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Title: CONTROLLED RELEASE FUMIGATION OF THE GREATER WAX MOTH, GALLERIA MELLONELLA (L.), A PEST OF STORED HONEYCOMB

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Examination and quantification of the controlled release behavior of ethylene dibromide (EDB) and paradichlorobenzene (PDB) in the polyethylene packet delivery system utilized by St. Clair et al. (1974) was undertaken. Effectiveness of these formulations against late instar larvae of the greater wax moth, Galleria mellonella (L.), under experimental conditions was determined.

Results showed that both EDB and PDB can be successfully formulated in a controlled release delivery system. Release rates are predictable over a range of temperatures. Control packets may be designed for toxicant emission over a specified time period. Various mixtures of EDB and PDB, with and without additional solvents, were examined in the controlled release delivery system and show potential for immediate wax moth control followed by long term protection. EDB controlled release packets showed 100% control of wax moth larvae in 48 hours under experimental conditions at temperatures of 26.6° and 32.2°C. PDB controlled release packets showed 13.3% and 17.2% control of wax moth larvae in 96 hours under the same conditions and temperatures.

A general discussion of the controlled release delivery system, and the history of the greater wax moth and its control is presented.

# Controlled Release Fumigation of the Greater Wax Moth, Galleria mellonella (L.), a Pest of Stored Honeycomb

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## INTRODUCTION

The greater wax moth, Galleria mellonella (L.) caused an estimated $4 million loss to beekeepers in the United States in 1976 (Williams, 1976). Damage is greatest in the southern United States where the moderate climate allows the wax moth to develop continuously throughout the year (Whitcomb, 1942; Smith, 1960; Oertel, 1969). The economic impact of these losses is felt through out the industry, however, since the northern United States and Canada rely heavily upon southern producers of young queens and packaged bees. Taking into account 4.2 million colonies in the U.S. in 1975 (USITC, 1976), that amounts to $0.95 per colony average cost for wax moth damage. Monies spent for control measures are not included.

It is the larva of the greater wax moth not the winged adult, which actually damages the honeycomb. The larva burrows through the combs, consuming the wax, pollen, cocoons and impurities present, and leaves behind a trail of debris and silken webbed tunnels (Paddock, 1930; Rybicki, 1960). Moderate infestations seriously weaken the structure of the combs and require energy consuming repairs by the house bees. Heavy infestations may remove all traces of comb, leaving only the wood and wires of bare frames exposed. Destroyed comb must be replaced by the beekeeper at an average cost of $1.40 per standard Langstroth frame (Williams, 1976). This includes the cost of foundation and salvage/assembly labor, as well as the ca. 1.5 pounds of honey metabolized by the bees while building each new comb (Whitcomb, 1946).

Most wax moth damage occurs while honeycombs are in storage. However, field infestations do occur in hives under stress from starvation, extended queeenlessness, disease, or pesticide damage (Townsend et al., 1965). In the southern United States, infestations in outyards which usually receive only ca. 10 inspections per year are particularly serious (Williams, 1976). Arizona beekeepers suffered a severe setback in 1967 when approximately 50,000 colonies were killed or damaged by pesticides applied for control of the pink bollworm, Pectinophora gossypiella Saunders. Over 1 million combs were subsequently destroyed by wax moth larvae which infested the dead and weakened colonies (Williams, 1976).

Extracted honeycombs must be protected from wax moth infestation prior to and during storage periods. To protect stored combs, it is necessary to destroy existing stages of the wax moth and prevent reinfestation. Chemical fumigation and temperature extremes are commonly used to destroy active stages of the wax moth (Monroe, 1961; Wilson, 1965). Storage of combs in moth-free, unheated, well-lighted, and well-ventilated facilities is presently the only preventative against reinfestation (Whitcomb, 1936).

Ethylene dibromide (EDB; $C_2H_4Br_2$) and paradichlorobenzene (PDB; $C_6H_4Cl_2$) are the fumigants commonly used for wax moth control (USDA, 1972). EDB is popular with commercial operators and is administered as a single application when combs are placed in storage. Except for reapplication, there is no residual protection from wax moth reinfestation with this method. EDB is a noxious chemical with a relatively high mammalian toxicity (acute $LD_{50}$ = 146 mg/kg, male adult rats; Von Oetingen, 1955; Spencer, 1968). It also can cause severe skin burns. Treatment with EDB consists of sprinkling several tablespoons of EDB onto an absorbant material lying in the top of a sealed stack of supers. A cover is added and fumigation continued for at least 24 hr. Commercial operators often

fumigate an entire airtight room of unsealed supers using several pounds of fumigant (USDA, 1972). Supers are then transferred to storage facilities. EDB is effective against all stages of the wax moth.

PDB is popular with small operators and hobbyists due to its availability, ease of application, and long-term protection. It has a lower mammalian toxicity ($LD_{50}$ = 500-5000 mg/kg; Von Oetingen, 1955; Spencer, 1968) and is not as irritating as EDB. Treatment with PDB resembles that for EDB. The fumigant is placed in the top of a stack of sealed supers. However, fumigation with PDB lasts for several months and thus the supers are treated directly in the storage facility. PDB is not effective on the egg stage of the wax moth.

Temperature extremes, i.e. intense heat or cold, are effective in destroying the greater wax moth in stored honeycombs. The need for large walk-in freezers and the obvious dangers associated with over-heating combs have prevented these methods from becoming widely practiced control techniques. In heat treatment, extracted supers are exposed to temperatures of 46°-54°C for 40-80 minutes (Pauli, 1932; USDA, 1972). Cold treatment requires 2-4.5 hr exposure at -18° to -7°C (USDA, 1972). Both treatments are effective in destroying all stages of the greater wax moth. Wax moth populations in the northern United States ordinarily remain small due to naturally occurring, subzero winter conditions.

There are a number of naturally occurring biological agents associated with the greater wax moth, none of which shows promise as a commercially useful control technique. For example, the predacious red fire ant, Solenopsis invicta Buren, and the braconid wasp, Apanteles galleria Wilkenson (Edwards et al., 1969), are known to attack the larvae and/or the pupae of the greater wax moth (Williams, 1976). At the microbial level, Bacillus thuringiensis Berliner (Johanson, 1968; Ali et al., 1973a, b; Burges, 1976a, b), an

entomophilus bacterium; and two viruses, a nuclear-polyhedrosis type (Gershenzon, 1957) and nonoccluded type (Lavie et al., 1965; Bergoin et al., 1968), are known to infect larvae of the wax moth.

Recently, the development of controlled release pesticide systems suggests some possible solutions to the problems of wax moth control. e.g., (1) long term protection against infestation, and (2) reduced operator exposure to noxious and potentially toxic insecticides.

Controlled release systems have been in use for nearly three decades in mosquito abatement programs and veterinary applications. As early as 1949 (Raley and Davis), DDT-Lindane impregnated briquettes were used against mosquito larvae, some with effective release periods of up to five years. Closer control of release rates caused researchers to look at organic polymers as possible substrates for controlled release systems. During the 1960's, Monontan wax, polyvinyl chloride (PVC), polyurethane foam, polyamide and rubber substrates were examined for effectiveness and subsequently utilized against various species of Culex and Anopheles mosquitoes (Knapp, 1977). Success in mosquito control began a new era in insect control in veterinary and medical entomology.

Dichlorvos (Vapona)-PVC (polyvinyl chloride) systems were developed for a multitude of uses. Flea control systems on dogs (Kibble, 1958) and cats (Fox et al., 1969) with impregnated plastic collars have been proven safe and efficient. Horse flies (Knapp, 1964) and horn flies (Harvey and Brethour, 1970) are effectively controlled with Vapona impregnated strips. Control of internal parasites in dogs, horses, and swine has also been accomplished with Dichlorvos-PVC formulations (Drudge et al., 1972; Beer et al., 1973; Hass, 1973; Hass and Young, 1973). More recently, pyrethrins, insect growth regulators, and sex attractants have been tested (Knapp, 1977; Miller et al., 1977).

A slightly different approach to formulation was taken by Toba et al. (1969) for control of cabbage loopers, and St. Clair et al. (1972) for control of wasps. Rather than impregnation, the chemical attractant was sealed inside a polyethylene packet, where the nature of the plastic, the surface area, thickness, and temperature controlled the rate of release. In 1975, it was suggested that a similar system might be applicable for controlling the greater wax moth in stored honeycombs. Preliminary unpublished work by St. Clair et al. (1974) and Burgett (1975) suggested that the two common wax moth fumigants, EDB and PDB, were indeed compatible with the polyethylene packet formulation; and that fumigant formulations of this type were toxic to wax moth larvae.

The present work, which is based upon these preliminary observations, has as its objective:

1. Examination and quantification of controlled release behavior of EDB and PDB in the delivery system devised by St. Clair et al. in 1974.
2. Determination through mortality studies of the effectiveness of these insecticides when delivered to live wax moth larvae via controlled release packages.

# LITERATURE REVIEW

Larvae of Galleria mellonella L. have been a nuisance to beekeepers since ancient times. The bee moth (Linnaeus: Tinea mellonella) was mentioned by Aristotle and Virgil as a formidable enemy of honey bees (Langstroth, 1859). In the seventeenth century, Swammerdam distinguished two species of "bee wolf," one much larger than the other (Langstroth, 1859). The smaller of the two may have been the lesser wax moth, Achroia grisella F. The wax moth was probably introduced into Europe from Asia (Morse and Liago, 1969), and from there it is likely that the wax moth, like the honey bee, was introduced to the Americas during early European settlement (Langstroth, 1859). It presently enjoys a worldwide distribution (Paddock, 1930).

Recently, the greater wax moth has been found parasitizing other species of Apis. Morse and Liago (1969) noticed wax moths infesting combs of A. dorsata F. in the Phillipines. Arkatanakul (1977) reported heavy infestations in combs of the dwarf bee A. florea F. in Thailand. It has been suggested that the exposed combs of A. dorsata and A. florea may be more attractive and/or vulnerable than the protected combs of A. cerana indica F. and A. mellifera L. (Akaratanakul, 1977). Extended queenlessness or disease is a contributing factor associated with the larger wax moth populations in nearly all cases. Oertel (1963) reported wax moths developing on bumble bee cells.

Nielsen and Brister (1977) described the behavior of the adult moth in an undisturbed environment. It appears that adult moths can emerge in, leave, and re-enter active colonies of bees without being molested. Eclosion generally occurs in the evening and adult moths leave the hive after dark, spending the daylight hours in nearby trees.

Mating can occur within the hive as well as outside. Adult male moths rarely return to the hive, but mated females return to a hive within 48 hr after eclosion to commence egg laying. The moth's movements are generally conducted during the twilight and dark of night.

It was previously thought that strong colonies prevented moths from infesting a hive (Paddock, 1918, 1930; Milum, 1940; Whitcomb, 1965) by expelling both the adults and larvae from the hive as soon as they were encountered. Nielsen and Brister (1977) found female moths capable of repeatedly entering even the strongest colonies, and many seem to prefer stronger colonies over weaker ones. Oviposition sites within the colonies include pollen cells, partially capped honey cells, empty cells, and at the juncture of top and end bars of the frames (Sichel, 1955). Moths usually exit from the hives just before dawn and return to the trees until nightfall. It appears that most colonies, particularly in the southern United States, normally support light infestations of wax moths. That these infestations remain suppressed is attributed to a healthy population of bees which in turn reflects the activities of a vigorous queen.

Numerous control methods have been suggested to combat the greater wax moth. The solution most often cited is the maintenance of strong colonies as a deterrent to infestation. In the 1800's, sulphuring of surplus combs and supers was a common practice. This involved immersing the combs in the "blueish fumes of burning brimstone" (sulphur dioxide). While quite effective on larvae and adults, sulphuring did not kill eggs, and reinfestation was common (Langstroth, 1919). Another common fumigant was carbon bisulphide; an extremely flammable (explosive) gas, which is also ineffective against eggs of the greater wax moth (Langstroth, 1919; Krebs, 1939). In northern climates, winter freezing was, and still is, its own

solution to wax moth infestation. All stages of the moth are susceptible to prolonged freezing (USDA, 1972).

In the twentieth century, fumigation has remained the most popular control technique. Toxicological advances have broadened the spectrum of chemicals available for control, most of which are effective against all stages of the wax moth, including eggs. Table 1 lists many of the compounds that have been commercially used and/or suggested for use against the greater wax moth. Unfortunately, of the compounds listed, many are extremely hazardous to the operator and are very flammable, if not explosive, once volatilized. Furthermore, the Environmental Protection Agency has restricted the use of any pesticide which could conceivably reach the consumer via residues left in honey (USDA, 1969). Of those listed, the two compounds in widespread use today are ethylene dibromide, an alkyl halide, and paradichlorobenzene, an aryl halide.

## Toxicology and Metabolism of EDB and PDB

Neifert et al. (1925) reported the insecticidal properties of EDB. It is about equal in toxicity to methyl bromide and carbon tetrachloride. Insects subjected to EDB treatment become moribund and remain so until death (Brown, 1951). Galleria mellonella larvae subjected to EDB lose locomotion. Musculature and internal organs become totally relaxed, so that the body is easily collapsed. At death, tissues appear to be disintegrating so that upon examination, the body resembles a sack full of spongy liquid. At this point, the larva begins to turn from a cream color to a dark brown hue. A putrid odor accompanies the change. The mode of action of EDB is not clear. Winteringham and Hellyer (1954) reported a slow depletion of phosphoglycerate (PGA) and a corresponding increase of free

Table 1. Fumigants Used for Wax Moth Control.

| Fumigant | Reference | Comments |
|---|---|---|
| Sulphur dioxide | Root, 1918; Langstroth, 1919; Borchert, 1938 | not proven effective |
| Camphor tetrachloroethane | Langstroth, 1919; Borchert, 1938 | not proven effective |
| Chlorosol--a 3:1 mix of ethylene dichloride & carbon tetrachloride | Krebs, 1939; Townsend, 1940 | not proven effective, stratifies, expensive |
| Monochloroethane Hexachloroethane | Borchert, 1938 | not proven effective |
| Carbon disulphide | Phillips, 1917 Langstroth, 1919 | ineffective on eggs, explosive/flammable |
| Calcium cyanide | Borchert, 1938; Root, 1945; Hive & the Honey Bee, 1949 | ineffective on eggs, high mammalian toxicity |
| Methyl bromide | Krebs, 1939; Townsend, 1940; Roberts et al., 1958; McKinley, 1963, Smirnov & Chanychev, 1965; Nazarov, 1969; Guy, 1970 | very effective on all stages, high mammalian toxicity |
| Proxate--a mix of 7% methyl bromide & 93% carbon dioxide | Townsend, 1940 | as effective as methyl bromide but more expensive |
| Paradichlorobenzene | Borchert, 1938; Becker, 1957; Vorwohl, 1965 | ineffective on eggs, good long term protection |
| Carbon dioxide | Cantwell et al., 1972a, b | very effective on all stages, difficult to administer |
| Ethylene dibromide | Krebs, 1957; Cale, 1958; Lehnert & Shimanuki, 1967 | very effective on all stages, high mammalian toxicity |
| Ethylene oxide | Michael, 1964; Smirnov & Chanychev, 1965; Lehnert & Shimanuki, 1968 | very effective on all stages, highly flammable |

phosphate ions by methyl bromide and EDB in Musca domestica. This suggests a common inhibition of triose phosphate dehydrogenase (Winteringham and Hellyer, 1954), which is an active enzyme in the glycolytic pathway of insects (Chapman, 1975). Disruption of glycolytic and respiratory enzymes would eventually exhaust the supply of high energy compounds, e.g. ATP, necessary for cellular maintenance and muscular activity. Further study is needed to determine whether EDB blocks the enzymes required for oxidation of triose phosphates to diphosphoglycerate in glycosis, and also the oxidation of $NADH_2$ to NAD in the extramitachondrial cytoplasm.

Metabolism of EDB in the insect body may follow that of animal metabolism. Aliphatic halogenated hydrocarbons generally undergo hydrolytic dehalogenation resulting in oxidized hydrocarbon and free halogen ions. For EDB, the result is 2-bromoethanol and free bromine (Matsumura, 1975).

PDB was mentioned by Moore (1916) as an insecticide. Of the three possible isomers, the para-configuration is the most insecticidal. This configuration has a dipole moment of nearly zero and a minimum total surface energy (Brown, 1951). Characteristics such as these make the molecule lipophillic and difficult to degrade, due to the lack of reaction sites. PDB is classified as a narcotic fumigant (Brown, 1951). Symptoms of narcotic fumigant poisoning are (1) excitation, (2) paralysis, and (3) death (Matsumura, 1975). In wax moth larvae, these three symptoms are present and also a convulsion stage (common to nerve poisons) prior to paralysis. Munson and Yeager (1945) reported symptoms of PDB poisoning as indistinguishable from DDT poisoning in roaches. DDT has all four stages present in its symptomatology.

