less than 1% by weight of the toxicant of a 1% formulation. Estimates of the concentration of propoxur in air that induced dispersal are an order of magnitude smaller than those of permethrin and phthalthrin that cause knockdown. They are also an order of magnitude smaller than the air concentration that would pose a human hazard as expressed in the TWV and STEL values. The filter paper emits a vapor cloud that "descends" down along the sides of the apparatus and rapidly fills Dish -1. The cockroaches are exposed to a rapid build up of vapors as the vapor pulse approaches equilibrium throughout Dish-1.

As this equilibrium is reached the more volatile components dissipate and the toxicant begins to condense on exposed surfaces of the apparatus, including the test cockroaches that were still in Dish-1. Once equilibrium is reached a constant rate of vaporization and condensation occurs over time until dryness.

Condensation of the toxicant occurs on all exposed surfaces including the tops of Dish-1 in all studies and the top of Dish-2 in the closed system study. The high DPM counts on the sides of Dish-1 in both the air scrub and no-air scrub studies probably reflects contamination of the sides of the dish by the wet formulation as the filter paper strip is attached.

The comparison of the system with and without air scrubbing indicates that the amount of redistribution can be changed by air flow. The actual amount of condensation was higher in the first dish with the air scrub system. This indicated that the air flow promoted vaporization of the formulation as the pesticide vapors continually sought equilibrium. However, the amount of redistribution in the second dish was lower in the air scrub system although little of the toxicant reached the second dish in either experiment. The difference was due to the drawing in of the fumes by the scrub system at the point of escape and the reduction of the overall amount that would "drift" into the second dish. The closed system study indicated that vapors built up in both dishes and vented away around the loose fitting Petri dish tops. The glass and mesh tube effectively vent away most of the formulation vapors. This prevents vapors from moving into the second dish and thus provides a "vapor free" habitat for the test cockroaches to move into.

The vaporization rates showed that a homogenous formulation tends to reach vapor equilibrium rapidly while a heterogeneous solution takes more time to reach equilibrium, during which the more volatile components evaporate. If the formulation contains a highly volatile component, as in the aerosol and EC formulations, a lot of material is lost in the first several minutes. Different equilibrium points are reached depending on whether the formulation is oil or water based. The pulse from the water based formulation at equilibrium is primarily water. It can be assumed that this would not induce dispersal, but on the other hand might enhance the quality of the cockroaches' environment by increasing the local relative humidity. The early loss of material in the aerosol formulation is probably the result of volatilization of the fluorocarbon propellant. In the EC formulation, the early evaporation of the volatile oil portion is also evident. However, in the EC, this did not appear to cause any significant amount of dispersal in the earlier experiments, even though a considerable amount of material was volatilized. The proportion of volatiles to nonvolatiles does not appear to alter the equilibrium time as it levels off at the level of the base compound.

A 10-fold difference in volume did not appear to affect the equilibrium time. However, it did change the equilibrium vaporization rates, with the larger volumes having a higher mg/minute loss of material. The largest vapor pulses per unit time were obtained with the use of acetone and TCE.

The above experiments demonstrate that a rapid build up of vapors occurs in Dish-1 and that vapor concentrations are maintained at equillibrium until dryness. Most of these vapors vent away from Dish-2, through the screen portion of the connecting tube. The equilibrium vapor pulses of water-based formulations are larger by weight than those of the oil based formulations. However, the dispersal rate induced by the oil based formulation (Part II.) was higher than that of the water based formulations. This was probably due to the "noxious" characteristics of vapors and of the oil formulations and the higher solubility of propoxur in the oil solvent as compared to water. In addition, the solvents in the oil and aerosol formulations have high liposolubility characteristics compared to water. Liposolubility might also be important in the area of perception as it increases a molecule's ability to be absorbed onto a sensilla membrane. On the other hand, the water in the EC and WP formulations would have little effect on dispersal. Propoxur is poorly solubilized in water so there would be less volatilization of the toxicant into the air from a water based formulation. In addition, by increasing the RH in the apparatus the water based formulation might enhance the harborage environment of Dish-1 and thus help explain the overall reduced dispersal response of the EC and WP formulations.

Impact of Sublethal effects: The fact that a pesticide formulation such as propoxur in oil will vaporize and recondense leaving low levels of toxicant is an important consideration. These "sublethal" concentrations could have an impact on the development of latent toxicity, reproductive potential, enzyme induction and behavior (Moriarty 1969). Luckey (1968) discussed another interesting if somewhat doubtful effect of "subharmful quantities of a stressing agent" and proposed the concept of insecticide "homoligosis". This concept implies that sublethal doses of a toxicant will be stimulatory to the target insect by increasing its sensitivity to respond to changes in its environment. He was able to demonstrate this phenomenon using insecticides against the house cricket Acheta domesticus with growth rate as a criteria.

