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Original article

The effects of sublethal exposure to diazinon, carbaryl and resmethrin on longevity and foraging in *Apis mellifera* L.

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Summary — Topical, sublethal applications of three insecticides, diazinon, carbaryl and resmethrin, were given to worker honey bees (*Apis mellifera* L.) of two age groups, 0 and 14 days. For newly emerged workers, carbaryl was the most hazardous in sublethal amounts, adversely affecting both longevity and foraging age. Resmethrin was intermediate in effect, and diazinon the least hazardous. This is different from mortality studies in which the effects of carbaryl and diazinon were similar, and resmethrin the most toxic.

For 14-day-old workers, there were only two statistically significant differences affecting foraging in the sublethal studies, and these were not conclusive. However, in mortality studies carbaryl was the least toxic, diazinon intermediate and resmethrin the most toxic pesticide.

Newly emerged worker honey bees were more sensitive to pesticide exposure, in both acute and sublethal effects, than older workers. In order to evaluate pesticide hazards to the honey bee, both laboratory and field tests should be used. A field bioassay involving sublethal exposure effects on longevity and foraging may be useful in this regard.

***Apis mellifera* — longevity — foraging — sublethal dose — insecticide**

Résumé — Action de doses sublétales de diazinon, de carbaryl et de resméthrine sur la longétivité et le butinage de l'abeille *(Apis mellifica* L.). *On a montré que de petites quantités de pesticides pouvaient causer des effets néfastes autant aux abeilles prises individuellement qu'à la colonie dans son ensemble. Cette étude évalue l'effet de l'exposition sublétale aux pesticides sur la longévité et le butinage des ouvrières de colonies standard de plein champ.*

On a fait des applications topiques sublétales de 3 insecticides, le diazinon, le carbaryl et la resméthrine, sur des ouvrières d'abeilles de deux groupes d'âge; 0 et 14 jours. On a testé les trois pesticides sur les abeilles naissantes et seulement le diazinon et le carbaryl sur les abeilles âgées de 14 jours. On a dilué chaque pesticide dans l'acétone pour obtenir des taux de mortalité d'environ 5, 10 et 25%, tels qu'ils sont déterminés dans les études de toxicité aigüe. Les ouvrières testées ont reçu 1 µl de la solution choisie et les témoins 1 µl d'acétone. La survie des ouvrières a été contrôlée tous les 10 à 14 jours. On a observé tous les troisièmes jours le butinage en bloquant l'entrée de la ruche pendant 30 minutes. Les abeilles testées trouvées hors de la ruche ont été classées comme butineuses.

Les 3 colonies diffèrent significativement par la longévité et l'âge des butineuses (Tableau II). Pour cette raison, dans les analyses suivantes les 3 colonies ont été considérées séparément.

Pour les ouvrières naissantes, le carbaryl est le plus nocif aux doses sublétales, affectant à la fois la longévité et l'âge des butineuses (Tableau III). La resméthrine a un effet intermédiaire et le diazinon est le moins nocif. Ces résultats sont différents de ceux obtenus avec la toxicité aigüe, pour laquelle le carbaryl et le diazinon produisent des effets semblables et la resméthrine est la plus toxique (Tableau I).

Chez les ouvrières âgées de 14 jours, on n'a trouvé que 2 différences statistiquement significatives pour les doses sublétales, mais elles ne sont pas concluantes (Tableau IV). Toutefois, en ce qui concerne la toxicité aigüe, la carbaryl s'est montré le moins toxique, le diazinon d'effet intermédiaire et la resméthrine la plus toxique (Tableau I).

Les relations entre les 3 insecticides ne sont pas les mêmes dans les tests de toxicité aigüe et dans les tests des effets sublétaux. La resméthrine, qui a la toxicité aigüe la plus élevée, produit des effets sublétaux moindres que ceux du carbaryl, qui a la toxicité aigüe la plus faible. Les ouvrières naissantes sont plus sensibles aux pesticides que les ouvrières plus âgées, tant dans les effets sublétaux qu'aigus. Afin d'évaluer la toxicité des pesticides pour les abeilles, il est nécessaire de faire des tests à la fois au laboratoire et sur le terrain. A cette fin, un test biologique prenant en compte les effets d'une exposition sublétale sur la longévité et le butinage serait utile.

