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Task-Dependent Effects of Dicofol (Kelthane) on Learning in the Honey Bee *(Apis mellifera)*

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Honey bees contribute substantially to the pollination of various wild plants and food crops; the annual value of agricultural crops benefiting from honey bee pollination is estimated at as much as $20 billion/year in the United States alone (Southwick & Southwick, 1992). Evidence exists that sublethal doses of insecticides may be decreasing the number of honeybee colonies available for pollination and reducing the honey bees' effectiveness as pollinators: Sublethal doses of deltamethrin, for example, disrupt the homing flight of honey bees (Vandame, Meled, Colin, & Belzunces, 1995), while parathion disrupts the communication dance of foragers (Schricker & Stephen, 1970). In addition to the disruption of natural behavior, it is known that sublethal exposure to permethrin retards learning as measured by the classical conditioning of proboscis extension (Mamood & Waller, 1990; Taylor, Waller, & Crowder, 1987).

The purpose of the present experiment is to examine the effects of dicofol, a chlorinated hydrocarbon insecticide and a chemical analog of DDT, on the learning ability of the honey bee, *Apis mellifera* .Dicofol is considered nontoxic to most insects. Its use is primarily as an acaricide, although it can be effective on other insects by altering their natural behavior (e.g., Walgenbach & Wyman, 1987). Previous research has shown that dicofol may represent a danger to a host of organisms other than its intended target. For example, Ramana Rao, Surendranath, and Kodavanti (1990) report evidence of toxic stress in the penaeid prawn, and it is known that dicofol results in the thinning of eggshells (Bennett, Dominguez, & Griffis, 1990). Given the wide use of dicofol (Clark, 1990), a noticeable gap in the literature exists given that no studies have been done assessing its effects on learning. In this study, classical conditioning of proboscis extension was used to measure dicofol's effect on learning. This method was used rather than a more naturalistic technique such as the free-flying paradigm because of greater control of training variables known to influence learning, In addition, by using a laboratory technique known to be effective in similar studies (Mamood & Waller, 1990; Taylor et. al., 1987) there is the greater possibility of relating dicofol's chemical and physiological effects (see Matsumura (1985) for review) directly to the animal's behavior.

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MATERIALS AND METHODS

The laboratory colony was obtained from a local apiary that maintains colonies in an insecticide free environment. A colony containing approximately 80,000 individuals was acquired and moved to the roof of a three story building on campus. The roof and area around the building are known to be insecticide free. Subjects were taken from the laboratory colony around 10:00 am on the day prior to use. They were captured individually in glass vials and taken to the laboratory, where the vials were placed in an ice water bath. When the bees became inactive enough to permit handling, they were removed from the vials and secured in individual restraining harnesses by a small strip of duct tape placed between the head and thorax and fastened to the sides of the harness. No attempt was made to determine the age of the subjects. Materials consisted of the metal harnessing tubes, a ventilation chamber to prevent the accumulation of the conditioned stimulus scent (CS) in the testing area, and plastic 20 cc syringes to present olfactory conditioned stimuli (for details see Smith, Abramson, and Tobin, 1991). Additionally, a 1 cc syringe was used to feed the honey bees sucrose solution (the unconditioned stimulus, or US) and a 25µL syringe to feed dicofol/sucrose solution to the group receiving treatment.

In Experiment 1, 64 bees were divided randomly into four groups of 16 subjects each. Two of these groups were fed 20 µL of 50% (by weight) sucrose solution, and served as controls; the remaining two groups formed the experimental portion of the design. Experimental subjects were fed 10 µL dicofol diluted in the proportion suggested on the manufacturer's label with 50% sucrose solution (to insure ready consumption) and an additional 10 µL 50% sucrose solution.

Twenty-four hours after feeding, one control and one experimental group were classically conditioned. The CS consisted of a 3 sec presentation of cinnamon odor, followed immediately by a US consisting of antennal touch to elicit proboscis extension and a subsequent 2 sec delivery of sucrose to the proboscis. The intertrial interval was 10 min. Twelve training trials were conducted, followed by 12 extinction trials in which the US was omitted. Learning was considered to have taken place if the bee extended its proboscis after onset of the CS stimulus, but before presentation of the US.

An unpaired conditioning procedure was conducted on the remaining two groups (one untreated and one treated with dicofol) to ensure that any increase in the probability of proboscis extension to the CS was the result of CS-US pairing and not a nonassociative effect such as sensitization. Unpaired CS-US stimuli were presented in pseudorandom order. For half the animals stimulus presentations consisted of three successive sequences of CS US US CS US CS CS US. For the remaining animals the sequences consisted of US CS CS US CS US US CS; the interval between stimulus presentations was 5 min.