The specific insecticidal action of PDB within the insect body has been likened to DDT (Brown, 1951). The exact mode of action of DDT is unknown. However, extensive studies have shown that the

nervous system is the site of action, and that repetitive discharge in impulse patterns is the result. Whether alteration of membrane permeability, or inhibition of nerve ATP-ases are at cause is still a matter of conjecture (Matsumura, 1975). For the insect, death by exhaustion has been suggested.

Metabolism of higher chlorinated benzenes in the animal body involves oxidation to monohydric phenols. Paradichlorobenzene is metabolized to phenols. The ortho- and meta- configurations are metabolized into mercapturic acids, as are monohalogen benzenes (Matsumura, 1975).

## Controlled Release Fumigation--A Model System

Toba et al. (1969) first used polyethylene packets for dispensing the sex pheromone of the cabbage looper, Trichoplusia ni Hubner. In that study, the effects of polyethylene film thickness were noted. However, release rates of the pheromone were not quantified. Unpublished work by St. Clair et al. (1972) suggested that the same dispensing system could be used successfully for trapping adult yellow jacket wasps (Vespula).

For controlled delivery systems, polymeric membranes are attractice by virtue of the dissolution and diffusion of molecules through the polymer. The rate at which this transport across the membrane occurs depends upon several factors:

(1) Thickness of the film. In diffusion theory (Crank, 1956) the rate of transport of an ideal substance is inversely proportional to the thickness of the polymer film. St. Clair et al. (1971) found this to be somewhat variable. This discrepancy is due in part to variability in commercial film composition and to non-ideal interactions between the permeating molecules and

the polymer matrix. The actual effect of the film thickness should be experimentally determined for each polymer system.

(2) Surface area. The release rate of the ideal substance is directly proportional to the surface area of exposed film. St. Clair et al. (1971) verified this.

(3) Chemical potential gradient across the membrane (Chandrasekaren, 1977). If an infinite sink prevails outside the membrane, then the release rate will depend upon chemical activity at the membrane, and will remain constant.

(4) Solute diffusion coefficient and solubility (Chandrasekaren, 1977). Solubility is a thermodynamic property governed by the chemical composition of the polymer and the chemical. Diffusivity is determined by the size and shape of the chemical and the matrix mobility of the polymer.

(5) Temperature. The most important factor affecting release rate (assuming an infinite sink) is ambient temperature. For heptyl and octyl buterates used in the wasp attractant system, release rate is approximately doubled for every 7°C rise in temperature in the range of 15-30°C (St. Clair et al., 1972). For wax moth control, this may be of great advantage since pesticide release may parallel increased larval activity as the temperature rises.

Larval development and activity are governed primarily by ambient temperature and the quality and quantity of the food supply. When reared on a natural food source, i.e. brood comb, temperature alone becomes the growth limiting factor. The temperatures most suitable for growth are between 29-35°C. while no activity is noted below 7°C. At ideal temperatures, the duration of egg, larval, and pupal stages are ca. 5, 28, and 8 days respectively (Flechtmann, 1964; USDA, 1972). At lower temperatures, e.g. 10-15°C, the eggs may require up to a month to develop; the larvae nearly five months; and the pupae two months. Rapid development at higher temperatures

may result in several generations per season in subtropical habitats and development can continue year round in the southern United States. Temperature regulated pesticide packages have the potential for releasing an amount of toxicant which corresponds to temperature controlled larval activity.

Duration of release is controlled primarily by the quantity of chemical enclosed within the system. Release rates will be constant until the atmosphere within the system becomes under-saturated (St. Clair et al., 1971).

The "model system" used in this study is patterned after the yellow jacket dispensing system used by St. Clair et al. (1972). That dispenser system can be described as "a volatile chemical sorbed on a fibreboard substrate which is enclosed within an envelop of thin plastic film which is permeable to the volatile chemical" (St. Clair et al., 1971). The rate of chemical permeation out of the package is steady and quantifiable. In the original work, these controlled release packets were loaded with specific attractants for Vespula wasps (heptyl and octyl butyrates). The packets were tested in conjunction with specially designed insect traps in the vicinity of Corvallis, Oregon. Results showed that controlled release packets could successfully attract wasps to the insect traps.

Preliminary unpublished work by Burgett (1975) using EDB on honey combs infested with G. mellonella larvae indicates that the controlled release delivery system could also be an effective technique for wax moth control. Such a controlled release fumigant package could be a significant pest management tool for many beekeepers. The continuous release nature of the packet could alleviate a potentially wasteful and hazardous beekeeping task.

# MATERIALS AND METHODS

## Polyethylene Test Packets

Plastic tubing and a heat sealing apparatus were obtained from the Harwil Company of Santa Monica, California. Layflat polyethylene tubing in 4 in. width and two differing thicknesses, 4 and 6 mils (actual measured thicknesses were 3.5 and 5.5 mils) were sealed with a T-bar Portable Heat Sealer (model T-7) to form the test packets. Inside dimensions of each test packet measured 8.89 by 10.16 cm, yielding a surface area of 180.64 $cm^2$. Larger "field size" packets, 35.56 by 10.16 cm with a surface area of 722.58 $cm^2$ were also constructed. Test packets were 1/4 the size of field packets in surface area. Dimensions for field packets were arbitrarily chosen with the assumption that all 10 frames of a standard Langstroth super should be covered by the packet.

Being heavier than air, vapors of EDB and PDB will settle to the bottom of a stack of supers. In time, the level of gas will rise, filling the entire stack. It was assumed that larval activity in the outermost frames of the upper supers would be disrupted if toxic gases were passing over these frames. A smaller packet would deliver adequate fumigation in time, but during the initial buildup of toxicant, some damage might be sustained in the uncovered frames of the upper supers. Field packets were constructed to lay across the top bars of all frames in a standard Langstroth super. The width of the Langstroth super is 37.47 cm and the field packets are 35.56 cm in length.

New test packets were constructed for every trial. First, 12.70 cm pieces of Layflat plastic tubing were cut. It was necessary to include a 3.81 cm allowance in length for overlap and heat shrinkage

in order to produce a packet with proper inner dimensions. Second, one end of the tube was sealed, removing 1.91 cm from the initial tube length. Third, the test compound was introduced either as free crystals or as a liquid sorbed onto a block of pressed fibreboard or paper towel. Fourth, the remaining open end of the tube was sealed, cutting off an additional 1.91 cm in length and producing a sealed packet with inner dimensions of 8.89 by 10.16 cm. Care was taken in heat sealing to insure that no bubbles or weak spots were included in the seal. Thin areas in either seal could act as over active diffusion sites, resulting in erroneous release rates. Additional care was exercised during sealing not to compress the packet. Initial trials revealed that diffusion is severely hampered when the inner walls of the packet were touching one another.

Once formed, test packets were normally used immediately. However, it was found that packets could be kept frozen for several days prior to use without adverse effects. A thawing and equilibration period of 45 min was allowed prior to use.

## Test Compounds

Ethylene dibromide (EDB, 1, 2, dibromoethane). Reagent grade. Mallinckrodt Chemicals, Saint Louis, Missouri.

p-Dichlorobenzene (PDB). Reagent grade. J. T. Baker Chemical Company, Phillipsberg, New Jersey.

Dichloromethane (Methylene chloride). Reagent grade. Mallinckrodt Chemicals, Saint Louis, Missouri.

Hexane, redistilled. Chemistry Department, Oregon State University, Corvallis.

## Insects

Live larvae of the greater wax moth, Galleria mellonella (Pyralidae) were obtained from Carolina Biological Supply Company, Burlington, North Carolina.

The larvae undergo eight instars (Smith, 1965), which appear morphologically identical except for size. The larvae may attain a length of 25-30 mm prior to pupation, and except for a few sclerotized structures, are practically devoid of any pigmentation. The heavily sclerotized head and body microspines (Peterson, 1948) are the only pigmented structures.

Late instar larvae were used exclusively in all tests. Work by Morrison and Perron (1963) and preliminary testing indicated that early instar larvae are more susceptible to pesticides than late instar larvae. Testing late instars, therefore, "guaranteed" control of all larval instars.

Larvae were shipped in jars containing a nutrient medium, which also served as a physical substrate. Upon arrival, larvae and medium were distributed in gallon jars of fresh medium and held at room temperature for several days prior to use (Beck, 1960; Allegret, 1963, 1964; Marston et al., 1973). The medium used contained the following ingredients:

| | |
|---|---|
| wheat bran | 260 g |
| Torula Type S yeast | 65 g |
| wheat flour | 162 g |
| cornmeal | 162 g |
| glycerine | 175 g |
| water | 175 g |

The proportions of glycerine and water were modified from the original formula given by Marston et al. (ARS, 1975), to produce a mixture with a relative humidity of 80% (Braun and Braun, 1958).

## Release Rate Determination

Release rates for the test compounds were determined over a range of temperature levels, 10-32.2°C, and in two differing thicknesses of polyethylene tubing, 4 and 6 mils. Samples were hung in Percival controlled environment chambers (± 1°C) and weight losses determined gravimetrically with a Metler H64 analytical balance. Three test packets were used for each test and a minimum of five weighings made per packet. With each subsequent weighing, a release rate was computed by dividing the weight loss by the elapsed time. A final averaged release rate, the mean of the averaged release rates for the three test samples in each temperature class, was calculated. Thus, a minimum of 15 observations contributed to each final release rate value. Testing was discontinued whenever test packet weight approached 1 g of empty packet weight. Early experiments revealed a rapid attenuation of release rate during the "runout" period; values, which if included, yielded erroneous averages.

## Gas Trapping and Analysis

A gas trap was constructed to collect the vapors emanating from a test packet containing a mixture of test compounds and solvent. Once collected, gas chromatographic analysis was used to identify the components of the vapor. The collection apparatus consisted of a sealed glass jar (3.5 liter capacity) containing the test packet, two liquid filled bubble traps packed in ice, an aquarium pump, and sufficient tubing to connect the lot in series (Figure 1). The pump and glass container were housed inside a controlled environment chamber, and the gas traps and ice packs placed immediately outside. Redistilled hexane was used as the trap solvent. Periodically, aliquots

Figure 1. Vapor collection apparatus.

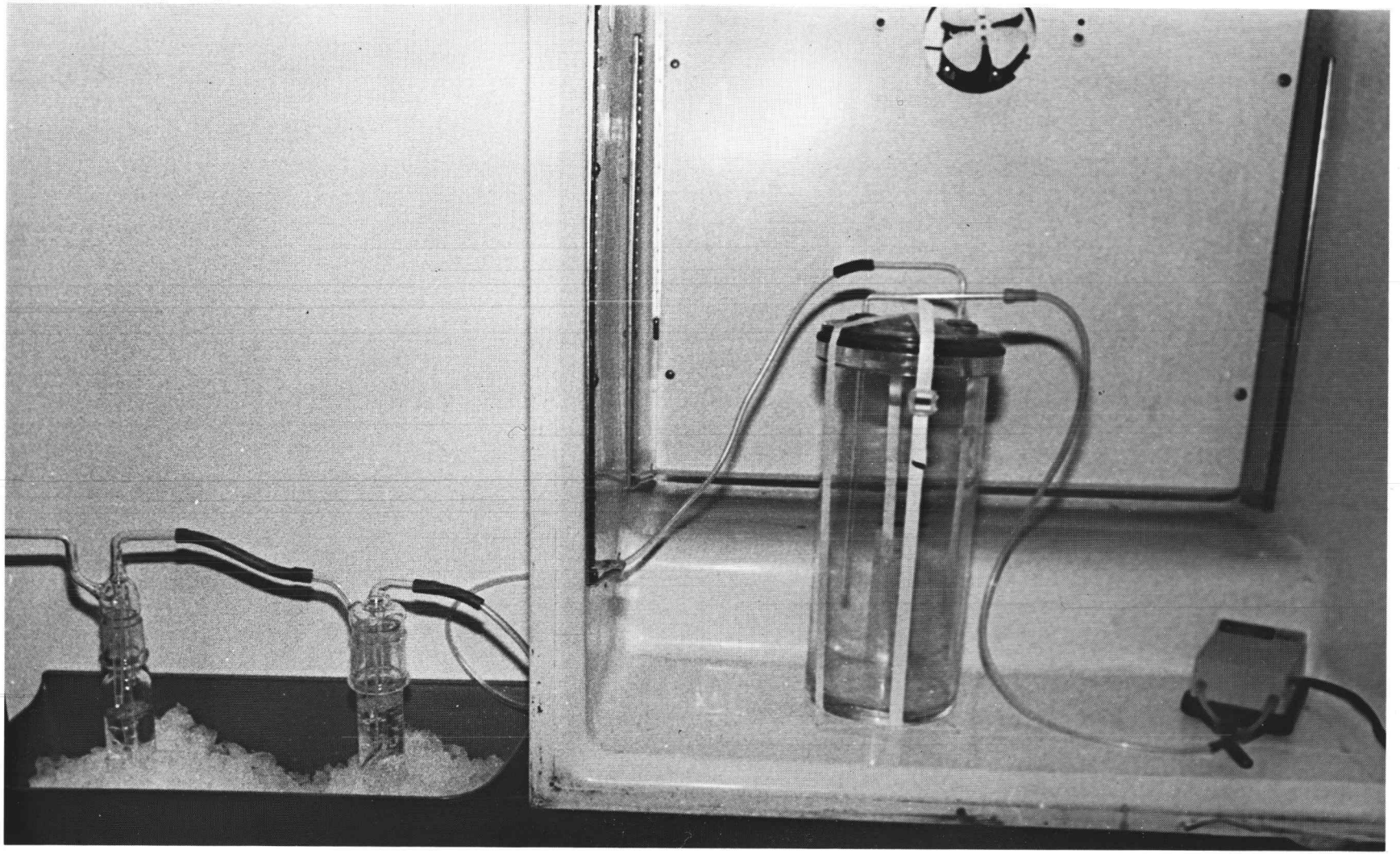


Figure 1

of each trap solvent were taken and the traps emptied, rinsed, and refilled with 100 ml of fresh hexane. Aliquots were refrigerated pending analysis. A Varian Aerograph (Series 2700) gas chromatograph with a 1.83 m by 3.2 mm glass column of 1.4:1 mixture (w/w) of 7% QF-1 and 7% DC11 coated on 100/120 mesh HP chromosorb W was used to identify compounds in the aliquot samples.

## Mortality Studies

Mortality studies were conducted in specially prepared frames inside a standard nuc box (inside dimensions were 46.36 by 19.05 by 25.4 cm) placed within a controlled environment chamber. An identical setup was run simultaneously for control comparison. Both nuc boxes had sealed entrances and flush fitting lids resulting in a relatively airtight test compartment. A glycerol-water humidifier was included in each box to maintain humidity at approximately 80% (Braun and Braun, 1958). Four numbered test frames were placed in each nuc. At specified times, i.e. 6, 12, 24, and 48 hr for EDB and 24, 48, and 72 hr for PDB, after the start of the experiment, a frame was randomly selected from each nuc box and the larvae contained therein examined for mortality. Lastly, each box contained a slow release packet placed on the top bars of the frames; the control packet was empty; the test packet held a pesticide. The amount of pesticide remaining in the test packet was measured each time a frame was removed.

Frames were constructed to hold nine circular cages, each containing five larvae and ca. 20 g of nutrient medium. Cages were made from two standard (Mason jar) screw caps with plastic screen in place of the metal sealing lid. Two of these caps, when placed together, open side inward, created a cage with two-way circulation

and a volume of 34.85 $cm^2$. The caps were taped together with duct tape, which was renewed periodically. Cages were stacked into each frame in tiers forming three depths with three cages per depth when the frame was hung vertically in a nuc box (Figure 2). Chicken wire secured the cages in place and prevented any shifting once the frame was moved to the nuc box. This arrangement of cages and frames created the opportunity to test each pesticide for exposure time and position or depth effect in the nuc box.

Ethylene dibromide in 6 mil test packets was tested over temperatures ranging from $10^{o}$ to $32.2^{o}C$. Paradichlorobenzene was tested in 4 mil test packets under identical conditions. Four mil test packets were used with PDB due to the extremely low insecticidal activity exhibited in preliminary tests with 6 mil packets.