Apis mellifica — *longévité — butinage — dose sublétale — insecticide*

Zusammenfassung — Wirkung subletaler Dosen von Diazinon, Carbaryl und Resmethrin auf Langlebigkeit und Sammelaktivität bei der Honigbiene (*Apis mellifera* L.). *Auch geringe Mengen von Pestiziden zeigen oft zerstörerische Wirkungen sowohl auf die einzelne Biene als auch auf die Kolonie als Ganzes. In dieser Untersuchung sollte der Einfluß von subletalen Dosen von Pestiziden auf die Langlebigkeit und das Sammelverhalten von Arbeitsbienen in Standard-Freilandvölkern beurteilt werden.*

Arbeitsbienen (Apis mellifera) *aus zwei verschiedenen Altersgruppen (0 und 14 Tage alt) wurden mit subletalen Dosen von 3 Insektiziden (Diazinon, Carbaryl und Resmethrin) topikal behandelt. Alle drei Chemikalien wurden bei frisch geschlüpften Arbeitsbienen getestet, aber nur Diazinon und Carbaryl bei den 14 Tage alten Bienen. Jedes Insektizid wurden in Aceton aufgelöst, und zwar so, daß Mortalitätsstufen von ungefähr 5%, 10% und 25% vorgegeben waren, die aus Untersuchungen der akuten Toxizität bestimmt wurden. Die Testbienen erhielten 1 µl der jeweiligen Konzentration und die Kontrollbienen 1 µl Aceton. Die Überlebensrate der Arbeiterinnen wurde 10-14täglich kontrolliert. An jedem dritten Tag wurde die Sammelaktivität protokolliert. Dazu wurde das Flugloch für 30 min geschlossen. Testbienen, die sich außerhalb des Stockes einfanden, wurden als Sammlerinnen gezählt.*

Die drei in diesem Versuch verwendeten Kolonien unterschieden sich signifikant in der Langlebigkeit und im Sammelalter (Tab. II). Dehalb wurden die drei Völker bei den folgenden Analysen separat betrachtet. Bei frisch geschlüpften Bienen war Carbaryl in subletalen Dosen das gefährlichste Gift. Es wirkte sich nachteilig sowohl auf die Langlebigkeit als auch auf das Sammelalter aus (Tab. III). Resmethrin war im Effekt ein mittelmäßiges und Diazinon das am wenigsten gefährliche Gift. Dies war bei den Tests über die akute Giftwirkung anders, dort waren Carbaryl und Diazinon gleich und Resmethrin das gefährlichste Gift (Tab. I).

Bei 14 Tage alten Bienen ergab die Untersuchung der Wirkung von subletalen Dosen nur zwei statistisch signifikante Unterschiede und diese waren nicht schlüssig (Tab. IV). Jedoch war bei der Untersuchung der akuten Wirkung Carbaryl das am wenigsten giftige, Diazinon ein mittelmäßiges und Resmethrin das giftigste Agens (Tab. I).

Das Verhältnis der drei Insektizide bei der Untersuchung der akuten Giftwirkung war also verschieden vom Verhältnis bei subletalen Dosen. Resmethrin, das am meisten akut giftige, hatte weniger nachteilige Effekte als Carbaryl, das am wenigsten akut giftige. Frisch geschlüpfte Bienen waren empfindlicher gegen Pestizide als ältere Bienen, sowohl bei akuten wie bei subletalen Giftwirkungen. Um die Gefährlichkeit von Pestiziden zu beurteilen, sollten daher Labor- und Feldversuche durchgeführt werden. Ein Bioassay zur Untersuchung der Effekte subletaler Dosen auf die Langlebigkeit und das Sammelverhalten wäre sinnvoll.

Apis mellifera — *Langlebigkeit — Sammelaktivität — subletale Dosen — Insektizide*

Introduction

The honey bee (*Apis mellifera* L.) is recognized as our most important crop pollinator (McGregor, 1976; NRCC, 1981; Crane and Walker, 1983). North American agricultural products valued at some $ 20 billion (U.S.) depend in some way on the honey bee (Levin, 1984; Winston and Scott, 1984). Pesticide use has also become an essential part of modern agriculture, and the honey bee is susceptible to many commonly used chemicals (Anderson and Atkins, 1968; Atkins, 1975; Johansen, 1977, 1979, 1983; NRCC, 1981). It is important, therefore, to understand how to protect this pollinator from pesticide exposure and to evaluate the hazards different chemicals pose.