In Experiment 2, a differential conditioning paradigm was employed. Sixty-four bees were randomly divided into a control and an experimental group, using treatment conditions described in Experiment 1. Groups were classically conditioned using a differential conditioning procedure which allows for the use of fewer animals per group and for each animal to serve as its own control (Abramson, 1994). For our purposes, differential conditioning represents a more complex learning task because the animal must now discriminate between two explicit cues -- a CS+, associated with feeding, and a CS-, which is not associated with feeding. The CS odors used were cinnamon oil and a generic copy of the fragrance “Poison”, chosen because of their similar salience. Acquisition and extinction phases were each composed of six CS+ and six CS- trials, so that a total of twelve acquisition and twelve extinction trials were conducted. For half the animals the presentation of the CSs consisted of three successive sequences of CS+ CS- CS- CS+ CS- CS+ CS+ CS-. For the remaining animals, CS+ and CS- presentation was reversed. Learning was considered to have taken place if the bee extended its proboscis afler onset of the CS+ stimulus but before presentation of the US, and if the bee stopped responding to the CS-, which was not paired with a feeding

Statistical analyses were conducted separately for each experiment using SAS. Data were examined for differences between treated and untreated groups over trials.

RESULTS AND DISCUSSION

Figure 1 shows the results of Experiment 1. The control group (not pretreated with dicofol) begins to respond to the CS by the second trial, reaching an 80% probability of response by the third CS-US pairing. In contrast, the group treated with dicofol reached only a 60% probability of response. This difference in acquisition carries over into the extinction phase as well, when the CS is no longer paired with a sucrose feeding; extinction is a common method used to measure the persistence of response (Abramson 1994) and is considered an important behavioral measure. It is important to note that in relation to the unpaired control measure (figure 2), both the control and experimental groups learned. However, the level of learning and its persistence differed greatly. In a one-tailed hypothesis, an ANOVA showed a significant difference between groups in the acquisition phase, $\underline{F}\,(1,30) = 4.087$, $\underline{p} < .05$, and in the extinction phase, $\underline{F}\,(1,30) = 3.641$, $\underline{p} < .05$. These results are corroborated by the ANOVA results for the unpaired groups, $\underline{F}\,(1,30) = 4.000$, $\underline{p} < .05$.

Experiment 2 found no differences between control and experimental groups in the differential conditioning paradigm (Figure 3). Acquisition of the CS+, $\underline{F}\,(1,62) = 0.017$, $\underline{p} = .448$, and the CS-, $\underline{F}\,(1,62) = .794$, $\underline{p} = .195$, reflects a similarity of acquisition response between control and experimental groups. This similarity in learning between groups is further supported by results of extinction of the CS+, $\underline{F}\,(1,62) = .0572$, $\underline{p} = .226$, and the CS-, $\underline{F}\,(1,62) = .026$, $\underline{p} = .436$.

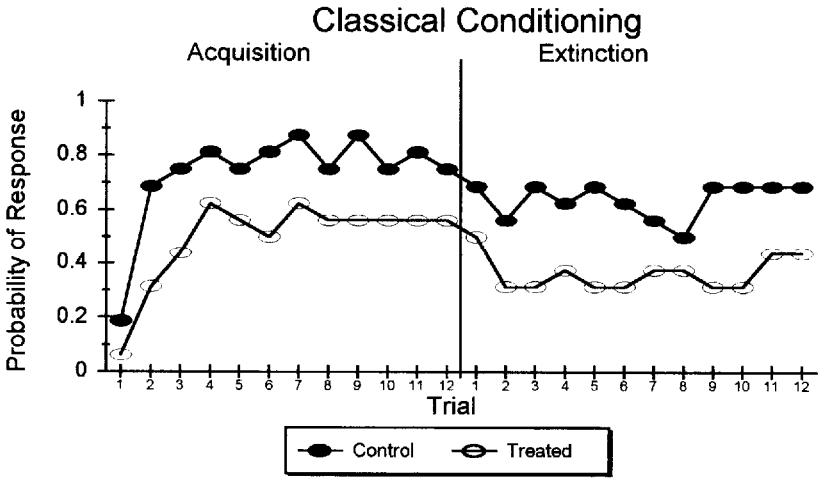

Figure 1. Classical conditioning of control vs. experimental groups: a significant difference in performance was found between control and experimental groups in acquisition ($\underline{p} < .05$) and in extinction ($\underline{p} < .05$),