He went on to state that the agent (insecticide) was stimulatory to the insect by providing increased sensitivity to respond to changes in its environment and increased efficiency to develop new or better systems to fit into a suboptimum environment. Field strains studied thus far do not show any consistent increase in the rate of insecticide-induced dispersal, although there were instances of faster dispersal than in the VPI strain (Part II.).

Operational Implications: The most obvious result of a formulation vapor pulse would be unintentional dispersal with the oil-based as compared to the water based product. Sublethal deposits could promote the developments of behavioral resistance. Whether sublethal exposure promotes physiological resistance is open to question. Sublethal deposits surrounding lethal deposits would probably increase the avoidance response to the lethal deposits and reduce overall cockroach mortality.

Table 8. Estimated vapor deposits from a 300 ul volume of a 1% propoxur solution, containing radiolabelled propoxur, applied to a filter paper collar in a dipsersal test apparatus (with air scrub 150 ml/min).

Location	Mean % of Total (DPM) (n=3) ± SD	total wt from 1% solution (ng)	area cm^2	projected propoxur conc. ng/cm^2
Dish-1 Top	0.039 ± 0.028	1170	113	10.39
Dish-1 Sides	1.146 ± 0.800	34380	226	152.12
Dish-1 Base	0.127 ± 0.001	3810	113	33.71
Dish-1 Screens*	0.016 ± 0.001	480	**	**
Connecting Tube	0.001 ± 0.0001	30	30	1.00
Glass Plate	<0.001 --	**	**	**
Dish-2 Top	<0.001 --	**	113	**
Dish-2	0.001 ± 0.0001	30	339	0.08
Dish-2	<0.001 --	**	**	**
Cockroaches	0.001 ± 0.0001	30	**	**
Total Dish-1	1.329 ± 0.20	39870	452	88.20
Total Dish-2	0.002 ± 0.0001	60	452	0.13
Filter Paper	61.61 ± 0.5	1848300	130	14217.69
Total Recovered	62.94 ± 5.6	**	**	**

* screen wire harborage
** not determined

Table 9. Estimated propoxur air concentrations in a dispersal test apparatus.

Time Interval (min)	Mean ±SD DPM (n=4)	% Total DPM	projected wt from 1% solutio n (ng)*	# ml air scrub	air conc pg/ml
30	2306 ± 1040	0.023	660	4500	146
60	613 ± 295	0.006	180	"	40
90	453 ± 391	0.004	120	"	26
120	370 ± 216	0.004	90	"	20
Total	3742 ± 1540	0.073	1050	18000	58

* 300 ul dose of 1% propoxur solution containing radiolabelled propoxur.

Table 10. Estimated vapor deposits from of a 300 ul volume of a 1% propoxur solution applied to a filter paper collar in a dispersal test apparatus (without air scrub).

Location	Mean % of Total (DPM) ± SD	total wt from 1% solution (ng)	area cm^2	projected propoxur conc. ng/cm^2
Dish-1 Top	0.035 ± 0.030	1050	113	9.29
Dish-1 Side	0.615 ± 0.182	18450	226	81.61
Dish-1 Base	0.084 ± 0.039	2520	113	22.30
Dish-1 Screens	0.023 ± 0.012	690	*	*
Connecting Tube	0.005 ± 0.003	150	30	5.00
Glass Plate	0.001 ± 0.0001	30	400	0.12
Dish-2 Top	<0.001 --	*	*	*
Dish-2	0.002 ± 0.0001	339	60	0.20
Dish-2 Screens	<0.001 ± --	*	*	*
Cockroaches	0.001 ± 0.0001	30	*	*
Total Dish-1	0.76 ± 0.22	22800	452	50.40
Total Dish-2	0.008 ± 0.005	240	452	0.50
Filter Paper	65.19 ± 3.5	1955850	130	15045.0
Total Recovered	66.729 + 2.7	*	*	*

* not determined

Table 11. Estimated vapor deposits from of a 300 ul volume of a 1% propoxur solution containing radiolabelled propoxur applied to a filter paper collar in a dispersal test apparatus with a modified connecting tube (without air scrub).