At each specified time interval, a frame was removed from both test and control nucs. Cages were disassembled by row and inspected for dead larvae. Remaining live larvae were placed in fresh medium, held for 72 hr, reinspected, and disposed of. Larvae of different time intervals were kept isolated from one another during the holding period, and not lumped in a common pool. Larvae dying during the holding period were later summed with the number of dead larvae found during the initial test inspection. Paralyzed larvae were not considered dead even though eventual death may have occurred. Control larvae were treated in identical fashion.

During the course of the experiments, all the equipment in contact with pesticides was isolated from the control equipment. At the end of both EDB and PDB series, an additional 96 hr experiment was run in which nuc boxes, frames, and cages were switched in the controlled environment chambers to test for residual buildup of toxic chemicals in the test equipment. Empty slow release packets were included in each nuc box.

Figure 2. Larval holding cages used in mortality studies.

Figure 2

## Statistical Methods

The accumulated data were subjected to statistical analysis. An arcsine transformation was performed on mortality, which had been recorded in percentages, to stabilize the variances. Analysis of variance and multiple regression analysis were performed on both EDB and PDB mortality data. Final results are recorded in Appendices I and II.

# RESULTS AND DISCUSSION

## Release Rate Studies

### Test Packets (8.89 x 10.16 cm)-- EDB and PDB

Release rates for EDB and PDB through 4 and 6 mil polyethylene film packets were determined. Emission rates for dichloromethane, described elsewhere as a solvent in EDB/PDB mixtures, were also established (6 mil only). Samples were weighed periodically until the fumigants were nearly exhausted. Rate of emission was calculated for each sample at every time interval and a grand mean over 15 data points calculated. The results can be seen in Table 2, and are graphically displayed in Figures 3a and 3b.

The curve in Figure 4 illustrates the constant release rate which is the principal advantage of this system. Emission continues uniformly until the internal vapor phase concentration within the test packet drops below saturation, whereupon release rate rapidly attenuates. Release rate depends upon diffusion through the polyethylene film, which may be considered as a membrane between two adjacent compartments. The juxtaposition of two compartments having different concentrations of a soluble chemical, creates a favorable gradient for diffusion, from high to low concentration, until equilibrium is reached or the system is exhausted. The mathematical principles describing diffusion across membranes were derived by a German physiologist, Adolph Fick, and are known as Fick's laws of diffusion (Crank, 1956). Fick's first law states that the transport rate of a solute across a plane of unit area is directly proportional to, (1) the difference in concentrations in the two adjacent

Table 2. Experimental Release Rates for EDB, PDB, and Dichloromethane in Test Sized 4 and 6 mil Polyethylene Packets.

| Temperature (°C) | EDB (g/hr) | PDB (g/hr) |
|---|---|---|
| | 6 mil | |
| 10.0 | 0.061[a] | 0.007[a] |
| 12.8 | 0.057 | 0.008 |
| 15.5 | 0.093 | 0.012 |
| 18.3 | 0.099 | 0.018 |
| 21.1 | 0.130 | 0.059 |
| 23.9 | 0.201 | 0.072 |
| 26.6 | 0.242 | 0.076 |
| 29.4 | 0.327 | 0.095 |
| 32.2 | 0.490 | 0.142 |
| 35.0 | 0.583 | 0.172 |
| | 4 mil | |
| 10.0 | 0.168[a] | 0.014[a] |
| 15.5 | 0.403 | 0.038 |
| 21.1 | 0.574 | 0.060 |
| 26.6 | 0.834 | 0.141 |
| 32.2 | 1.556 | 0.253 |
| | 6 mil | |
| | Dichloromethane (g/hr) | |
| 10.0 | 0.231[a] | |
| 15.5 | 0.364 | |
| 21.1 | 0.625 | |
| 26.6 | 1.147 | |
| 32.2 | 1.429 | |

[a]Mean values of 15 observations over three replicates.

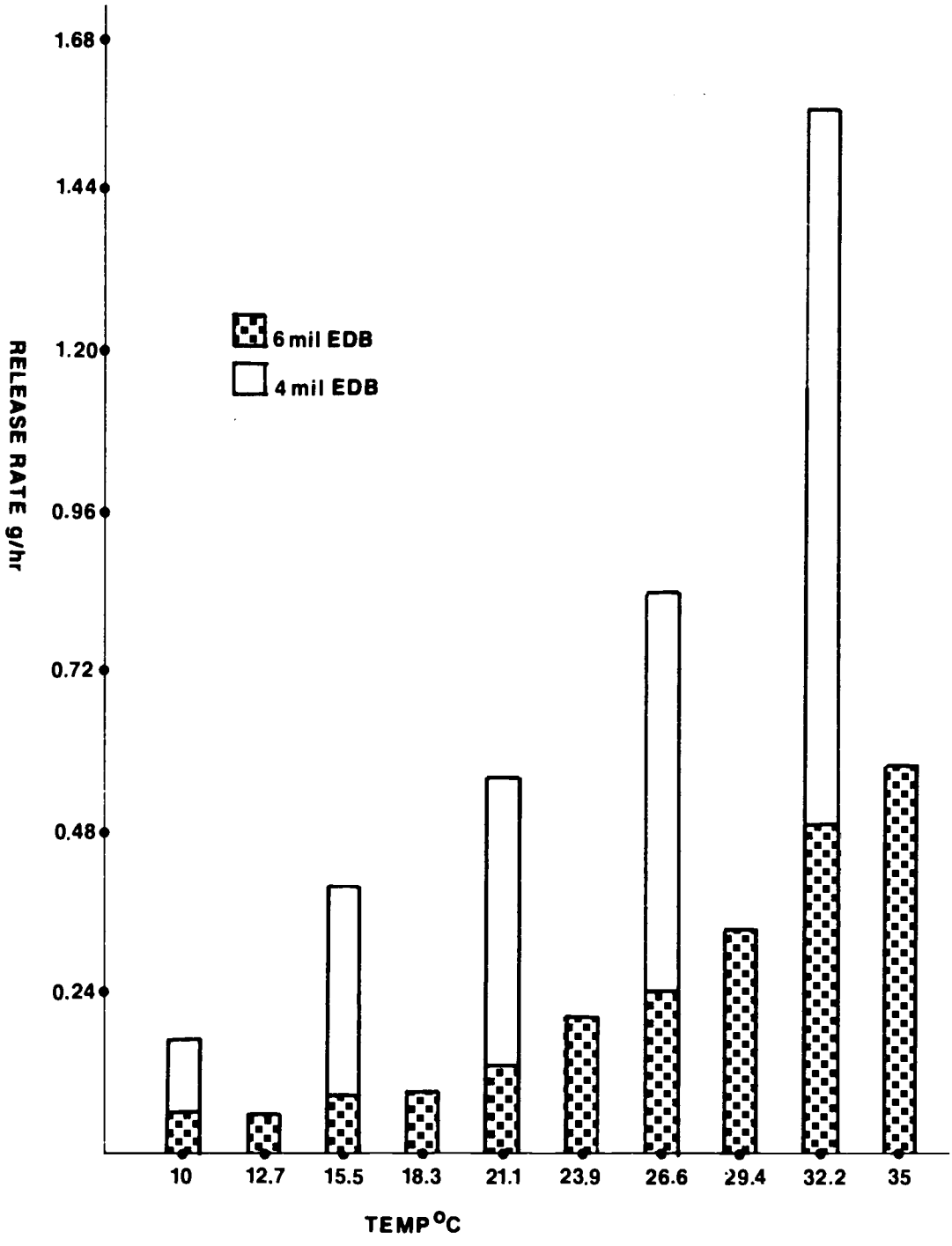



Figure 3a. Release rates of ethylene dibromide in 4 and 6 mil packets.

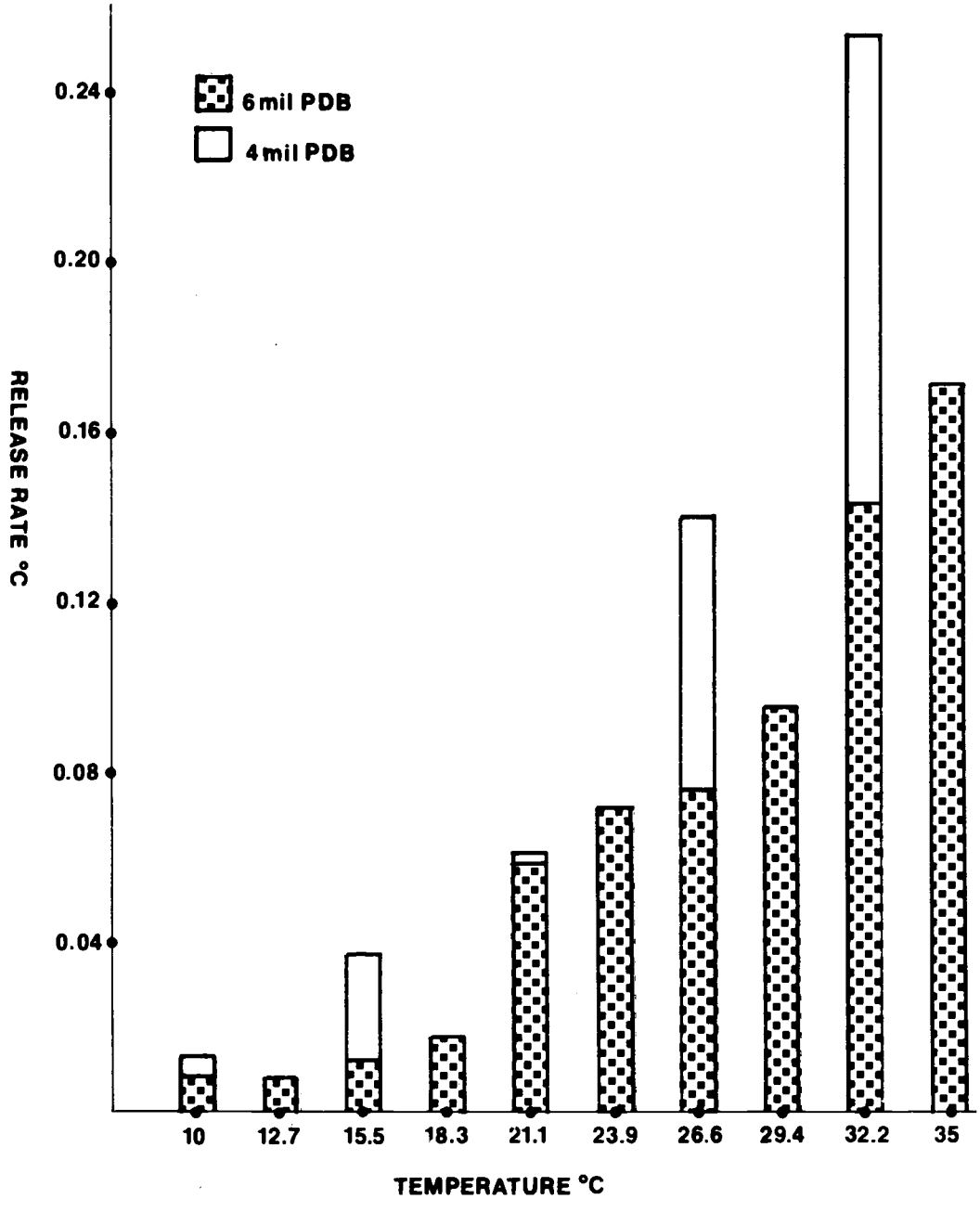



Figure 3b. Release rates of paradichlorobenzene in 4 and 6 mil packets.

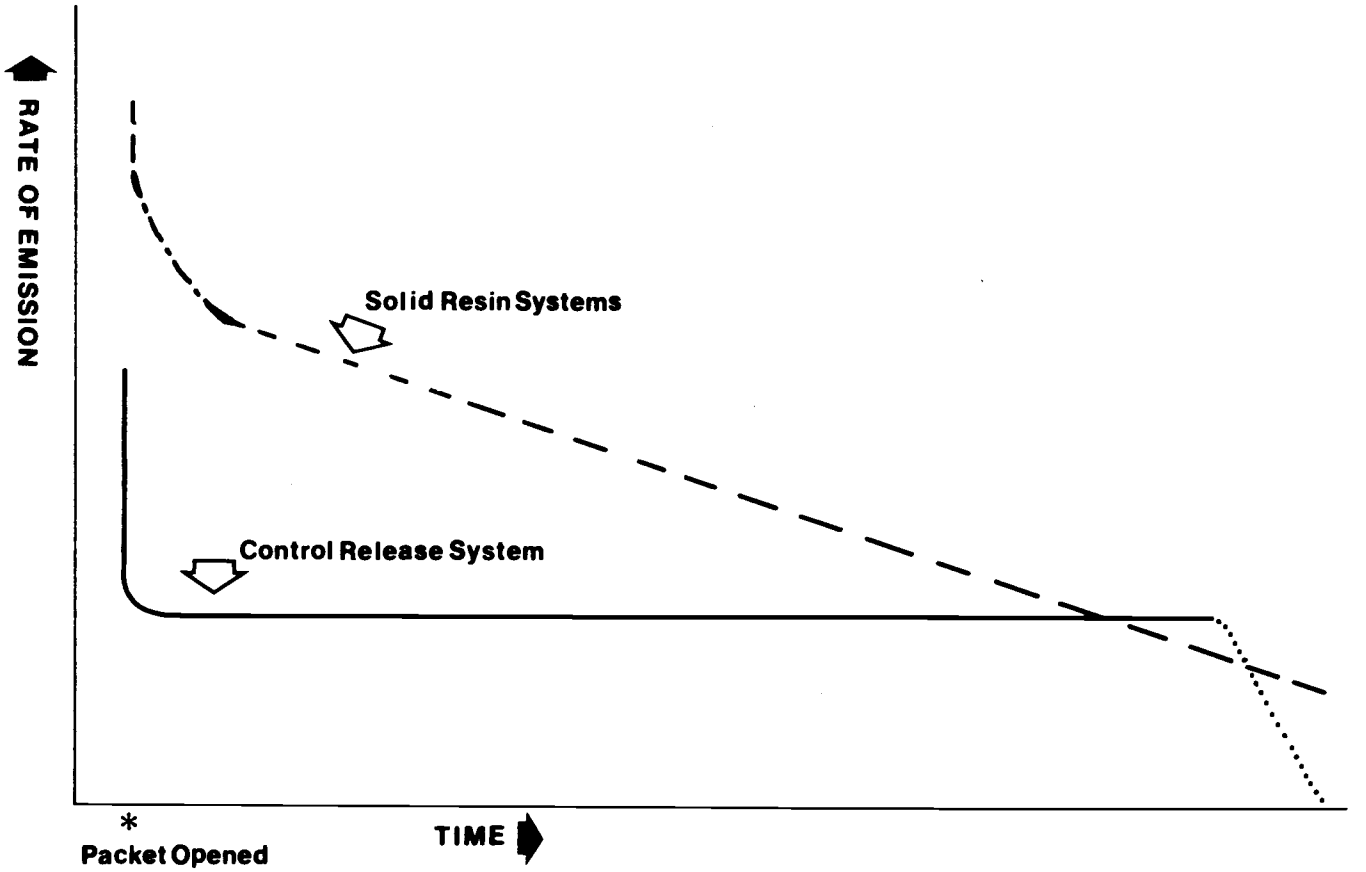



Figure 4. Uniform emission of the controlled release delivery system versus other systems (adapted from St. Clair et al., 1971).

compartments ($C_{inside} - C_{outside}$), and (2) the diffusion coefficient of the solute (D); and inversely proportional to the thickness of the membrane. This may be expressed as:

$$F = \frac{D(C_i - C_o)}{\ell}$$

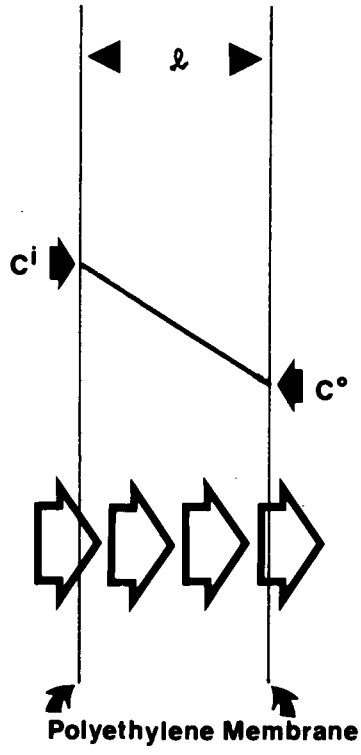
where F represents transport flux and $\ell$ is membrane thickness (Figure 5). If the internal vapor phase (donor compartment) is saturated, i.e. a constant source, and if the external vapor phase (receptor compartment) acts as an infinite sink, i.e. zero concentration, then the previous equation may be simplified to:

$$F_{max} = \frac{D\,C_i}{\ell}$$

The mean release rates in Table 2 are equivalent to $F_{max}$.