A number of different factors should be considered when pesticides are evaluated for their hazard to the honey bee. Acute toxicity studies which assess mortality have been used almost exclusively in these considerations. However, exposure to sublethal concentrations of pesticides in the field also could have adverse effects on colony success, honey production and crop pollination (NRCC, 1981). Chronic feeding of such commonly used pesticides as acephate (Stoner *et al.*, 1985), carbaryl (Winterlin and Walker, 1973), dimethoate (Waller and Barker, 1979; Waller *et al.*, 1979; Barker *et al.*, 1980; Stoner et al., 1983) and carbofuran (Stoner *et al.*, 1982) to whole colonies reduced honey production, brood rearing and worker population size. Sublethal topical treatments to individual workers resulted in impairment in the dance language with methyl-parathion (Schricker and Stephen, 1970), reduced lifespan with malathion or diazinon (Smirle *et al.*, 1984; MacKenzie, 1986); and altered foraging patterns with diazinon (MacKenzie, 1986) and permethrin (Cox and Wilson, 1984). It is important therefore to evaluate both acute toxicity and sublethal effects of a pesticide when gauging its hazard to the honey bee.

A bioassay using longevity as a measure to evaluate sublethal pesticide exposure to the honey bee has been suggested (Smirle *et al.*, 1984). This study was designed to examine the sublethal effects of three insecticides, and to evaluate the effect of treatment age on the results. In addition to longevity, foraging was also studied, since increasing foraging activity and earlier foraging age have both been correlated with reduced longevity (Free and Spencer-Booth, 1959; Sekiguchi and Sakagami, 1966; Winston and Katz, 1981, 1982; Winston and Fergusson, 1985). Inclusion of this task, foraging, may increase the sensitivity of the bioassay.

Materials and Methods

Chemicals

Commercial formulations of the three insecticides were used. The organophosphorous insecticide diazinon was formulated as a 12.5% emulsifiable concentrate (Later's Diazinon, PCP Act. No. 11437, Later Chemicals Ltd., Richmond, BC), the carbamate carbaryl as a 22.5% concentrate (Wilson Liquid Sevin Carbaryl insecticide, PCP Act. No. 17971, Wilson Laboratories Inc., Dundas, Ontario), and the pyrethroid resmethrin as a 0.25% solution (House Plant Insect Killer, PCP Act. No. 16219, Later Chemicals Ltd., Richmond, BC). The formulations were diluted in acetone to the required concentrations. The formulation of carbaryl was first diluted (1:10) with distilled water to facilitate further mixing with acetone.

The colonies

Test colonies were located at Simon Fraser University, Burnaby, BC and studies conducted from June to September 1985. Workers to be treated were obtained from a single colony (not one of the experimental colonies) to minimize genetic variation. Combs containing emerging workers were placed overnight in an incubator at 34°C and 50—70% RH. Newly emerged workers were marked on the dorsal surface of the thorax with coloured and numbered plastic labels (Opalithplattchen, Chr. Craze, KG, Endersbach, FRG), and either treated immediately or introduced into a test hive and removed and treated at the desired age.

Three colonies in standard Langstroth deep equipment were used. These colonies consisted of 2 boxes (supers) with 10 frames each, enough workers to cover most of the frames, and a healthy laying queen. A third super was added 10 July to alleviate colony crowding. This super was removed on 16 August for honey extraction. Experimentation began 18 June and continued until 5 September for colonies 1 and 3, and 1 August for colony 2. Queen problems occured in colonies 1 and 3. Colony 1 was found to be queenless on 10 August and was requeened on 16 August. A virgin queen was found and removed from colony 3 on 27 June. Subsequently, the original queen appeared to be laying normally, but was superseded sometime before 18 July.

Treatments

Honey bee workers were treated with a topical insecticide application on the dorsal surface of their abdomens. An SMI Micro/Pettor-A® was used to dispense 1 µl treatment solution to each insect. Workers were held by the hind leg with forceps while the insecticide was applied. A group of workers were treated with 1 µl acetone as a control in all experiments. Concentrations causing approximately 5%, 10% and 25% mortalities were used for each pesticide and each age group in the sublethal experiments.