Location	Mean % of Total (DPM) (n = 3) ± SD	total wt from 1% solution (ng)	area cm^2	projected propoxur conc. ng/cm^2
Dish-1 Top	0.041 ± 0.013	1230	113	10.88
Dish-1 Side	0.775 ± 0.039	23100	226	102.20
Dish-1 Base	0.065 ± 0.048	1950	113	17.20
Dish-1 Screens	0.014 ± 0.003	420	*	*
Connecting Tube	0.001 ± 0.0002	30	5	7.20
Dish-2 Top	0.002 ± 0.0004	60	113	0.53
Dish-2 Sides	0.001 ± 0.0001	30	226	0.13
Dish-2 Base	<0.001 ± --	*	113	*
Dish-2 Screens	<0.001 ± --	*	*	*
Cockroaches	0.001 ± 0.0003	30	*	*
Total Dish-1	0.890 ± 0.315	26700	452	59.07
Total Dish-2	0.006 ± 0.0001	180	452	0.39
Filter Paper	65.195 ± 4.5	1955850	130	15045.00
Total	65.59 ± 5.5	*	*	*

* not determined

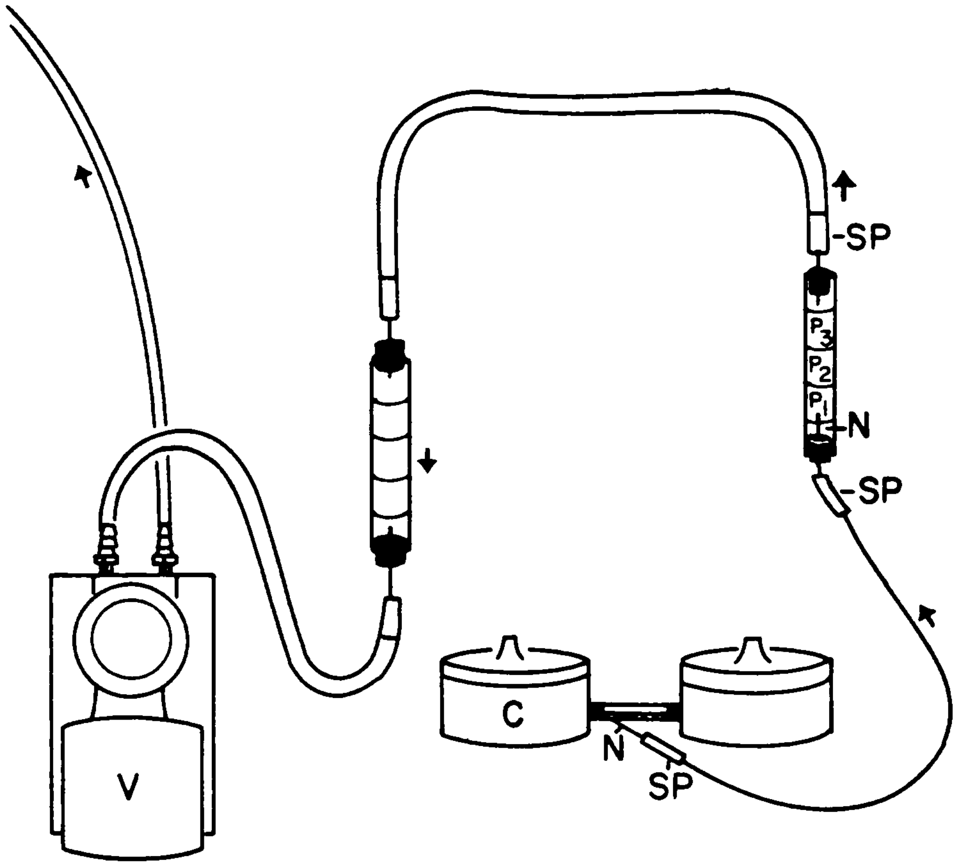

Figure 24. Diagram of experimental air scrub apparatus. V-vaccum pump; SP-syringe parts; P1,P2,P3-polyurethane foam plugs; C-Dish-1(aggregation dish); N-needle. Arrow indicated the direction of air flow.