In the following pages, conditions will be described where release rates did not approximate $F_{max}$. When in the course of an experiment the magnitude of concentration difference in adjacent compartments decreased, e.g. receptor compartment other than zero concentration, a marked decrease in release rate occurred. Such conditions appear to have existed during packet testing, gas trapping, and mortality studies. Low release rates were experienced whenever airtight conditions prevailed during an extended test. Subnormal rates were particularly evident when testing in small enclosures such as a nuc box or gas trap. This "environmental" control of emission may ultimately have advantages in field application. If fumigant emission responds to external concentration inside a stack of stored hive bodies, then emission may effectively keep pace with leakage of fumigant out of the stack. Prolongation of control packet life would result.

Release rate of EDB and PDB as a function of temperature is graphically represented in Figures 6a and 6b. An exponential gain in




$$F = \frac{D(C^i - C^o)}{\ell}$$

F = Transport Flux
D = Diffusion Coefficient
C = Chemical Concentration
(at inside$^i$ & outside$^o$ of membrane)
$\ell$ = Membrane Thickness

Figure 5. Solution diffusion (adapted from Chandrasekaren, 1977).

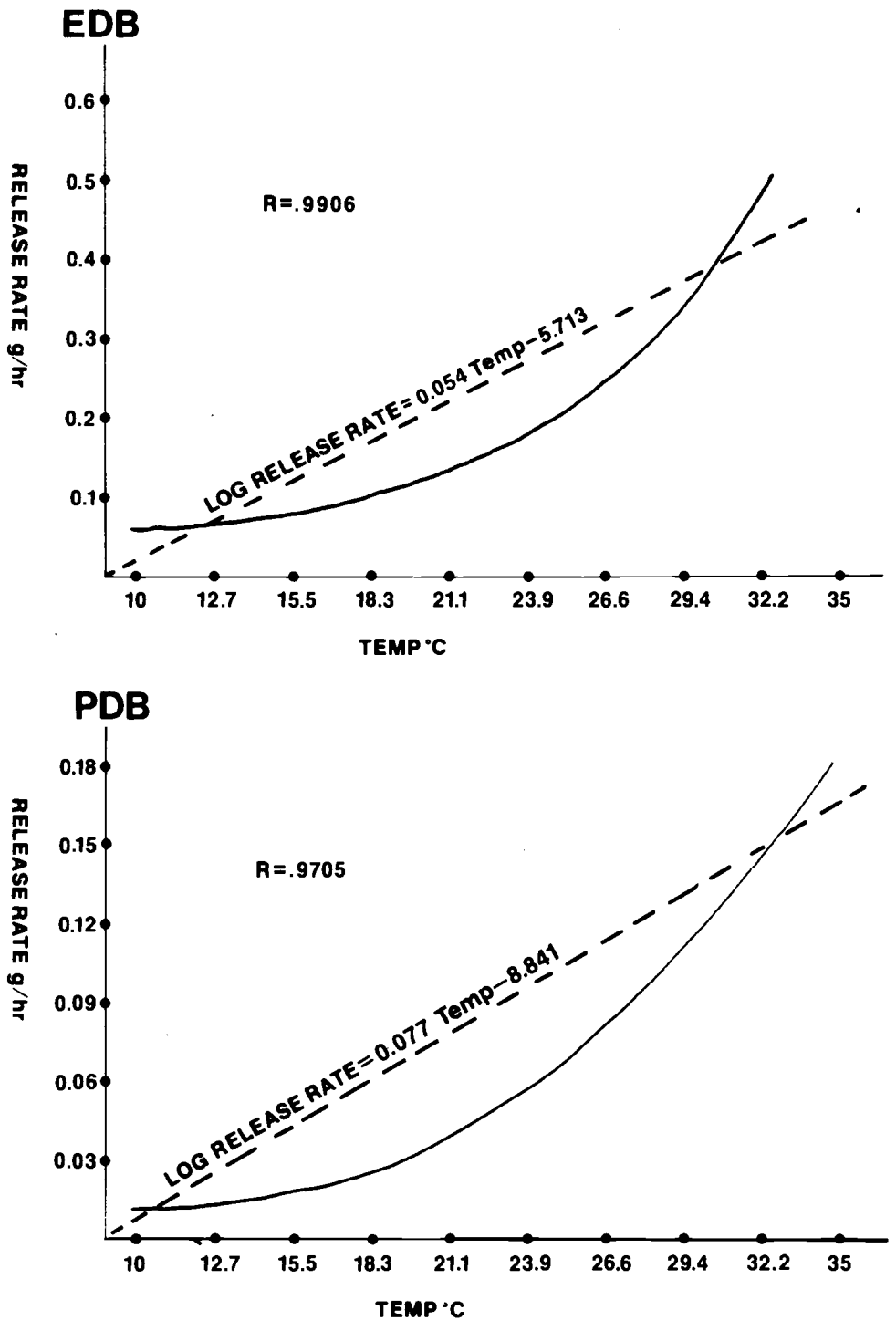



Figure 6a. The effect of temperature on release rate of EDB and PDB in 6 mil packets.

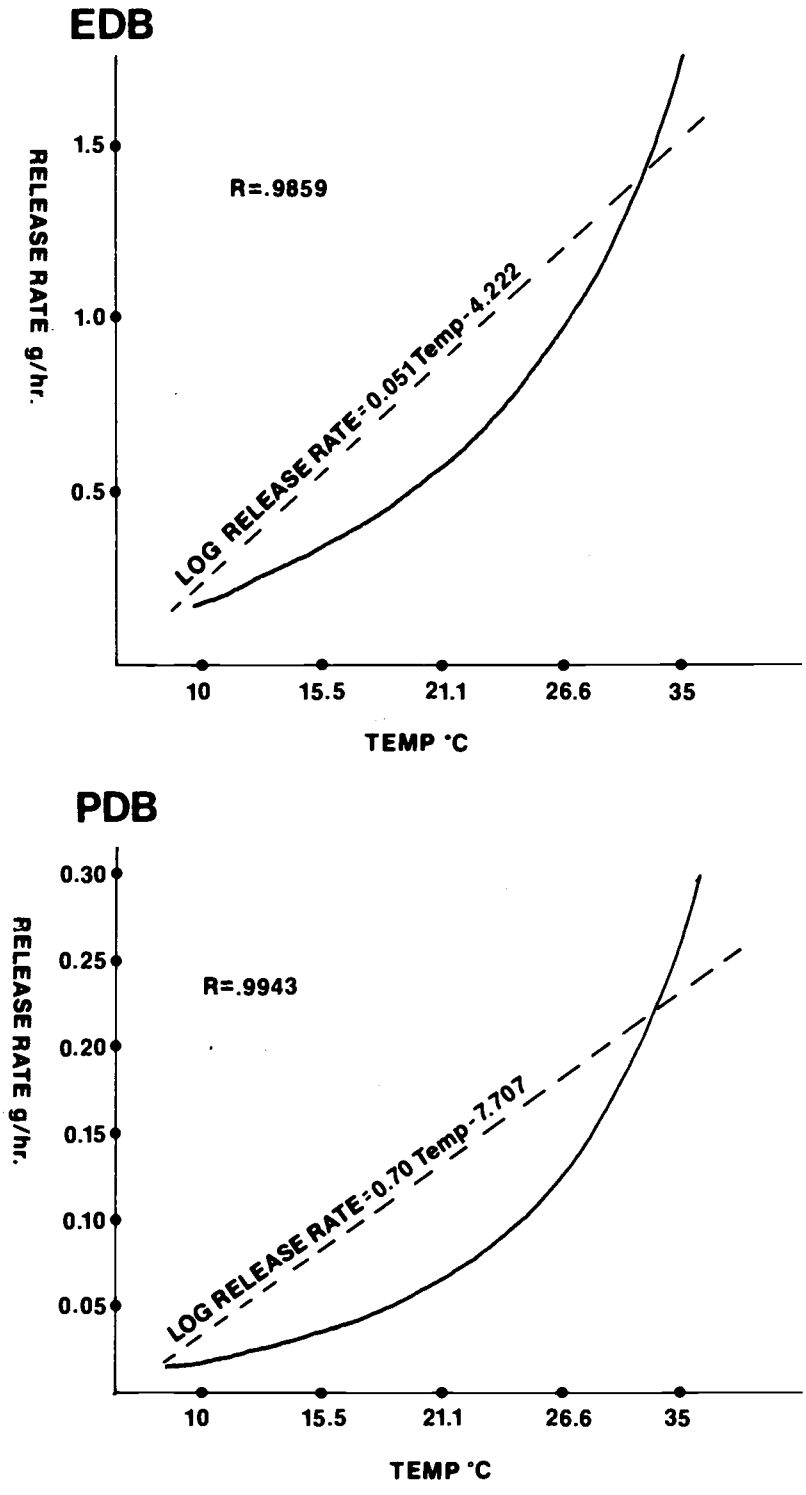



Figure 6b. The effect of temperature on release rate of EDB and PDB in 4 mil packets.

release rate with increasing temperature is suggested. A natural logarithmic transformation of release rate yields a first order equation and a straight line relationship between emission and temperature. Via these equations, dosage estimates (the amount of chemical sealed into the packet) to insure fumigation for a specified time period are possible. For example, four months of PDB fumigation protection in a 6 mil packet at 29.4°C would require at least 273.60 g of PDB crystals (120 d x 24 hr/d x 0.095 g/hr). Conversely, knowing packet dosage, a minimum period of effectiveness can be predicted. The PDB concentration conditions inside stored supers, as mentioned above, would probably affect release rate and might allow for dosage alteration. Quantification of such factors is needed for commercial preparations. The predictive power of these equations is satisfactory for emission at constant temperatures between 10° and 35°C.

The usefulness of these equations under changing environmental conditions, such as temperature fluctuation in commercial storage facilities, is questionable. Dosage requirements differ drastically when temperature fluctuation affects release rate. An experiment was designed to examine whether a mathematical weighted average approach, e.g. estimating dosage by the proportional amount of time spent at different temperature (emission) levels, could approximate the actual observed weight loss when a packet was subjected to different temperature levels for a specified time period. During the test three 6-mil packets containing EDB underwent 12.8°C for 12 hr, 32.2°C for 5 hr and 23.9°C for 20 hr. A weighted average of release rates ($R_1$, $R_2$, and $R_3$) over times ($T_1$, $T_2$, and $T_3$) predicts that 7.394 g of PDB should be released at an average rate of 0.200 g/hr ($\Sigma RT/\Sigma T$). The results of the trial packets were averaged and a mean weight loss and release rate computed. Mean weight loss was 7.40 g and mean release rate was 0.200 g/hr. While much more

rigorous testing is necessary, these results appear to indicate that environmental fluctuations could be compensated for in the design and construction of the control packet. If commercial production becomes a reality it might be advantageous to divide the U.S. into climatic regions by temperature records and design packets with dosages tailored to specific regions.

In addition to monitoring duration of release, it is of paramount importance that the amount of fumigant being delivered is sufficient to insure protection against wax moth damage during the specified period. Lehnert and Shimanuki (1967) showed that 0.02 ml/l (0.0436 g/l) of EDB was effective in destroying all stages of the greater wax moth in 24 hr at 24°C, 22% RH. No such data have been reported for PDB, although it has long been in use as a wax moth fumigant.

Recommended fumigation procedures (USDA, 1972) call for treatment of stacks of no more than eight standard Langstroth supers having a calculated volume of 340.80 liters (42.6 liters/super). Not accounting for possible wax absorption, a minimum level of 14.86 g (340.80 liters x 0.0436 g/l) of EDB vapor would have to be released at any temperature over a 24 hr period to insure eradication. Field sized packets (35.56 cm x 10.16 cm) are capable of delivering such quantities, although at lower temperatures several days might be required before the critical concentration was reached. Leakage out of the stack would compound the problem, particularly with lower release rates, which might not compensate quickly enough for the loss. Lehnert and Shimanuki (1967) showed that lower concentrations of fumigant are also reasonably effective for control purposes. In that work, 1 ml/l (0.021 g/l) of EDB maintained for 24 hr killed all but the egg stage of the moth, and of these, 50% hatched. Perhaps a minimum level of 7.43 g of EDB vapor maintained for several days might suffice.

Estimating the toxic dosage of PDB for wax moth larvae is more difficult. Literature on the toxicity of PDB to Galleria is lacking. Toxicological studies of PDB on larvae of the webbing clothes moth, Tineola biselliella, were conducted in 1931 by Herrick and Griswold in airtight chambers. Their experiments showed good results (greater than 80% mortality in most cases) with dosages of 0.12-0.16 g/l (12-16 oz/100 $ft^3$) of sublimated PDB. Temperatures in their experiments ranged from 62-80°F (mean 71.7°F) and duration from 3-7 days (mean 5.1). While Tineola and Galleria are only distantly related at best, for purposes of discussion, it could be assumed that the dosage rate for Tineola might serve as a baseline for estimates on Galleria. With this assumption in mind, the low release rates for PDB in 6 mil packets suggests that the 4 mil preparation could better maintain the minimum dosage level required. Both 6 mil and 4 mil packets were later tried in preliminary mortality tests against active larvae. The 4 mil packet outperformed the 6 mil packet, which showed minimal mortality (6.1%) at 26.6°C over a 96 hr period.

Temperature fluctuations, concentration buildup in the receptor compartment, and leakage compensation are problems which affect PDB in much the same way as they do EDB. Depressed release rates were noted under conditions where concentration buildup in the receptor compartment occurred. As the compartment was aired, which simulated leakage, release rates returned to normal. A temperature fluctuation test identical to that for EDB was conducted with similar results. Three PDB test packets were subjected to 10°C for 68.5 hr, 32.2°C for 23 hr, and 23.9°C for 5 hr. Expected release differed from observed release by less than 0.5 g. Averaged release rate varied by 0.01 g/hr.

Ethylene dibromide in 6 mil packets and paradichlorobenzene in 4 mil packets present an obvious contradiction where fumigant mixtures are considered. Enclosing an appropriate amount of EDB in

a 4 mil packet to insure 24 hr fumigation poses problems in adsorption and also defeats the purpose of frugality. Whether concentration buildup in the receptor compartment could regulate release to the point that dosage could be practically decreased is not yet determined.

Field Sized Packets (35.56 cm x 10.16 cm--EDB and PDB

To verify the assumption that field sized packets would quadruple release rate, tests were run using EDB and PDB in field sized packets for two temperature levels and two tubing thicknesses. Actual and predicted results are shown in Table 3. It is apparent that the assumption of quadrupled release rate is not altogether accurate. Results were uniformly below predicted values. Also, test results for the higher temperature deviated from predicted values by much more than the lower temperature results. PDB deviates much less than EDB.

It is not surprising that deviations from predictions are present, since the predictions themselves are derived from experimental data. However, the magnitude of deviations in the higher temperatures (and lower temperature in 4 mil) of ethylene dibromide is questionable.

During the experiments, three large packets were tested simultaneously in an environmental chamber with a volume of 78.5 liters. The odor of EDB was extremely strong when the chamber was opened. Those conditions showing greatest deviation (EDB in 4 and 6 mil packets) had receptor compartment concentrations calculated at 0.03-0.09 g/l. Depression of release rate due to high receptor compartment concentration of EDB is suspected. Repeating the experiments in larger volume controlled environment chambers might yield more accurate results.

Table 3. Experimental and Predicted Release Rates for EDB and PDB in Field Sized 4 and 6 mil Polyethylene Packets.

| | Temperature (°C) | Release Rate (g/hr) | | Difference |
|---|---|---|---|---|
| | | Observed | Predicted | |
| | | 6 mil | | |
| EDB | 15.5 | 0.30[a] | 0.37[a] | -0.07 |
| | 26.6 | 0.69 | 0.97 | -0.28 |
| PDB | 15.5 | 0.04[a] | 0.05[a] | -0.01 |
| | 26.6 | 0.17 | 0.30 | -0.13 |
| | | 4 mil | | |
| EDB | 15.5 | 1.21[a] | 1.61[a] | -0.40 |
| | 26.6 | 2.48 | 3.34 | -0.86 |
| PDB | 15.5 | 0.14[a] | 0.15[a] | -0.01 |
| | 26.6 | 0.53 | 0.56 | -0.03 |

[a]Mean values of 15 observations over three replicates.