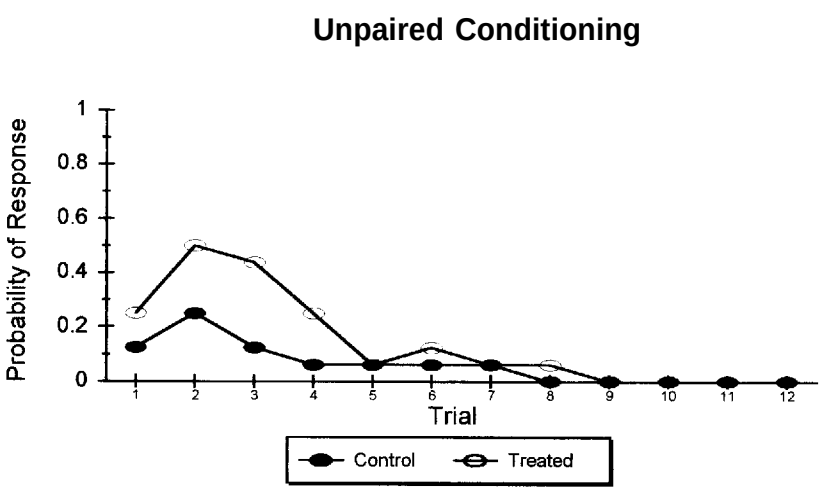

Figure 2. Unpaired conditioning of control vs. experimental groups: experimental animals differed significantly from controls ($\underline{p} < .05$), showing high sensitization after treatment with dicofol. This indicates that the experimental group's probability of response measured in the classical conditioning procedure may be artificially high due to sensitization effects.

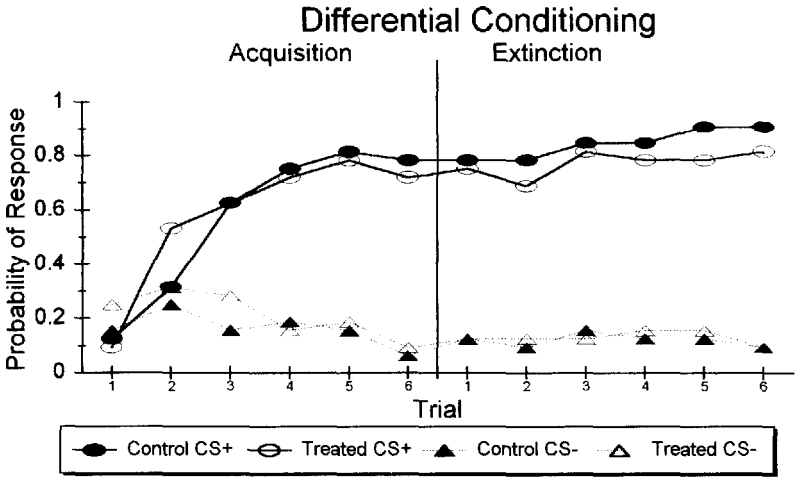

Figure 3. Differential conditioning of control vs. experimental groups: no differences between control and treated groups were found in acquisition on CS+ ($\underline{p} > .05$) or CS- ($\underline{p} > .05$) trials, or in extinction on CS+ ($\underline{p} > .05$) or CS- ($\underline{p} > .05$) trials.

The retardation of acquisition and the lower asymptotic performance seen in the simple Pavlovian situation in which the animal must associate a single CS with a US, and the lack of such an effect when the animal must distinguish between two CSs -- only one of which is paired with a US -- represents an interesting interpretive problem. At the outset of these experiments we had expected findings similar to those of Medved, Spynu, and Kagan (1964) in which increased latency of conditioned reflexes was observed to various toxicants, even though our study involved a chemical considered to be less toxic and delivered in far smaller amounts. Unexpectedly, performance on the differential conditioning task was unaffected.