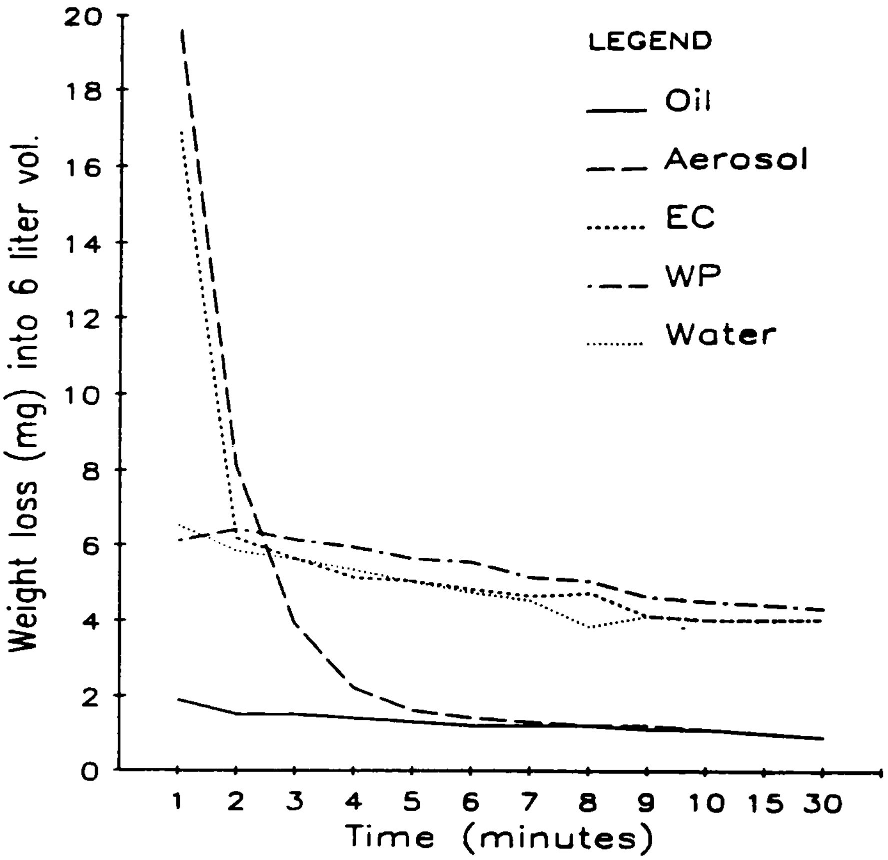

Figure 25. Mean vaporization rates of four formulations of propoxur into a 6 liter closed volume (n =3).

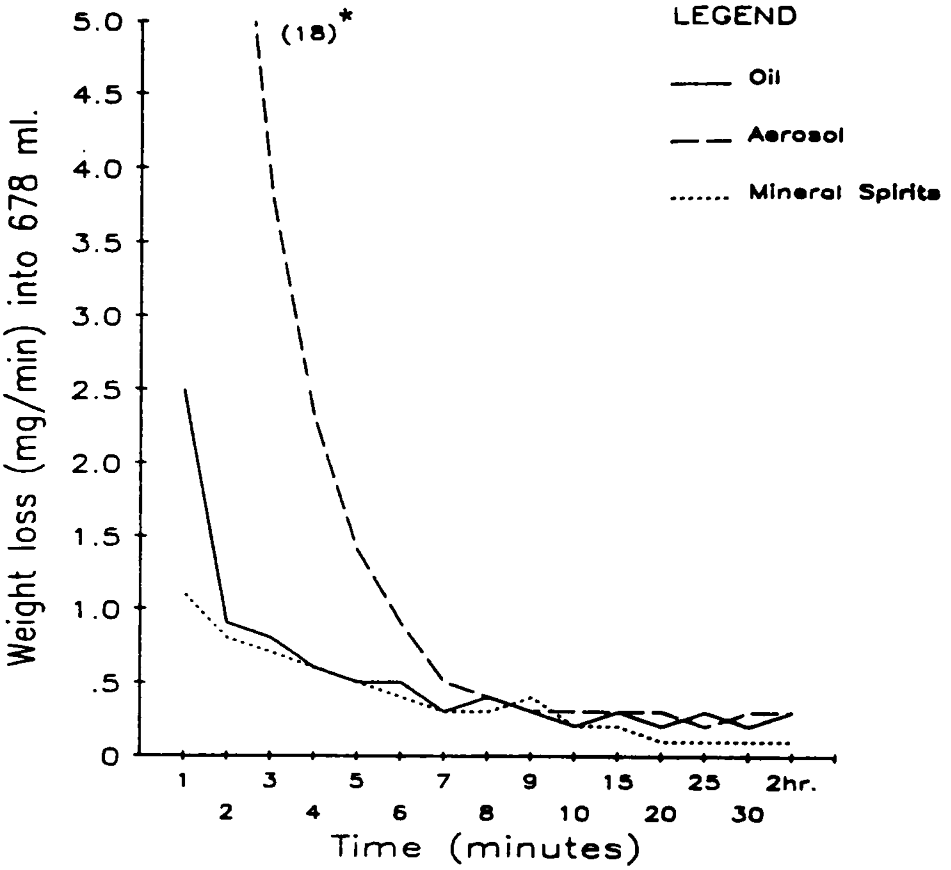

Figure 26. Mean vaporization rates of an oil and aerosol formulation of propoxur compared to a mineral spirits treatment in a dispersal test apparatus (n = 3). *indicates values are off scale.