Test Packets (8.89 x 10.16 cm--
Fumigant Mixtures

Experimentation was conducted with various mixtures of EDB, PDB and solvents. The creation of a mixture packet which would deliver a 24 hr dose of EDB followed by several months of PDB emission was proposed. It was expected that the initial fumigation would destroy 99% of active stages of the wax moth. Followup protection would then control those individuals which survived, and also discourage reinfestation.

It was determined that EDB and PDB are compatible in the delivery packet with and without solvents. The two chemicals are somewhat miscible, and have several common organic solvents. Neither chemical appreciably degrades or complexes with the polyethylene tubing from which the test packets are constructed. The tubing is permeable to both substances. The chemicals appear to retain their chemical integrity when mixed. Separated peaks in chromatographic analysis showed that EDB emission did not deter PDB emission; however, release rates computed from packet weight loss revealed that emission was not simply additive. By this, two concepts are implied: (1) that a mean emission rate for a mixture packet cannot be computed due to chemical differences in vapor pressures, diffusion coefficients, and mixture proportions which cause release rate to vary over time, and (2) that release rate at any single point in time is not the sum of the individual release rates of the chemicals remaining in the packet.

EDB/PDB Mixture. Preliminary tests with an EDB/PDB mixture (1:2) at 15.5$^{o}$ and 26.6$^{o}$C revealed two effects. Compared to hypothetical release rate curves based on the activities of the independent chemicals, permeation through the film was abnormally elevated. In the hypothetical model, release rates were assumed to

be additive, suggesting independent activity by both fumigants. Thus, when the more volatile chemical (EDB) was exhausted, release would rapidly drop to the lower level of the less volatile chemical (PDB). Observed release rates for the 1:2 EDB/PDB mixtures in 4 mil packets were uniformly above the hypothetical model throughout the experiment. During the first 10 hr of each experiment, abnormally high release rates were observed:

| Temperature | Release Rate (observed) | Release Rate (predicted) | Difference |
|---|---|---|---|
| 26.6°C | 1.412 g/hr* | 0.975 g/hr | +0.437 g/hr |
| 15.5°C | 0.697 g/hr | 0.441 g/hr | +0.256 g/hr |

*Mean of 15 observations from three replicates.

Furthermore, the rapid dropoff in release rate predicted by the model was not realized. Release rate did not fall rapidly but gradually decreased over time to a level slightly higher than that for PDB alone. It was not possible to determine what contribution each fumigant made to the total rate of permeation during most of the tests.

The cause for deviation from predicted values in the mixture tests is not known. Chemical interaction inside the packet and at the film surface may play an important part. Codistillation of PDB with EDB is likely to have occurred and may account for higher emission rates. After the initial permeation flux, attractions between EDB and PDB may have caused the remaining EDB to resist volatilization, delaying release until the PDB slowly sublimated. This would explain the slow decline in release rate, and perhaps the slightly elevated final rates. The behavior of these chemicals when mixed is certainly not independent.

It was noted the EDB solvent capacity for PDB was low. Much of the PDB included remained crystalline. Organic solvents were examined which could be added to the mixture to dissolve more PDB, and therefore provide a longer protection period. Ethyl ether, chloroform, carbon tetrachloride, dichloromethane, benzene, toluene, and various alcohols were tried. Dichloromethane was later chosen rather than ethyl ether or chloroform, because in subsequent vapor analysis, it would be easier to separate from EDB and PDB than either chloroform or ether. The proportion of dichloromethane required to create a 1:2 mixture of EDB/PDB was found to be 1.41 times the amount of EDB. The final solution was, therefore, one part EDB, two parts PDB and 1.41 parts dichloromethane. At 21°C, PDB did not crystallize out of this solution.

EDB/PDB/Dichloromethane Mixture. Tests were continued with an EDB/PDB/dichloromethane mixture in 6 mil packets. Hypothetical release curves were constructed for comparison. These curves were again based on the sum of the independent release rates for each chemical. Duration of release for each component of the mixture was computed from the independent release rates and the amount of each material included. In the model, overall release rate declined in two distinct steps, corresponding to the sequential depletion of dichloromethane, and EDB. The final rate of release was anticipated to be equivalent to that of PDB alone. Such performance would indicate independent chemical activity in liquid and vapor phase, as well as within the polyethylene film. Figure 7 demonstrates an ideal release rate curve based on independent release at 12.7°C of a 1:2:1.41 mixture. This may be compared to the observed curves for the EDB/PDB mixture and an EDB/PDB/dichloromethane mixture.

The results (Figure 8) were similar to those for the EDB/PDB tests in some respects. Lack of agreement with the hypothetical model indicated that chemical activity within the packet is not independent.

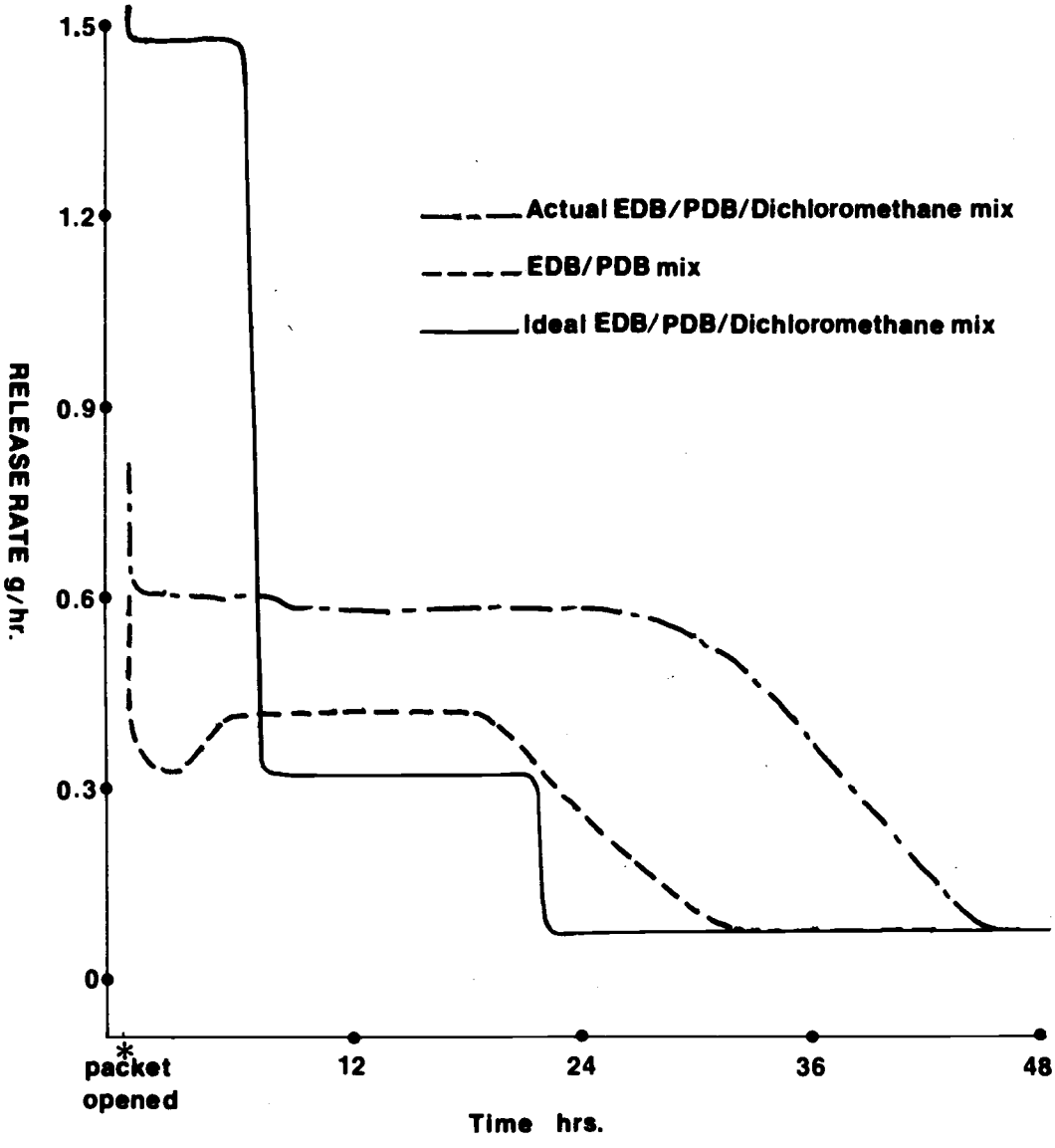



Figure 7. A comparison of mixture release rate behavior at 26.6°C.

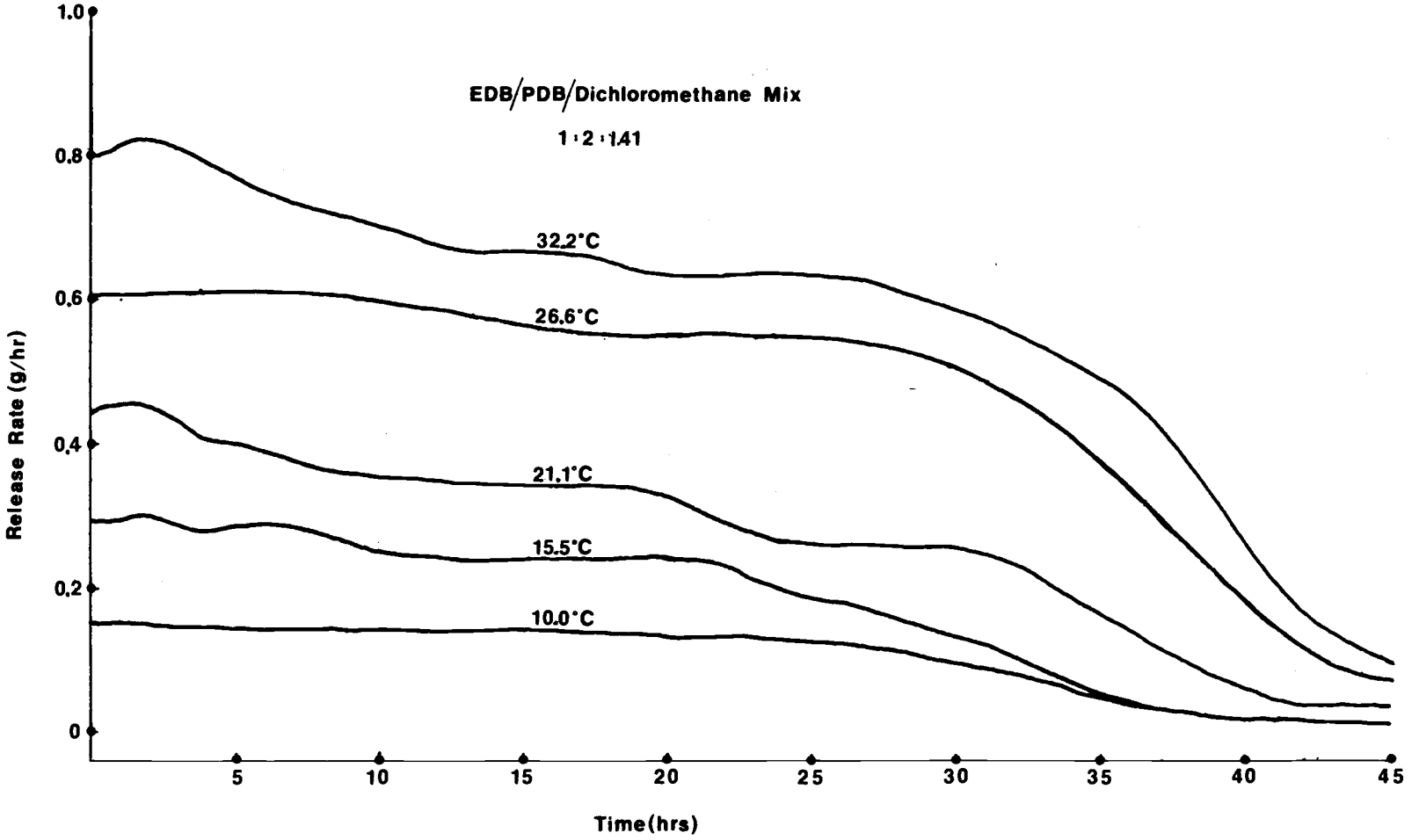



Figure 8. Emission behavior of EDB/PDB/dichloromethane mixtures at five temperature levels.

Release rate tended to be lower than predicted at first, disagreeing with both the model and the previous EDB/PDB results. As in the previous experiments, release rates declined gradually, consistently remaining above the rates predicted by the model. Observed release rates eventually arrived at the rates recorded for PDB alone, but did so much later than predicted by the model.

What interactions are responsible for the observed activity are not yet determined. It is likely that the same factors affecting the EDB/PDB mixture also affect the EDB/PDB/dichloromethane mixture. The overall behavior of the two mixtures is similar. Chemical interactions could certainly influence permeation. Transport capabilities, and chemical-polymer interaction within the polyethylene film, could also be involved.

## Chromatographic Analysis

An experiment was designed to capture the vapors emanating from an EDB/PDB/dichloromethane packet. A gas trapping apparatus was constructed for this purpose and aliquots of trap solvent taken every hour. The experiment was performed twice, at 23.9°C and 29.4°C. Standard peaks for PDB, EDB, and dichloromethane were obtained on the chromatograph (Figure 9), and the aliquots run through for comparison. The purpose of this analysis was to (1) determine whether chemical interactions had occurred between the fumigants, resulting in hybrid molecules, and (2) to examine the relative proportions of fumigants present in the vapor.

Results indicated that all three chemicals were being emitted from the packet intact; no evidence of hybrid molecules appeared in chromatographic separation. Figure 10 shows typical scans from aliquots taken at various times during the gas trapping. Considerable amounts of both EDB and PDB were found in the first bubble trap (A).

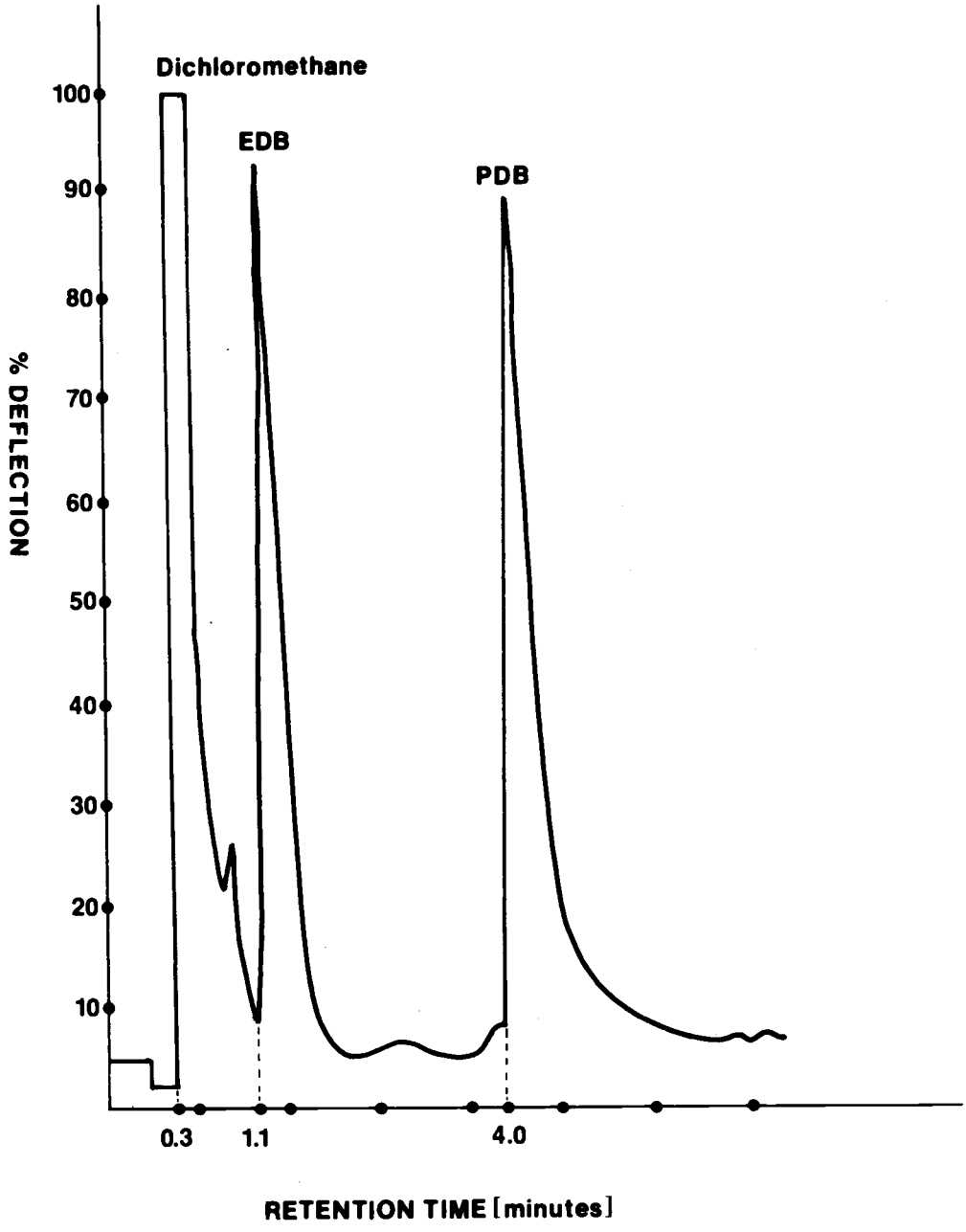



Figure 9. Standard GC peaks for dichloromethane (hexane), EDB and PDB.