Acute toxicity tests (LD_{50}) for the 3 chemicals were conducted to choose the dose range for sublethal exposure. Between 40 to 50 workers were treated at each of the 4 to 7 doses in the mortality tests. Newly emerged workers (0 days) were treated and placed into holding cages, while the older age group (14 days) was marked at emergence, placed into standard colonies, removed at the appropriate age (grasped by their hind legs with forceps and placed into cages) immobilized with carbon dioxide and treated, and placed into holding cages. Sugar syrup (50%) and water were supplied *ad libitum via* gravity feeding bottles. Mortality counts were taken at 24 h and data analyzed following the WHO for insecticide susceptibility tests (Swarrop, 1966). The resultant regression lines were used to calculate doses corresponding to the desired mortality levels.

For experimental purposes, newly emerged honey bee workers (0 days of age) were treated with all 3 pesticides, while 14-day old workers were treated with diazinon and carbaryl only, due to time constraints. This left a number of untreated workers, exposed to neither pesticide nor acetone, in each of the 3 colonies. Newly emerged bees were treated on 18—20 June with the desired dosage of pesticide, and placed in the hive through the feeding hole in the top board. Treatments for the 14-day old bees were carried out on 3 July for colonies 1 and 3, and 4 July for colony 2. The hives were taken apart and frames examined one by one. Marked workers were picked up by the hind leg, treated, held in a cage until all treatments for the hive were completed, and reintroduced through the feeding hole. The number of workers treated per dose ranged from 50—65.

Observations

Entrance observations began on 22 June and continued every third day until most of the bees were dead. Entrances were blocked with wire screen for 15 min, preventing any workers from leaving or entering, then the numbers of marked workers at the entrance were recorded for another 15 min. All returning workers were considered to be foragers (Winston and Katz, 1982). Every 10—14 days the colonies were checked for surviving workers by removing and examining carefully each frame and recording all marked workers.

Data analyses

Acute toxicity data were analyzed using the WHO method for insecticide susceptibility tests (Swaroop, 1966). The criterion of nonoverlapping 95% confidence limits was used to determine significant differences between LD_{50}s for each age group.

For sublethal studies, longevity and the first day and duration of foraging were analyzed. Longevity in days was determined as the midpoint between the last day seen and the subsequent day survivorship was examined. Foraging was considered to commence on the first day a worker was seen outside the hive in the entrance observations. Duration of foraging was the number of days between the first and last time a worker was seen outside the hive. The treated controls and the 3 dosages of each pesticide in each age group initially were compared by one-way analysis of variance and Duncan's multiple range test (Zar, 1984). Then to examine the effects of different pesticides, all pesticide treatments in one age group were analyzed by two-way analysis of variance (*i.e.*, pesticide and dosage) and the Student—Newman—Keuls multiple comparison test (Zar, 1984).

To evaluate the possible effects of differences in colony conditions, data from the marked, untreated workers in the colonies were compared by one-way analysis of variance and Student—Newman—Keuls multiple comparison test (Zar, 1984) for each of the 3 categories examined.

Results

Acute toxicity tests of the 3 chemicals to newly emerged workers showed carbaryl and diazinon to be of similar toxicity and resmethrin the more acutely toxic (Table I). The relationships between the 3 chemicals for the 14-day old workers were : carbaryl was the least toxic pesticide, resmethrin the most, with diazinon in between. In addition, all chemicals were found to be significantly more acutely toxic to newly emerged workers than 14-day old workers.

Marked, untreated workers in the 3 test colonies were significantly different in longevity and the first day of foraging (Table II). Colonies 1 and 2 were similar in duration of foraging, but statistically different from colony 3. Therefore, further analyses of sublethal data were carried out separately for the 3 colonies.

Sublethal exposure to carbaryl had adverse effects on longevity and foraging

Table I. Acute toxicities of 3 pesticides[1] and dosages used to treat 2 age groups of worker honey bees.

Worker age (days)	*Pesticide*	*N*[2]	*LD_{50}*[3]	*95% CL*[3]	*Dosages used to treat workers*[3]		
					LD_5	*LD_{10}*	*LD_{25}*
0	Diazinon	200	0.052b[4]	0.049 — 0.056	0.035	0.040	0.045
	Carbaryl	280	0.055b	0.050 — 0.060	0.023	0.028	0.037
	Resmethrin	320	0.045a	0.041 — 0.049	0.020	0.025	0.032
14	Diazinon	175	0.115b	0.108 — 0.123	0.085	0.090	0.100
	Carbaryl	515	0.142c	0.133 — 0.151	0.084	0.094	0.115
	Resmethrin	350	0.076a	0.070 — 0.082	0.045	0.050	0.060