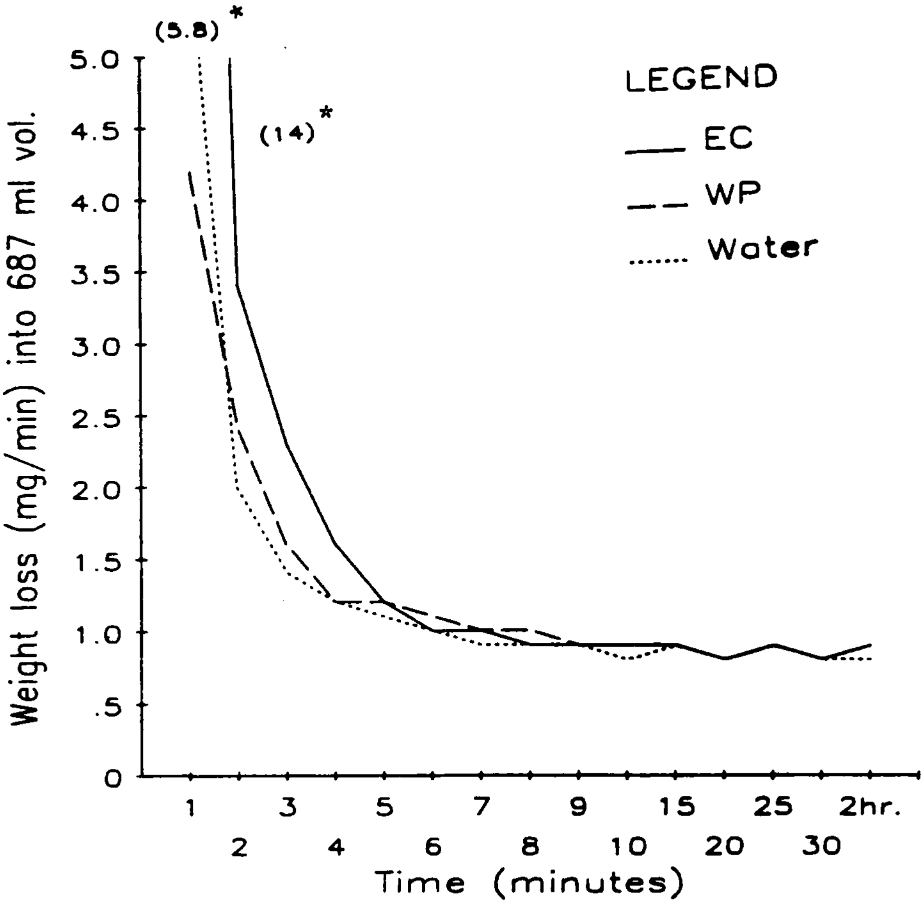

Figure 27. Mean vaporization rates of two water-based propoxur formulations compared to a water treatment in a vapor dispersal test apparatus (n =3). *indicates values are off scale.

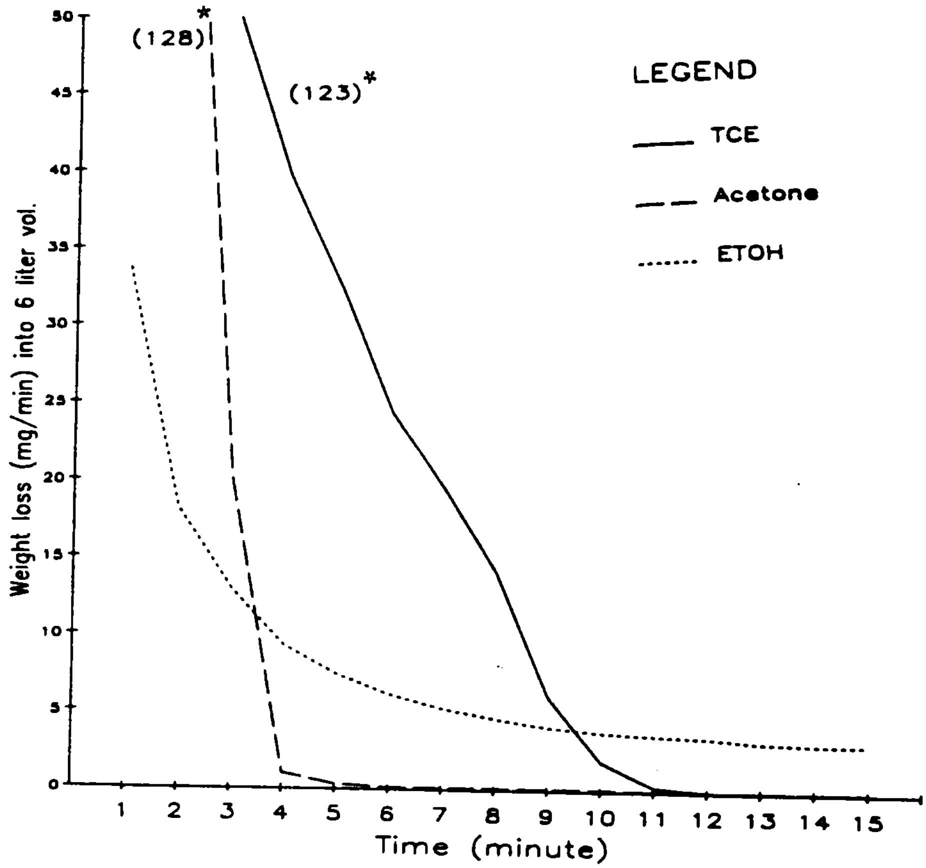

Figure 28. Mean vaporization rates of several highly volatile compounds (n = 3). *indicates values are off scale.