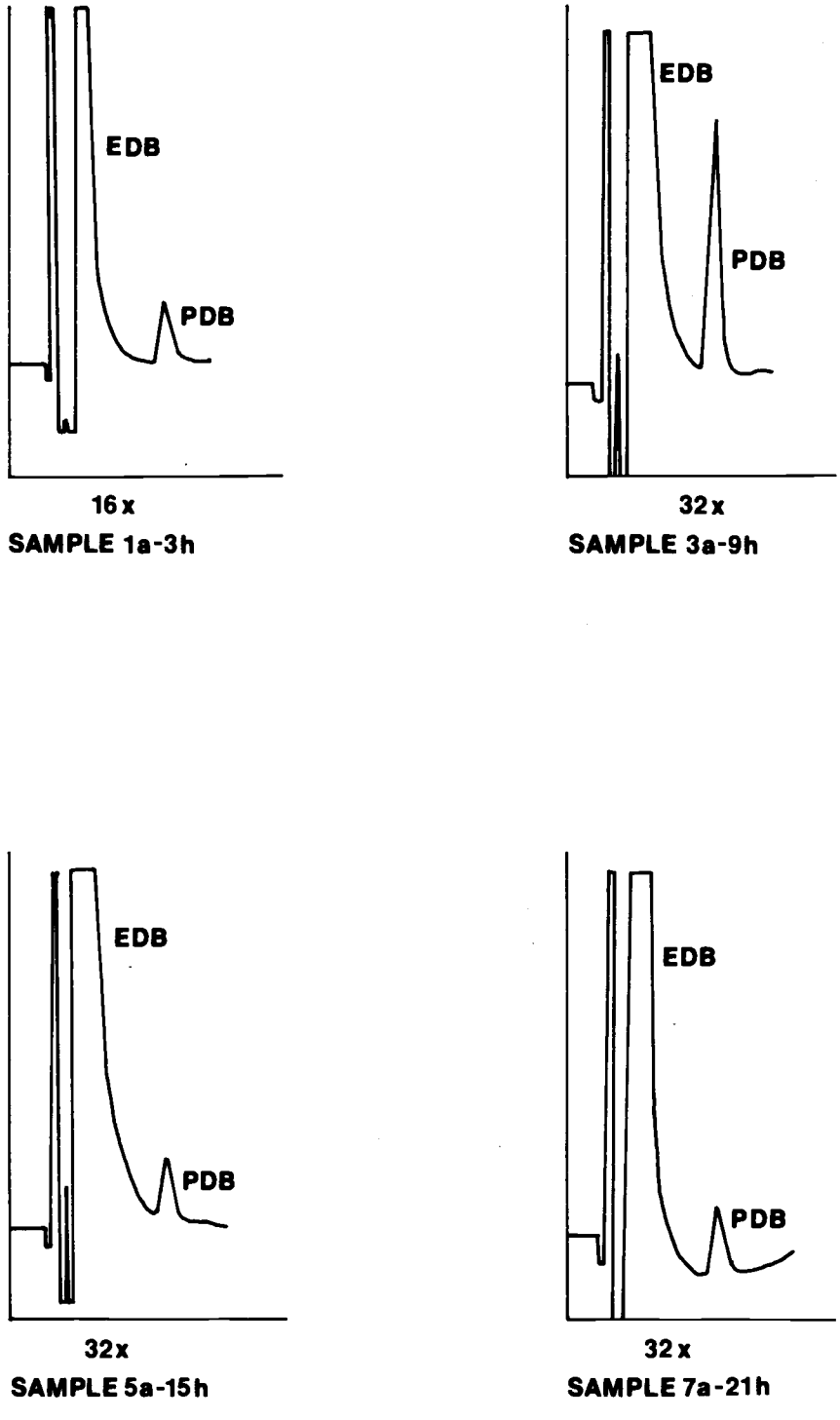
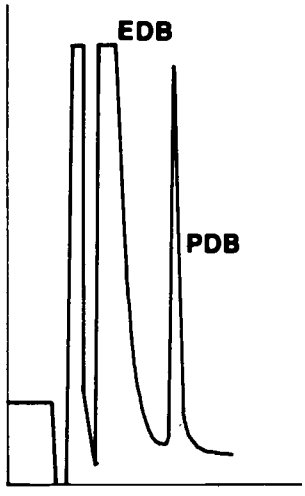
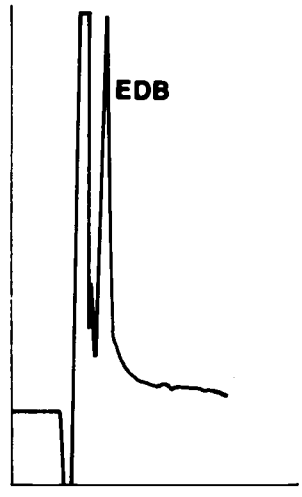


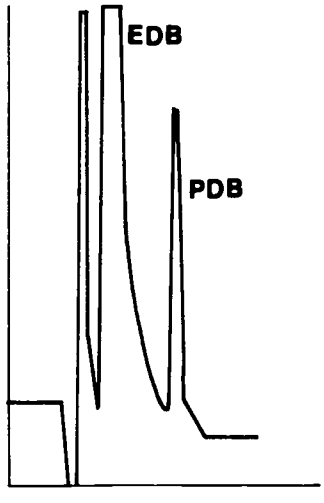

Figure 10. GC separation of vapor trap samples at increasing attenuations.



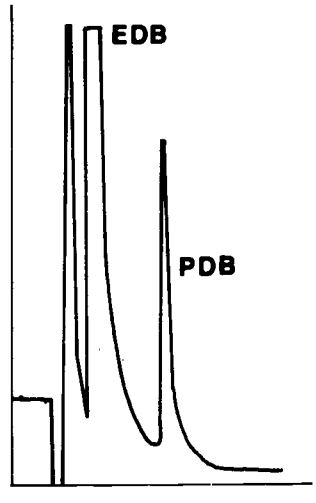

64x
SAMPLE 9a-27h




64x
SAMPLE 9b-27h




128x
SAMPLE 10a-30h




128x
SAMPLE 11a-33h

Figure 10. (Continued)

However, in the second trap (B) no trace of PDB was ever found, even when tested full strength in the chromatograph. EDB was always present in the second trap. It is assumed that dichloromethane was present in both, but since its peak merges with that of the carrier solvent (hexane), the assumption cannot be proven through this analysis.

Examination of peak heights and widths suggests the EDB is present in much greater proportion than PDB in the emitted vapor. It was not possible to determine quantitatively the exact proportions present, because dilutions aimed at the critical range of EDB effectively washed out the PDB present. Another question arose concerning the machine's sensitivity to PDB after a massive does of EDB. The nature of the electron capture chromatograph renders it prone to oversaturation which could cause dampening of the following peaks. It is possible that the PDB peaks shown in Figure 10 may not represent the true proportion of PDB present, but rather only that amount which the machine could detect while recovering from the EDB. It was noted that EDB emission continued throughout the trapping period rather than abating as might be predicted by the behavior of EDB alone. However, it is difficult to assess the magnitude of EDB compared to PDB late in the test due to sensitivity of the machine as mentioned above.

Several conclusions are possible. Given that mixture components retain their identity through the controlled release process, the factors affecting release rate must exert their influence while the components are in solution, or diffusing through the plastic film. The choice of solvent is of paramount importance. Addition of solvent reversed the initial trend observed in sans-solvent mixtures, i.e. an elevated emission curve. It would appear that chemical and/or mechanical interactions heretofore unnoticed may be affecting the release rate. Chemical interactions, ionic and Van der Waals

attractions between fumigants and solvent while in solution, could be involved. The carrying capacity of the plastic film and chemical competition for diffusion channels are unexplored areas which might suggest plausible reasons for the observed activity.

Further examination of mixture emission will be necessary before attempting mortality studies with two-phase packets. The question of whether to use a solvent and if so, which one, is important. Dichloromethane was found to be toxic to wax moth larvae. Quantification of release rate, and fumigant proportions in the emitted vapor need to be examined.

## Mortality Studies

Using live Galleria mellonella larvae, and EDB or PDB controlled release packets, mortality studies were conducted to measure the efficacy of the delivery system previously described. Over 3600 larvae were tested: 1800 with ethylene dibromide, and 1800 with paradichlorobenzene. Six mil polyethylene packets were chosen for EDB tests, and 4 mil packets for PDB tests. Reducing EDB film thickness to 4 mil accelerated the rate of mortality in time, reaching 100% mortality 24 hr sooner than with 6 mil packets. Four mil EDB packets were not chosen because such rapid mortality could conceivably mask the effect depth or position within the frame could have upon survival.

In the 48 hr ethylene dibromide tests, mortality was assessed at 6, 12, 24, and 48 hr by randomly removing a frame from the nuc box and counting the dead larvae present in the nine replicates. These time replications were further subdivided into three depths and horizontal positions, and recorded accordingly. Control frames, from an identical nuc box in an adjacent environment chamber, were treated similarly. Once counted, all the remaining live larvae from

nine replicates were placed in a jar containing new medium and held for 72 hr at 22°C for re-examination. Controls were similarly treated. Paradichlorobenzene was tested in much the same manner, except the total time was extended to 96 hr with mortality assessed at 24 hr intervals.

At the end of these experiments, the equipment was reversed and the cages, frames, and nuc box used in the test chamber were exchanged for those from the control chamber. A final trial was made without fumigant to determine whether any residual toxicity had accumulated in the equipment which could have biased results. In 96 hr, mortality was less than 2% in both set-ups.

## EDB Studies

Analysis of variance (Appendix I) shows a significant difference in mortality between the fumigated larvae and the non-fumigated larvae. The temperature at which fumigation is conducted and the duration of exposure also have a significant effect on mortality as compared to survival in the control larvae.

Regression analysis (Appendix I) dismissed both position and depth within a frame as having affected mortality. The independent effects of chemical, temperature and time are also insignificant at the 5% level. The interaction of time and temperature did not significantly affect survival. The factor responsible for the large difference in mortality between the fumigated larvae and controls is the interactions of chemical, temperature and time. This includes the interactions of chemical and temperature and also chemical and time. All three interactions are significant at the 1% level. These three interactions plus chemical account for 81% ($R^2 = 0.8109$) of the variation in mortality between fumigated larvae and controls.

The effect of the interaction of chemical and temperature on mortality is shown in Figure 11. Mortality nearly triples when temperature rises to 32.1°C from 21.1°C. This temperature corresponds to the exponential increase in packet release rate noted in Table 2 and Figure 6a. Higher temperatures cause greater dosages of EDB to be released from the packet, which increases mortality. The nature of the delivery system, which includes temperature as a controlling variable, is responsible for this effect.

The interaction of chemical and time on mortality of wax moth larvae is shown in Figure 12. Mortality triples after the initial 12 hr of fumigation, and continues to rise through the end of the tests. As will be clarified later, mortality at the end of the 48 hr period does not accurately reflect the efficiency of the system. Seventy-two hours after the close of the experiment, the number of dead larvae often increased 50% or more. In this system, time and dosage are intrinsically linked. An ever greater proportion of the packet dosage is being liberated as time progresses. Hence, the atmospheric toxicity around the larvae is magnified through time. The correlation of increasing mortality over time reflects the larval response to rising levels of toxicant.

It appears, then, that in the physics of the controlled release delivery system, temperature and time represent convergent approaches to dosage-mortality problems commonly dealt with in insect toxicology. Both time and temperature affect the amount of toxicant released into the atmosphere. The level of toxicant in the atmosphere directly influences survival of the larvae. Both of the mortality curves for time and temperature approximate the S-shaped curves of cumulative frequency distributions. In insect toxicology, such curves are generated from mortalities encountered in a succession of tests utilizing increasing levels of toxicant. In effect,

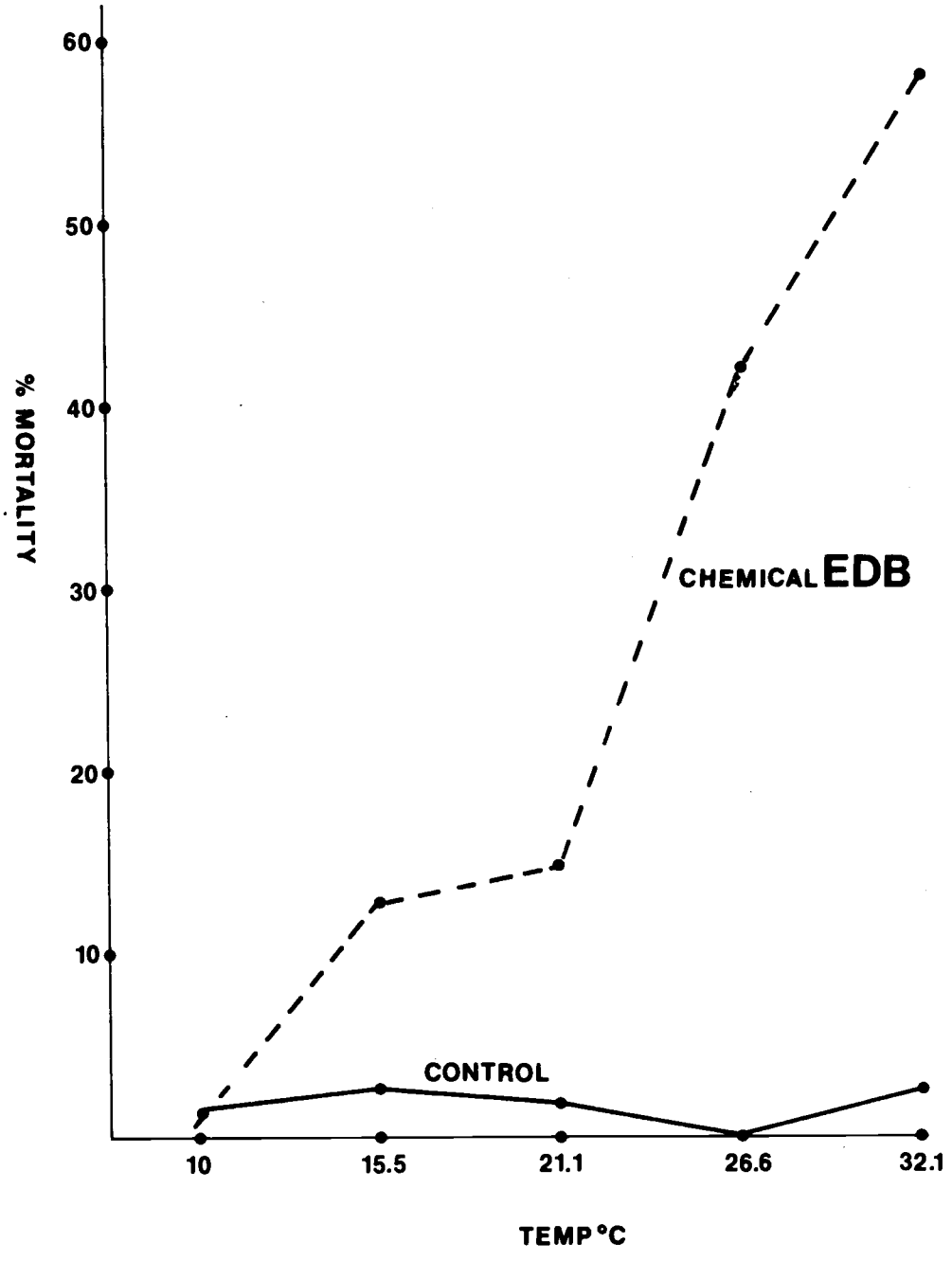



Figure 11. The effect of chemical (EDB) x temperature on mortality of wax moth larvae. (Data points represent all times.)