[1] Based on 24 h mortality, bees held in dosage groups in cages; analyzed by the WHO method for insecticide susceptibily tests (Swaroop, 1966).
[2] Total number of insects treated to estimate concentration/mortality regressions.
[3] LD_{50}, CI and dosages expressed as μg per bee.
[4] For each age group, LD_{50}s followed by the same letter are not significantly different based on the criterion of non-overlapping 95% CL.

in newly emerged workers (Table III). In colonies 1 and 2, control workers lived longer than those treated with carbaryl, and began foraging later in colony 1 and earlier in colony 2. In colony 3, the only significant difference between control and pesticide-treated bees was that bees treated with carbaryl LD_{25} continued foraging longer than either of the other 2 dosages or the control.

When the toxicities of all 3 pesticides were compared for newly emerged workers, carbaryl caused more adverse effects than the other two chemicals, with a general pattern of decreased longevity in all 3 colonies, and the latest foraging in colony 1 (Table III). Longevity was intermediate in bees treated with resmethrin, and longer in those treated with diazinon. There were no effects on foraging due to resmethrin or diazinon treatment.

When workers were treated at 14 days of age with carbaryl or diazinon, there were 2 statistically different results (Table IV). The control group continued foraging longer than any carbaryl treatment in colony 2. In colony 3, foraging began earliest in those workers treated with diazinon LD_5, latest in those treated with carbaryl LD_5, and was intermediate in the other treatment groups.

Discussion

This paper has evaluated both lethal and sublethal effects of 3 different insecticides on the honey bee. Carbaryl was found to be more harmful to newly emerged workers than diazinon or resmethrin in sublethal tests, although resmethrin was the most acutely toxic pesticide. There were few effects on 14-day old workers in sublethal experiments; but once again, in acute tests resmethrin was the most toxic, while carbaryl was the least toxic insecticide. These toxicity rankings are the same as those previously published (NRCC, 1981), and all 3 chemicals have been placed into groups with high hazard to the honey bee (Johansen, 1979; Crane and Walker, 1983).

In rating pesticide hazard to honey bees, acute toxicity values of mortality are often relied on (Anderson and Atkins, 1968; Atkins, 1975; Johansen, 1977; NRCC, 1981), even when field experimentation is incorporated into the hazard rating. The relationships between chemicals in laboratory mortality and field studies have been shown to vary in some cases. For example, the synthetic pyrethroids, such as permethrin, have high

Table II. Comparison of longevity and foraging of marked, untreated worker honey bees in the 3 standard colonies.

Category		*Mean ± SE*			*Significance*[1]
		Colony 1	*Colony 2*	*Colony 3*	
Longevity		22.2 ± 0.7 a[2]	19.5 ± 0.4 b	39.6 ± 1.2 c	0.01
Forage	- 1st day	23.5 ± 0.7 a	20.6 ± 0.6 b	43.1 ± 1.2 c	0.01
	- Duration	2.8 ± 0.3 a	2.4 ± 0.3 a	4.2 ± 0.6 b	0.01

[1] Probabilities of analysis of variance F-test.
[2] Means followed by the same letter are not significantly different (Student — Newman — Keuls multiple comparison test [Zar, 1984]).

Table III. Longevity, foraging age and duration of worker honey bees treated at emergence with 3 concentrations of diazinon, carbaryl and resmethrin, and acetone-treated control.