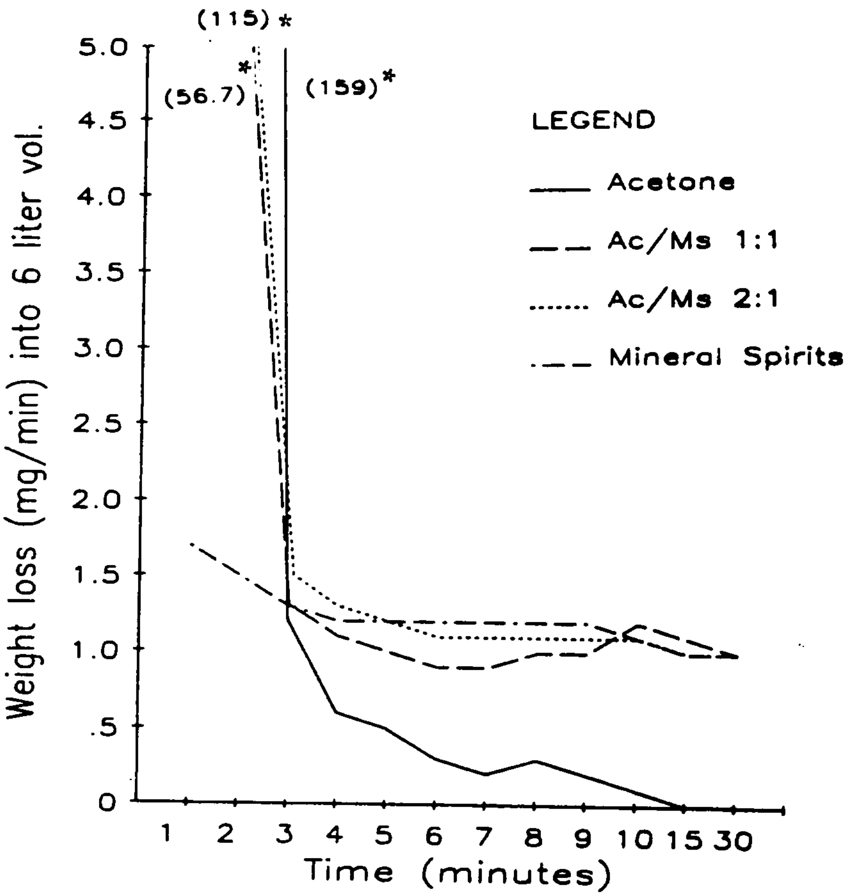

Figure 29. Mean vaporization rates of a mixture of several ratios of a highly volatile and moderately volatile compounds (n = 4). *indicates values are off scale.

SUMMARY

It has long been recognized that insecticide-induced dispersal is a problem in the control of the German cockroach, Blattella germanica. Vapors from insecticide formulations may cause control failures as cockroaches are dispersed from a harborage before the insect receives a lethal dose. Research was undertaken to investigate several aspects of the dispersal response induced by vapors of some commonly used insecticides.

It was first necessary to find a method of testing vapor-induced dispersal. An apparatus was developed that allowed the exposure of cockroaches to insecticide vapors in an enclosed "harborage" (Dish-1) and their subsequent movements into a vapor free area (Dish-2). The apparatus was designed to simulate dispersal of cockroaches from field harborages and to reduce the dispersal response from any cause except that induced by the test formulations.

A series of experiments was conducted with large nymphs (5th and 6th instars) exposed to vapors of several commercial propoxur formulations and their blanks. The type of formulation, as shown in the results of these experiments, indicated that vapor-induced dispersal has two formulations components: (1) dispersal responses induced by only the inert ingredients as represented by the blank formulations and (2) dispersal induced by the

complete formulation with the toxicant. Little or no dispersal occurred from exposure to the wettable powder formulations (WP), emulsifiable concentrates (EC), or their blanks. In contrast, significant and comparatively rapid dispersal occurred in the experiments with propoxur in oil, propoxur aerosol and the oil and aerosol blanks.