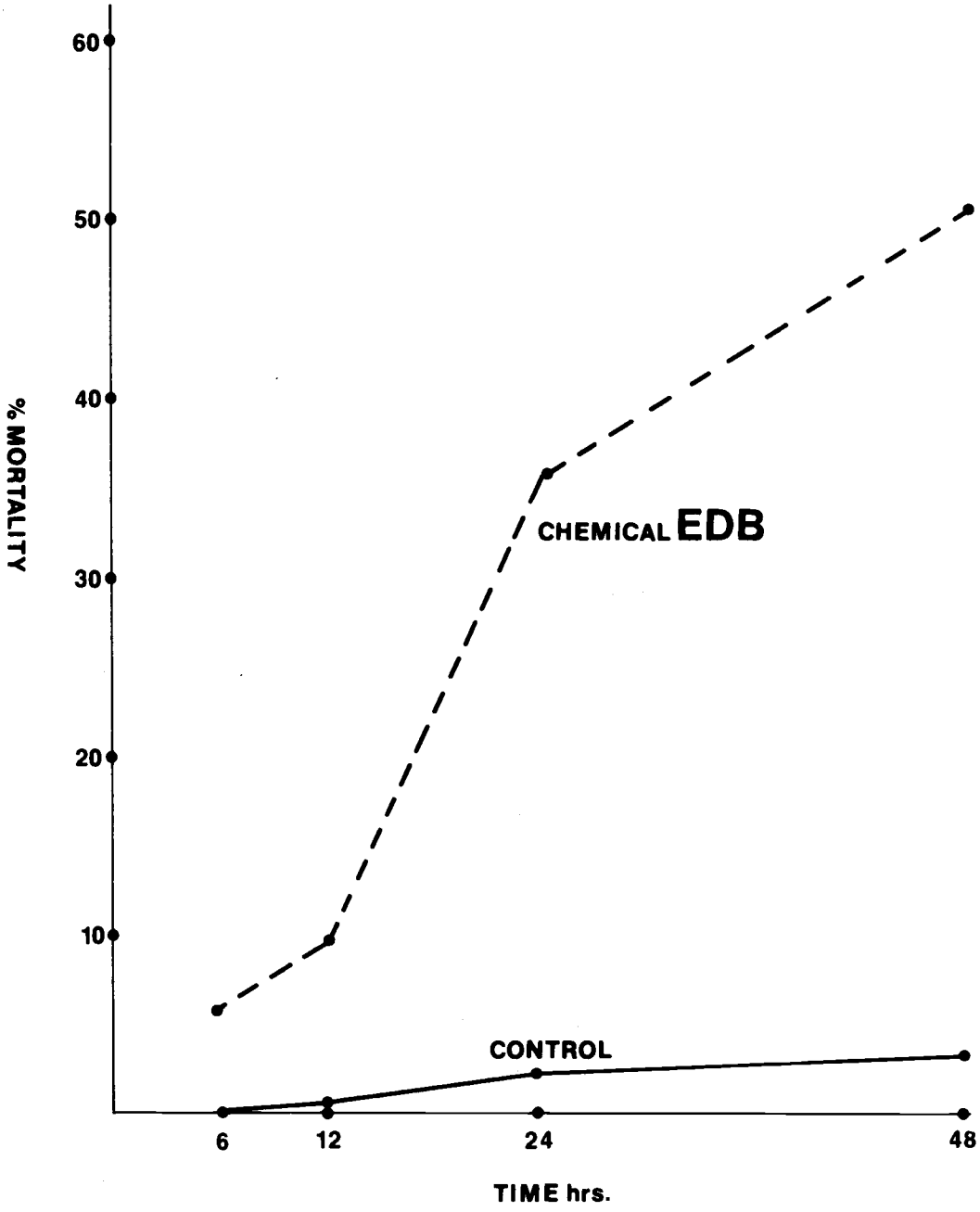



Figure 12. The effect of chemical (EDB) x time on mortality of wax moth larvae. (Data points represent all temperatures.)

the experimental setup and the physics of the delivery system accomplish the same results in a single experiment.

It is not accurate to use either interaction alone to explain the mortality encountered in these experiments. Mortalities much higher than 60% were encountered in tests. It is the interaction of chemical, temperature and time which best fits the experimental results. Figure 13 shows the intermeans of this three-way interaction. Each column represents a time interval, the black area in each of four columns represents the chemical, temperature and time interaction, or total mortality. Control mortality is not shown, but the mean value was approximately 1.8%. The characteristic variability in population response to the toxicant is demonstrated by the difference in time peaks within the group. Some individuals are particularly sensitive and die after only a few hours exposure. Others are tolerant and can withstand longer exposure and thus larger doses. Resistance to EDB did not appear to be present in the population tested.

Also shown, bridging each temperature level, is the post-treatment mortality total (listed in Table 4). It is the sum of mortality during the test and mortality in the remaining live larvae 72 hr after the test. It appears that mortality is actually much higher than is evidenced by a 48 hr examination. The mean mortality in survivors of the 48 hr test (81.60) is nearly twice the mean mortality for the test itself (45.80). The physiological damage caused by EDB may be irrevocable, or at best the detoxification system of the larvae may be overwhelmed by the magnitude of the task. If EDB does block enzymes involved in metabolic energy transfers, detoxification systems existent may remain inactive due to lack of energy.

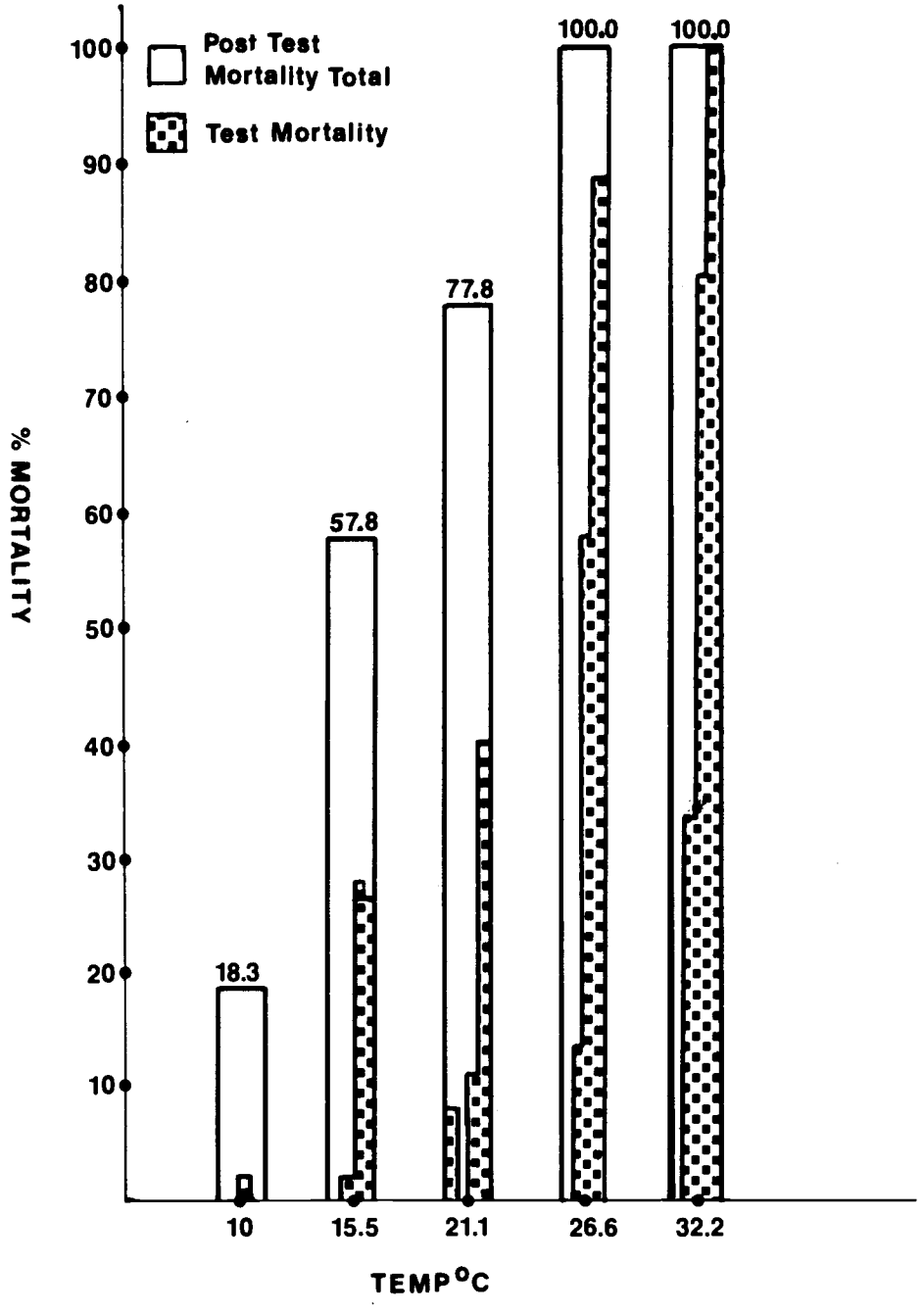



Figure 13. The effect of chemical (EDB) x temperature x time on mortality of wax moth larvae.

Table 4. Larval Mortalities during and after Testing with EDB and PDB.

| Temperature (°C) | Total larvae | Mortality during 48 hr test | | Mortality within survivors 72 hr later | | Total mortality | | % Control |
|---|---|---|---|---|---|---|---|---|
| | | test | control | test | control | test | control | |
| | | | | EDB (6 mil) | | | | |
| 10.0 | 180 | 1 | 5 | 32 | 8 | 33 | 13 | 18.3 |
| 15.5 | 180 | 23 | 4 | 81 | 6 | 104 | 10 | 57.8 |
| 21.1 | 180 | 26 | 3 | 114 | 2 | 140 | 5 | 77.8 |
| 26.6 | 180 | 75 | 0 | 105 | 2 | 180 | 2 | 100.0 |
| 32.1 | 180 | 104 | 4 | 76 | 0 | 180 | 4 | 100.0 |
| $\bar{x}$ | 180 | 45.80 | 3.20 | 81.60 | 3.60 | 93.40 | 6.80 | |
| | | | | PDB (4 mil) | | | | |
| 10.0 | 180 | 2 | 2 | 1 | 1 | 3 | 3 | 1.7 |
| 15.5 | 180 | 4 | 3 | 3 | 3 | 7 | 6 | 3.9 |
| 21.1 | 180 | 4 | 0 | 1 | 1 | 5 | 2 | 2.8 |
| 26.6 | 180 | 7 | 2 | 17[a] | 1 | 24 | 3 | 13.3 |
| 32.1 | 180 | 13 | 2 | 18[a] | 2 | 31 | 4 | 17.2 |
| $\bar{x}$ | 180 | 6.00 | 1.80 | 8.00 | 1.80 | 14.00 | 3.60 | |

[a] >50% Larvae alive but paralyzed or in convulsions.

PDB Studies

Mortality tests for PDB were conducted in much the same manner as for EDB with two notable exceptions: (1) 4 mil packets were used, and (2) time was extended to 96 hr with mortality assessed at 24 hr intervals.

As before, mortality was significantly affected by fumigation. The temperature and duration of fumigation again significantly affected mortality (ANOVA, Appendix II). Position and depth within a time frame were not significant in regression analysis (Appendix II), nor were the independent effects of chemical, temperature, time and the interaction of temperature and time. The interactions chemical and time, and chemical, temperature and time were both significant at the 5% and 1% levels, respectively. The interaction chemical and temperature missed by 0.11 being significant at the 5% level. These three interactions and the variable chemical accounted for 21% ($R^2 = 0.2108$) of the variation between fumigated larvae and controls.

The best fitting regression models for EDB and PDB both contain identical variables. The obvious difference in correlation coefficients is attributed to the rapid killing action of EDB. PDB sublimates much more slowly than EDB volatilizes, and the modes of action are quite dissimilar. Because of the overall similarity in regression models, and considering the delayed action of PDB as compared to EDB, the interaction, chemical and temperature, will be included in discussion, despite its lack of significance at the 5% level. The chemical and temperature interaction is suspected to contribute more to larval mortality than is evidenced by the 96 hr tests. Judging instead from the mortality, and morbidity within survivors examined 72 hr after the test, the numbers belie PDB's potential for control. Lengthier testing periods are recommended for a more accurate representation.

The effects of the significant interactions between chemical and temperature and time are displayed in Figures 14-16. While the PDB mortality percentages are not nearly as impressive as those for EDB, the trends which they represent are similar. There appears to be a critical temperature and exposure time, above which mortality rises rapidly. For EDB, these are 21.1°C and 12 hr exposure. For PDB, temperature is again 21.1°C, but exposure time is now greater than 72 hr. The difference in timing probably reflects differences in volatility and activity between EDB and PDB. The similar temperature factor suggests two possibilities: (1) that at 21.1°C both fumigants begin to volatilize rapidly enough to be lethal to the larvae, and/or (2) at 21.1°C larval metabolism increases to a point where the toxic effects of the fumigants become realized. A metabolic survey, perhaps testing larvae in a simple vial respirometer under various temperature regimens, might clarify the role of metabolism.

As with EDB, parallels may be drawn between the PDB mortality interaction curves (Figures 14 and 15) and S-shaped cumulative frequency distribution curves used in dosage/mortality studies. Cumulative mortality increases in proportion to toxicant dosage. In the design of this system, dosage increases in proportion with time and temperature. It is encouraging to see mortality response to the interaction of chemical, temperature and time (Figure 16) mirror the exponential release rate curves shown in Figure 6a and 6b. In Table 4 and Figure 16, mortality within survivors 72 hr after the test is shown. The increase in mean mortality over test results suggests a delayed action for PDB. It was apparent from the large number of moribund larvae (>50%) that mortality increased as time passed. In fact, although several days were required, nearly all larvae which had reached the convulsion stage eventually died.

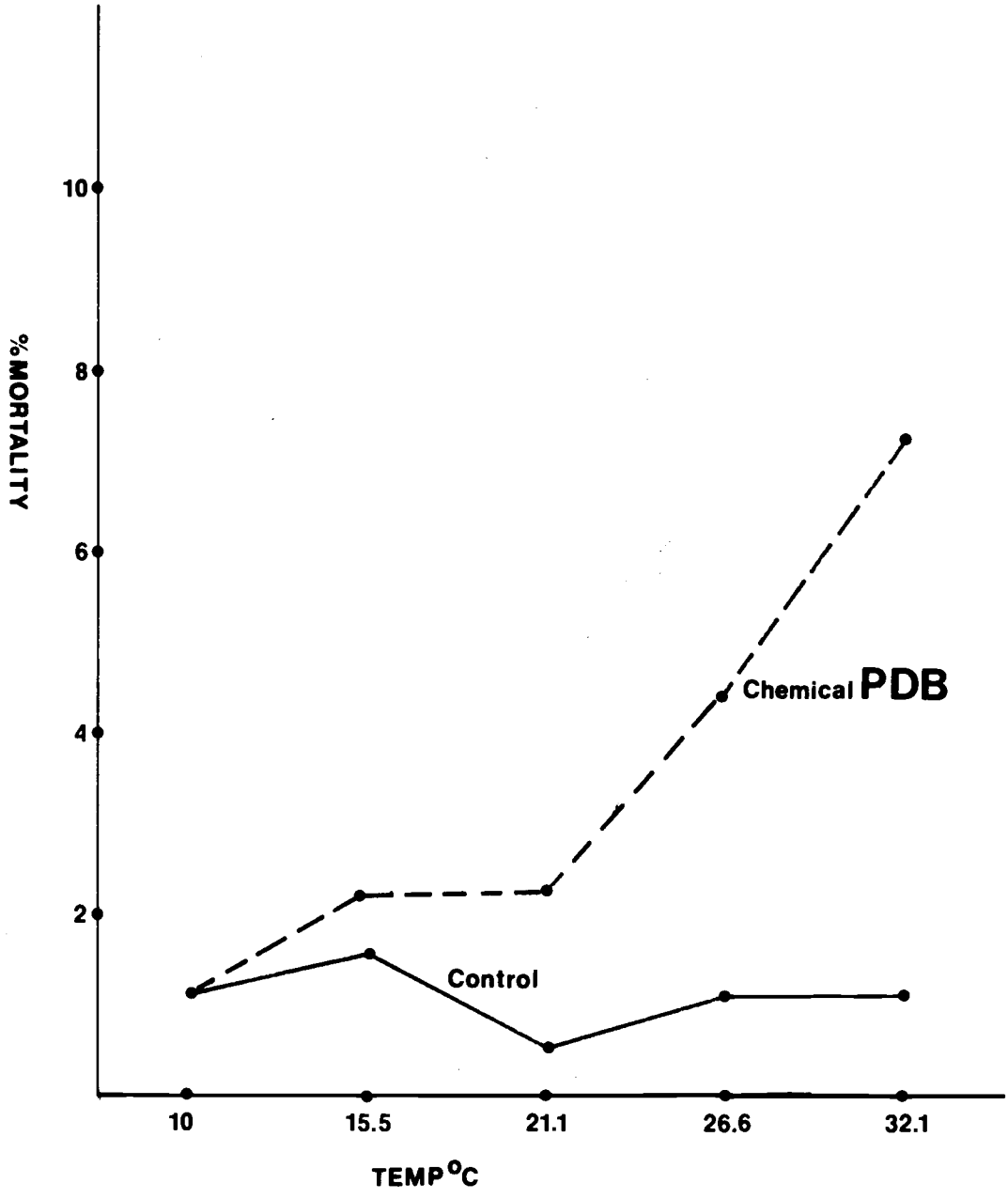



Figure 14. The effect of chemical (PDB) x temperature on mortality of wax moth larvae. (Data points represent all times.)