Hive No.	*Insecticide*	*Mean ± SE at specified dose* [1]			*Control*
		LD_5	LD_{10}	LD_{25}	
		Longevity			
1	Diazinon	25.2 ± 1.6 bc	25.8 ± 1.3 c	26.0 ± 2.4 c	
	Carbaryl	20.6 ± 5.0 a	20.8 ± 3.4 a	20.1 ± 1.8 a+	27.2 ± 1.5
	Resmethrin	25.4 ± 1.1 c	24.5 ± 1.2 bc	23.4 ± 1.2 b	
2	Diazinon	20.8 ± 1.1 b	22.0 ± 0.8 c	19.1 ± 2.1 b	
	Carbaryl	14.2 ± 1.6 a+	14.0 ± 1.8 a+	20.2 ± 1.4 bc	20.7 ± 0.9
	Resmethrin	19.6 ± 1.1 bc	20.5 ± 1.0 bc	19.7 ± 0.8 bc	
3	Diazinon	37.8 ± 3.1 f	38.1 ± 2.1 f	33.0 ± 4.4 d	
	Carbaryl	23.0 ± 3.5 a	30.0 ± 3.8 c	35.2 ± 3.4 e	35.1 ± 3.0
	Resmethrin	27.8 ± 3.1 b	28.1 ± 2.9 b	38.4 ± 2.4 f	
		Foraging Age			
1	Diazinon	22.5 ± 1.3 ab	23.0 ± 1.1 ab	24.0 ± 2.5 b	
	Carbaryl	24.4 ± 8.2 d+	24.5 ± 4.4 c	21.3 ± 1.6 a	23.1 ± 0.9
	Resmethrin	22.2 ± 1.0 ab	22.2 ± 1.2 ab	20.8 ± 1.1 a	
2	Diazinon	22.3 ± 1.2	21.3 ± 1.1	22.0 ± 2.3	
	Carbaryl	17.0 ± 1.0 +	17.3 ± 0.9 +	21.7 ± 1.6	20.5 ± 0.6
	Resmethrin	21.9 ± 1.3	21.1 ± 1.0	20.9 ± 1.0	
3	Diazinon	40.4 ± 3.0	35.7 ± 2.6	39.6 ± 5.3	
	Carbaryl	32.0 ± 4.9	40.3 ± 4.6	37.0 ± 6.4	37.0 ± 2.7
	Resmethrin	43.6 ± 2.5	38.4 ± 2.7	41.3 ± 2.3	
		Foraging duration			
1	Diazinon	1.8 ± 0.3	3.6 ± 0.8	4.6 ± 1.6	
	Carbaryl	2.1 ± 1.1	2.2 ± 0.8	3.3 ± 0.8	3.9 ± 1.0
	Resmethrin	3.9 ± 0.7	3.8 ± 0.8	3.2 ± 0.6	
2	Diazinon	2.2 ± 0.8	3.1 ± 0.7	1.4 ± 0.4	
	Carbaryl	1.8 ± 0.8	2.4 ± 0.9	2.0 ± 0.7	2.6 ± 0.5
	Resmethrin	1.6 ± 0.4	2.8 ± 0.8	1.9 ± 0.4	
3	Diazinon	1.5 ± 0.4	4.2 ± 1.4	9.8 ± 4.0 +	
	Carbaryl	3.0 ± 1.5	1.4 ± 0.3	1.5 ± 0.3	3.6 ± 1.0
	Resmethrin	3.2 ± 1.4	2.7 ± 0.8	5.5 ± 1.5	

[1] Mean ± SE includes workers seen at least 2 days after treatment, $P \leq 0.05$ from analysis of variance F-test; for each hive and each category, different letters indicate significant differences in means by the Student—Newman—Keuls multiple comparison test, and + indicates significant differences between the treatment mean and the control mean by Duncan's multiple range test (Zar, 1984).

Table IV. Longevity and foraging age and duration of worker honey bees treated at 14 days of age with diazinon and carbaryl and acetone-treated control.

Hive No.	*Insecticide*	*Mean ± SE at specified dose* [1]			*Control*
		LD_5	LD_{10}	LD_{25}	
		Longevity			
1	Diazinon	26.0 ± 0.8	28.3 ± 1.2	28.6 ± 1.4	28.4 ± 1.3
	Carbaryl	28.8 ± 1.3	28.4 ± 1.2	26.7 ± 1.0	
2	Diazinon	23.7 ± 0.4	23.0 ± 0.2	23.6 ± 0.4	24.2 ± 0.5
	Carbaryl	23.0 ± 0.2	23.3 ± 0.3	23.3 ± 0.3	
3	Diazinon	40.7 ± 1.8	45.0 ± 2.0	45.5 ± 1.4	44.8 ± 2.0
	Carbaryl	45.6 ± 1.7	45.6 ± 1.4	46.8 ± 1.5	
		Foraging age			
1	Diazinon	23.0 ± 1.1	23.9 ± 0.9	23.4 ± 0.9	24.7 ± 0.9
	Carbaryl	22.1 ± 1.2	25.0 ± 1.0	23.3 ± 1.3	
2	Diazinon	21.6 ± 0.8	20.7 ± 0.9	19.9 ± 0.8	21.5 ± 1.1
	Carbaryl	19.6 ± 0.8	20.2 ± 0.6	19.8 ± 0.6	
3	Diazinon	37.1 ± 2.1 a	42.6 ± 1.9 c	43.3 ± 1.8 c	41.1 ± 2.6
	Carbaryl	46.0 ± 1.8 d	41.1 ± 2.1 b	43.1 ± 1.9 c	
		Foraging duration			
1	Diazinon	4.0 ± 0.7	3.7 ± 0.7	3.9 ± 0.7	3.7 ± 0.6
	Carbaryl	4.9 ± 1.0	4.8 ± 0.9	4.9 ± 1.4	
2	Diazinon	2.6 ± 0.6	1.9 ± 0.4	2.2 ± 0.5	3.8 ± 0.9
	Carbaryl	1.7 ± 0.5 +	2.2 ± 0.5 +	1.8 ± 0.3 +	
3	Diazinon	5.4 ± 1.2	4.5 ± 0.9	4.5 ± 0.9	5.4 ± 1.3
	Carbaryl	4.1 ± 1.0	6.2 ± 1.3	4.4 ± 0.9	