Strain differences were also discovered in the experiments with large nymphs. One of the resistant strains (Kenly) showed a more rapid response to propoxur-in-oil compared to a susceptible strain (VPI). Another resistant strain (Carver) showed a slower response to propoxur aerosol, aerosol blank, and an oil blank formulation compared to the susceptible strain. The overall vapor-induced response of any wild type strain to a specific formulation is probably a reflection of the degree of expression by either a perception/avoidance response and/or an intoxication/ hyperactivity response as a result of "field" selection.

Large nymphs were also exposed to the vapors of diazinon, malathion and cyfluthrin. Results indicated that the class of insecticide might also be important in the final dispersal response. It appears that the dispersal response to the pyrethroid, cyfluthrin, is different from that induced by the other pesticide classes tested. The dispersal response of the VPI susceptible strain exposed to this pyrethroid was extremely slow compared to a very fast dispersal response demonstrated by the two resistant strains.

The propoxur formulations and their blanks were also used in experiments with mixed age groups of the VPI and three field strains. There were fewer clear cases of significant differences than in the single age class study. Nevertheless, certain patterns that were found in the experiments with the large nymphs were also evident in the mixed age class studies. In general, dispersal of all age classes was faster in the experiments with propoxur-in oil, followed by the aerosol, its blank and the oil blank. Inter-strain differences were also similar to the single age class study with the Carver strain, in general responding more slowly than the VPI strain. Also, individual age classes of the Kenly strain responded faster than the VPI strain to propoxur-in-oil. Differences from the experiments with the single age class include intra-strain variations in one strain that was unlike those in another strain. For example, in one field strain a DT_{50} response to the oil blank occurred within the 2 hour period among all age classes. A second field strain strain differed in that dispersal induced by the oil blank was less than 50% for all age classes. Comparisons between age classes showed that the males and females were similar in their response and dispersed in a consistent manner. The mixed age class experiments suggested that the dispersal response of an individual cockroach might be changed by different population levels within the harborage and/or by different response patterns of other age class occupants of the same harborage.

Examination of the dose transfer process showed that the total air concentration of the toxicant in the oil solution was less than 1/10 of 1% of the actual amount of toxicant applied. It was also demonstrated by the use of ^{14}C that the propoxur vapors were vented away from Dish 2 of the test apparatus. The amount of additional dispersal induced by the complete formulation as compared to the blank formulation is most likely a function of the amount of toxicant that is vaporized into the air. The results of the dispersal studies seemed to indicate that the amount of toxicant vaporized is a function of solubility of the toxicant in the base solvent. Since propoxur is virtually insoluble in water, it would not be vaporized in the water based EC and WP formulations. However, propoxur is soluble in organic solvents like those found in the oil and aerosol formulations and a high rate of propoxur volatilization would be expected.

Total vapor concentration from the complete formulation also differed by formulation type. Surprisingly, the actual weight of volatiles in the air from the complete formulation in the test apparatus was higher in the water based formulations as compared to the oil-based formulations. It must be remembered that most of this vaporization was water, particularly after vapor equilibrium had been achieved. Another interesting aspect of vapor pulse formation was that those formulations that had homogeneous volatiles (oil and WP) rapidly reached vapor equilibrium in the test apparatus. In those formulations where the volatile component was a mixture of two or more materials (aerosol oil solvent:fluorocarbon propellant; EC xylene:water), the vapor pulse took several minutes to reach equilibrium, during which time a large amount (weight) of material was vaporized from the filter paper into the air.

Figure 30 is a flow diagram attempting to summarize some of the results found in the above studies of vapor-induced responses. The schematic is divided into two halves. The first half describes the dose transfer process best exemplified by the studies in Part IV. The combination and solubility of the toxicant with the inert ingredients are shown in a box marked "solubility". The formulations are divided into four boxes representing the four formulations tested. The pesticide application process is represented by two dotted lines. One line represents pesticide surface deposits. These surface deposits could be the result of a pesticide application or, in these studies, the application of 300 ul of 1% test formulation to a filter paper strip. The formation of the vapor pulse and its impact on the cockroach are represented by other dotted line originating from the formulations. The formation of the vapor pulse and its relative proportion of toxicant to inert ingredient are shown in the large box labeled "vapors". The modification of the vapor pulse, including condensation of the toxicant by environmental factors (outer rectangle) or the physical aspects of the harborage (inner rectangle), are shown as the vapor line penetrates the harborage and impinges upon the cockroach (central oval).

The second half of the diagram represents the response of the cockroach to the pesticide vapors. Modifications of the dispersal response are shown by a dotted line going through boxes labeled "strain", "age class" and "sex". For simplicity, the modifications caused by strain, sex or age class are reduced to changes in perception/avoidance (P/A) and/or intoxication/hyperactivity (I/H).