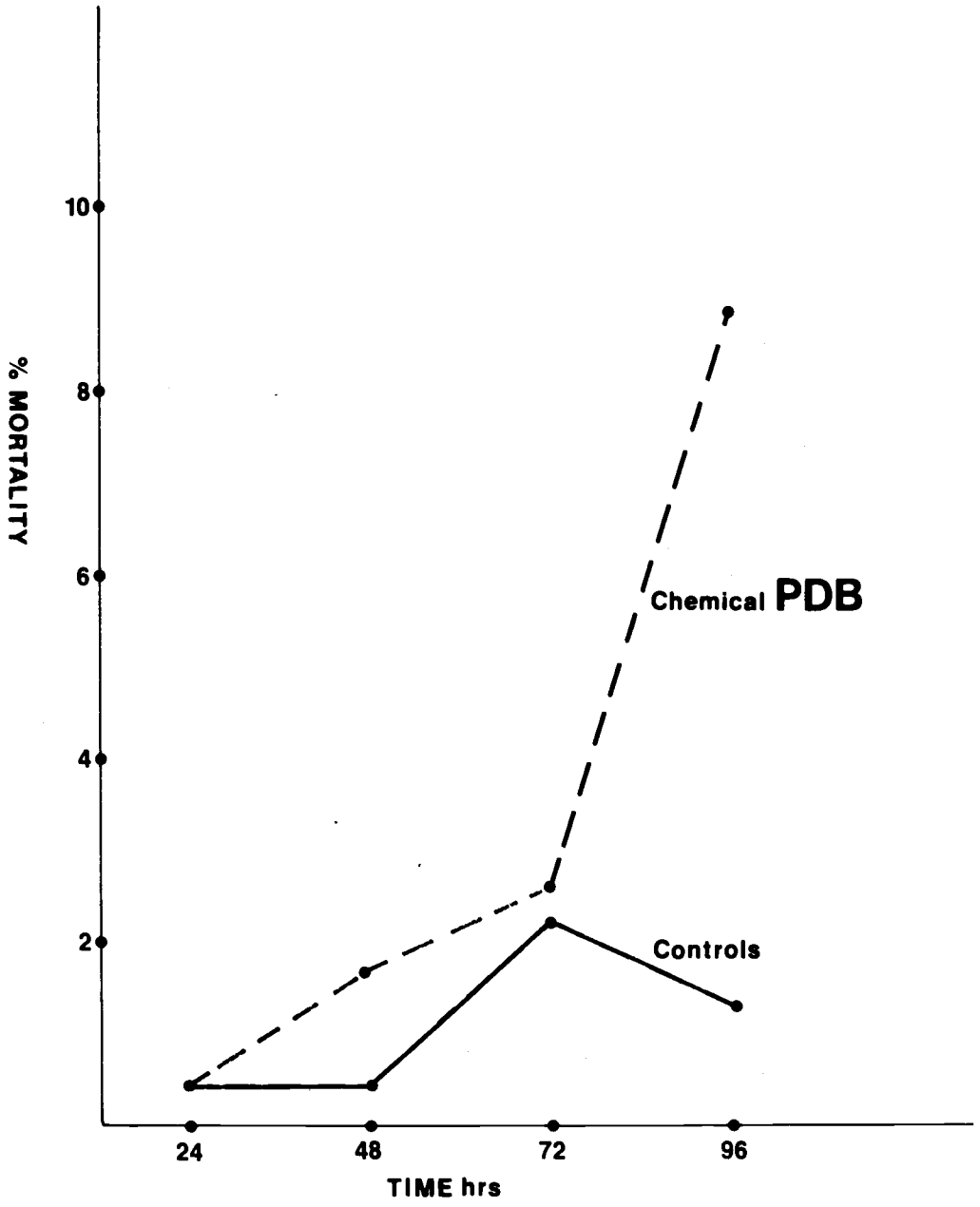



Figure 15. The effect of chemical (PDB) x time on mortality of wax moth larvae. (Data points represent all temperatures.)

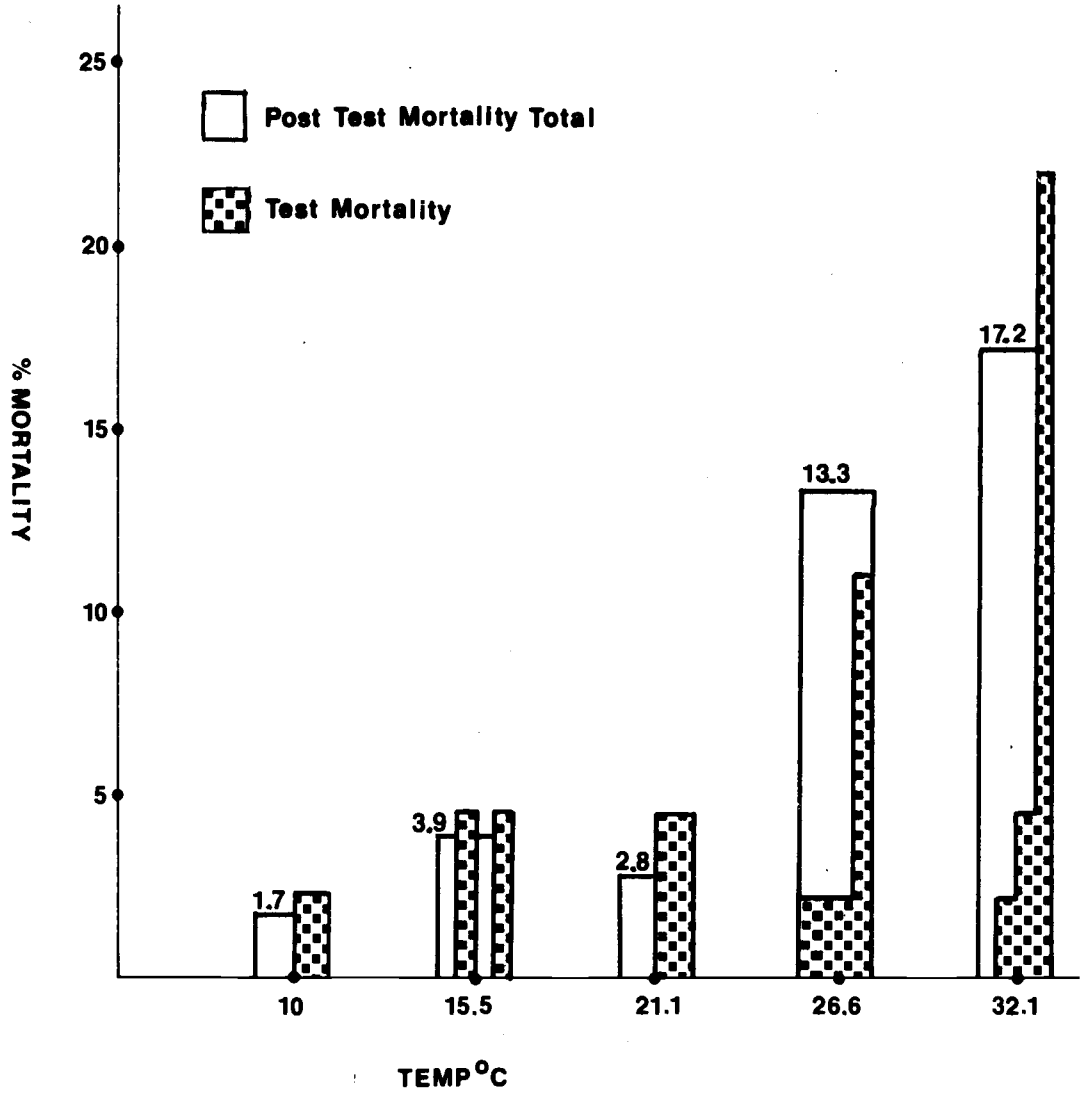



Figure 16. The effect of chemical (PDB) x temperature x time on the mortality of wax moth larvae.

# SUMMARY AND CONCLUSIONS

Controlled release delivery systems exhibit ideal characteristics for insect pest control and abatement programs. They are especially suited for control where multiple generations and/or chance of reinfestation are common. The wax moth is commonly multivoltine particularly in the southern U.S. and causes persistent infestations. This current work indicates that a controlled release system may be suitable for wax moth suppression.

Rates of release of ethylene dibromide and paradichlorobenzene from 4 and 6 mil controlled release packets are quantitative and predictable. Methods of prediction within specified limits permit construction of control packets designed for use under determinate conditions and time periods. In fumigant mixtures, the mixture components retain their chemical identity throughout the release period. Component release is not independent, however, indicating physical interactions within the packet. More work is necessary to clarify the behavior and expose the control possibilities inherent in fumigant mixtures.

Larval mortality in wax moths is significantly affected by controlled release fumigation with EDB and also PDB. Cumulative mortality is proportional to increasing dosage. Dosage corresponds to rising temperature and emission time in the controlled release packet. EDB is faster acting than PDB, and delivers complete control in 48 hr at higher temperatures from 6 mil packets. PDB is slow acting and shows greater effectiveness as exposure time increases. Its slow disappearance from 4 mil packets might make it suitable for long term protection.

The incorporation of EDB into a controlled release system appears to have several advantages. It protects the applicator from

contact with a potentially noxious chemical. It regulates the release of EDB according to the external concentration of vapor within the stack of supers, thus providing a longer fumigation period. The system is temperature sensitive, emitting EDB readily when ambient temperature and larval activity are high, and conserving fumigant when they are low.

The advantages of a PDB controlled-release packet do not appear great enough to warrant the extra cost of material and preparation. Sublimation of PDB is naturally slow, several months usually being necessary to exhaust the 200 g (± 25 g) dosage recommended for control. It is not a particularly noxious chemical to handle and is easily applied.

Ethylene dibromide/paradichlorobenzene mixtures in a controlled-release system offer some attractive possibilities for wax moth control. The initial release of EDB followed by a long term release of PDB offers immediate control plus prolonged protection against reinfestation. All the advantages of the EDB system also apply. Since the mixture components retain their chemical integrity, the development of this packet, with or without solvent, appears to be a technical problem.

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# APPENDICES

# APPENDIX I

## EDB

(Mortality = arcsin mortality)

$$\sin^{-1}\sqrt{P}$$

Regression Analysis:

```
MORTALIT= 6.0951E-02          +4.3735E-01 CHEM
          -9.0287E-03 C*T     -5.1999E-01 C*TIM
          +9.9947E-03 C*T*TIM

:AVTABLE

            ANALYSIS OF VARIANCE TABLE

SOURCE      DF   SUM OF SQUARES        MEAN SQUARE
TOTAL      359   3.29869932E 01      9.18857749E-02

REGRESSION   4   2.67500930E 01      6.68752326E 00

RESIDUAL   355   6.23690016E 00      1.75687328E-02

            R SQUARED =   .81092850

:RCOEFSE,T

VAR          S.E. OF REGR. COEF.          T
CONSTANT       9.87947728E-03      6.16943125E 00
CHEM           1.22601088E-01      3.56728890E 00 Chemical
C*T            1.71117565E-03     -5.27634101E 00 Chemical*Temp
C*TIM          4.46220015E-02     -1.16532001E 01 Chemical*Time
C*T*TIM        6.24833000E-04      1.59957664E 01 Chemical*Temp*Time
```

Analysis of Variance:

```
ANALYSIS OF VARIANCE FOR MORT
 LINE  SOURCE OF VARIATION          DF  MEAN SQUARE
(   1) CHEM                          1  5.82649E 00
(   2) TEMP                          4  1.25449E 00
(   3) CHEM*TEMP                     4  1.25720E-00
(   4) TIME                          3  1.42034E 00
(   5) CHEM*TIME                     3  1.18167E 00
(   6) TEMP*TIME                    12  2.23729E-01
(   7) CHEM*TEMP*TIME               12  2.35752E-01

(   8) ERROR                       320  1.18560E-02

       TOTAL                       359

:F,1,8

                                              5%     1%
DEGREES OF FREEDOM      F VALUE
(     1,    320):     491.4374      3.84   6.63

:F,2,8

DEGREES OF FREEDOM      F VALUE
(     4,    320):     105.8108      2.37   3.32

:F,3,8

DEGREES OF FREEDOM      F VALUE
(     4,    320):     106.0393      2.37   3.32

:F,4,8

DEGREES OF FREEDOM      F VALUE
(     3,    320):     119.7994      2.60   3.78

:F,5,8

DEGREES OF FREEDOM      F VALUE
(     3,    320):      99.6698      2.60   3.78

:F,6,8

DEGREES OF FREEDOM      F VALUE
(    12,    320):      18.9705      1.75   2.18

:F,7,8

DEGREES OF FREEDOM      F VALUE
(    12,    320):      19.9847      1.75   2.18
```

# APPENDIX II

## PDB

(Mortality = arcsin mortality)

$$\sin^{-1}\sqrt{P}$$

Regression Analysis:

```
MORTALIT= 5.8428E-02          +9.1307E-02 CHEM
        -1.8075E-03 C*T       -6.3526E-02 C*TIM
        +1.2190E-03 C*TIM*T

:AVTABLE

            ANALYSIS OF VARIANCE TABLE

SOURCE      DF    SUM OF SQUARES        MEAN SQUARE
TOTAL      359    1.24606443E 00       3.47093156E-03

REGRESSION   4    2.62671512E-01       6.56678779E-02

RESIDUAL   355    9.83392919E-01       2.77012090E-03

            R SQUARED =    .21080091

:RCOEFSE,T

VAR          S.E. OF REGR. COEF.          T
CONSTANT       3.92295304E-03       1.48940023E 01
CHEM           4.86825670E-02       1.87555393E 00Chemical
C*T            6.79475398E-04      -2.66011096E 00Chemical*Temp
C*TIM          1.77195505E-02      -3.58539411E 00Chemical*Time
C*TIM*T        2.48109335E-04       4.91319605E 00Chemical*Temp*Time
```

Analysis of Variance:

```
ANALYSIS OF VARIANCE FOR MORTALIT
  LINE  SOURCE OF VARIATION          DF   MEAN SQUARE
(   1)  CHEM                          1   3.34691E-02
(   2)  TEMP                          4   8.20341E-03
(   3)  CHEM*TEMP                     4   8.20341E-03
(   4)  TIME                          3   2.63926E-02
(   5)  CHEM*TIME                     3   2.01251E-02
(   6)  TEMP*TIME                    12   7.44478E-03
(   7)  CHEM*TEMP*TIME               12   5.41856E-03

(   8)  ERROR                       320   2.66573E-03

        TOTAL                       359

:F,1,8
                                          5%     1%
DEGREES OF FREEDOM      F VALUE
(     1,     320):      12.5629       3.84   6.63

:F,2,8

DEGREES OF FREEDOM      F VALUE
(     4,     320):       3.0774       2.37   3.32

:F,3,8

DEGREES OF FREEDOM      F VALUE
(     4,     320):       3.0774       2.37   3.32

:F,4,8

DEGREES OF FREEDOM      F VALUE
(     3,     320):       9.9007       2.60   3.78

:F,5,8

DEGREES OF FREEDOM      F VALUE
(     3,     320):       7.5496       2.60   3.78

:F,6,8

DEGREES OF FREEDOM      F VALUE
(    12,     320):       2.7928       1.75   2.18

:F,7,8

DEGREES OF FREEDOM      F VALUE
(    12,     320):       2.0327       1.75   2.18
```