Dicofol's mode of action is thought to involve the suppression of octopamine, an important neurotransmitter in the honey bee. However, because dicofol is metabolized by insects within a few hours after exposure, and octopamine levels are known to return to normal in a similar time frame, it may be that dicofol affects some other physiological system involved in learning as well. One possible hypothesis is that simple reflexes, such as that exercised in the simple Pavlovian paradigm, are more affected by the prolonged negative neuronal afterpotential, symptomatic of dicofol exposure (Matsumura, 1985), than are sensory discrimination functions. Consider that in any classical conditioning situation with a single conditioned stimulus, the organism is required to discriminate that CS from all background signals and noise that might serve as potential CSs. With the increased neuronal "noise" induced by dicofol exposure, an animal that relies on its olfactory ability as much as the honey bee may find the single CS situation a more difficult problem than discriminating between two CSs, which under normal circumstances is considered the more complex task.

Accordingly, our results indicate task dependent behavioral effects of sublethal amounts of insecticides generally considered innocuous to the honey bee (Johansen, 1979) and point to the importance of continued investigation into behavioral effects of agricultural chemical use.

Task-dependent effects have also been found when operant conditioning methodology is used to examine the behavioral effects of drugs. For example, effects of pentobarbital on stimulus control of responding are different when free operant or discrete trials procedures are used (Katz, 1982). The importance of task specificity in the interpretation of results is also found in the behavioral contrast literature; rats demonstrate successive negative contrast in lever press tasks but not in locomotion tasks (Rashotte, 1979). The importance of task specificity in evaluating the effects of chemicals on behavior suggests that in addition to determining dose response curves for a given chemical, "task response curves" should be constructed as well, particularly in instances of sublethal exposure. In the construction of such a measure, the dosage of a pesticide is held constant while the type of task is manipulated as the independent variable. Clearly, a complete picture of the effects of agricultural chemicals can only be obtained by considering behavioral as well as physiological measures.

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REFERENCES

Abramson CI (1994) A primer of invertebrate learning: The behavioral perspective. American Psychological Association, Washington, DC.

Bennett JK, Dominguez SE, Griffis WL (1990) Effects of dicofol on mallard eggshell quality. Arch Environ Contam Toxicol 19:907-912

Clark DR, Jr. (1990) Dicofol (Kelthane) as an environmental contaminant: A review. Fish and Wildlife Technical Report 29, United States Department of the Interior Fish and Wildlife Service, Washington, DC.

Johansen CA (1979) Protecting honey bees from pesticides. Bee World 60: 109-127,

Katz JL (1982) Effects of drugs on stimulus control of behavior. I. Independent assessment of effects on response rates and stimulus control. J Pharmacol Exp Ther 33:255-262

Mamood AN, Wailer, GD (1990) Recovery of learning responses by honeybees following a sublethal exposure to permethrin. Physiol Entomol 15:55-60

Matsumura F (1985) Toxicology of insecticides. Plenum Press, New York

Medved LI, Spynu EI, Kagan, IuS (1964) The method of conditioned reflexes in toxicology and its application for determining the toxicity of small quantities of pesticides. Residue Rev 6:42-74

Ramana Rao KV, Surendranath P, Kodavanti PRS (1990) Levels of transaminases in tissues of the penaeid prawn, *Metapenaeus monoceros* (Fabricius) following sublethal Kelthane exposure. Bull Environ Contam Toxicol 44:114-120

Rashotte ME (1979) Reward training: Contrast effects. In: Bitterman ME, Lolordo VJ, Overmier JG, and Rashotte ME (eds) Animal Learning: Survey and Analysis. Plenum, New York, p 195-239.

Schricker B, Stephen, WP (1970) The effect of sublethal doses of parathion on honeybee behaviour. I. Oral Administration and the communication dance. J Apic Res 9:141-153

Smith BH, Abramson CI, Tobin TR (1991) Conditional withholding of proboscis extension in honey bees *(Apis mellifera)*during discriminative punishment, J Comp Psychol 105:345-356

Southwick EE, Southwick L (1992) Estimating the economic value of honey bees (Hymenoptera: Apidae) as agricultural pollinators in the United States, J Econ Entomol 85:621-633

Taylor KS, Waller GD, Crowder LA (1987) Impairment of a classical conditioning response of the honey bee *(Apis mellifer a*L.) by sublethal doses of synthetic pyrethroid insecticides. Apidologie 18:243-252

Vandame R, Meled M, Colin M, Belzunces LP (1995) Alteration of the homing-flight in the honey bee *Apis mellifer a*L. exposed to sublethal doses of deltamethrin. Environ Toxicol Chem 14:855-860

Walgenbach JF, Wyman JA (1987) Dicofol as a feeding inhibitor of the Colorado potato beetle (Coleoptera: chrysomelidae). J Econ Entomol 80:1238-1245