It must be kept in mind that exposure to vapors of either the toxicant and/or inert ingredient could cause sublethal effects regardless whether the cockroach actually dispersed or not. This is indicated by a line running from the P/A-I/H box to a box marked "sublethal". The box marked "lethal" indicates that pesticide vapors might indeed kill those individuals exposed to them. The actual manifestations of sublethal effects are best explained in the reviews of Moriarty (1969) and Haynes (1988). However, in this diagram, they are represented as boxes marked: "LT" (latent toxicity), "RP" (reproductive potential), "EI" (enzyme induction), and "B" (behavior). The "choice" of dispersal or non-dispersal is indicated with the non-dispersed cockroach remaining in the original harborage. The flow diagram for the dispersed cockroach, however, can be drawn a little further. The possibility of a dispersed cockroach crossing the surface deposit from the current or a past pesticide application is shown with the addition of further sublethal effects if it survives. The line is then extended off the chart to indicate the questionable future of a dispersed cockroach.

The persistence of the German cockroach as a pest species is a tribute to its flexibility in response to man made selective pressures of cockroach control programs. That there are differences in response from strains from different localities with different exposure histories and from different environmental arenas is not all that surprising. What the differences in dispersal response suggest, however, is that techniques that work in one area might not work in another. Thus it can be concluded that effective cockroach control programs probably will have to be tailored made for each area.

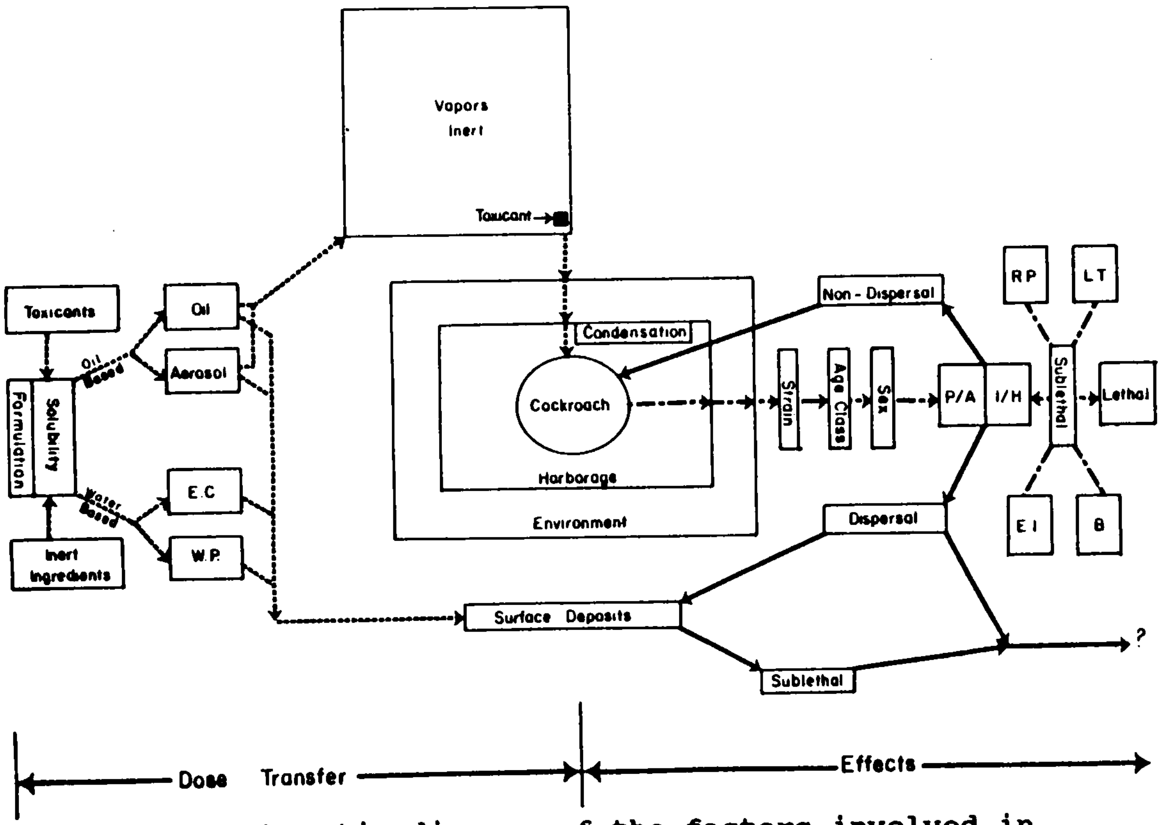

Figure 30. Schematic diagram of the factors involved in dose transfer of vapors and cockroach vapor-induced dispersal. P/A-perception avoidance; I/H-intoxication hyperactivity; RP-reproductive potential; LT-latent toxicity; EI-enzyme induction; B-behavior; EC-emulsifiable concentrate; WP-wettable powder.

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