1 Mean ± SE includes workers seen at leat 2 days after treatment, $P \leq 0.05$ from analysis of variance F-test; for each hive and category, different letters indicate significant differences in means by Student—Newman—Keuls multiple comparison test and + indicates significant differences between the treatment mean and control mean by Duncan's multiple range test (Zar, 1984).

acute toxicities in mortality tests and are of low hazard in the field due to low application rates and repellent action (Pike *et al.*, 1982; Shires *et al.*, 1984).

In this study the relationships between acute toxicity and sublethal effects also varied. Resmethrin, the most acutely toxic compound, was only intermediate in its sublethal effects, while carbaryl, which caused the most sublethal effects in this study, was not the most acutely toxic. Carbaryl has been found to be a serious problem for beekeepers, causing high mortality to field colonies (Johansen and Brown, 1972; Anderson and Glowa, 1984; Melksham *et al.*, 1985). In studies on residual toxicity of 10 pesticides, carbaryl was rated as the most hazardous to honey bees due to its persistence (Mansour *et al.*, 1984; Mansour and Al-Jalili, 1985). These 2 characteristics, adverse effects of sublethal exposure and a residual nature, may account for the beekeeping problems caused by carbaryl. These results suggest that, in addition to laboratory and field experimentation, sublethal exposure studies should be used when rating the hazards of chemicals to the honey bee.

Foraging and longevity are both categories that have been shown to be affected by sublethal exposure to pesticides (MacKenzie, 1986; and this study). Foraging and longevity are related, since earlier foraging activity results in reduced lifespan (Free and Spencer-Booth, 1959; Sekiguchi and Sakagami, 1966; Winston and Fergusson, 1985). Reduced longevity was a more consistent indicator of adverse sublethal pesticide exposure than age of first foraging, but neither of these categories were affected in all cases. Considering the number of factors, including worker activity, internal colony conditions and forage availability (Maurizio, 1950; Woyke, 1984; Winston and Fergusson, 1985), that can influence lifespan and foraging in the honey bee, perhaps this is to be expected. In an earlier study, Smirle *et al.* (1984) also found reduced longevity due to sublethal pesticide exposure. Similar results were obtained due to single sublethal exposure to diazinon in observation hives (MacKenzie, 1986). Probably other factors are more important in determining longevity and regulating temporal division of labour than pesticide exposure, which makes uncovering sublethal pesticide effects difficult.

Newly emerged workers were found to be more sensitive to pesticides, including both acute toxicity and sublethal exposure, than older workers. Previous studies on acute toxicities agree with this result (Koch, 1958/1959; Mayland and Burkhardt, 1970; Smirle *et al.*, 1984; MacKenzie, 1986). Differences in toxicities could be related to a number of factors, including differences in enzyme levels or activities and absorptivity of the cuticle. Other insects have been found to be more susceptible after moults and at emergence (Busvine, 1971). Enzymes which metabolize xenobiotics are found in the honey bee. Smirle and Winston (1988) found that the concentrations and activities of 2 important detoxification enzymes, glutathione S-transferases and the polysubstrate monooxygenases, were very low in newly emerged workers compared to any other age from 1 to 7 weeks. This alone could account for the great susceptibility of newly emerged workers to various insecticides.

Stress may also have been a factor in this study. Honey bees, especially newly emerged workers, are sensitive to stress in many forms such as narcosis (Beckmann, 1974; Ebadi *et al.*, 1980), sublethal pesticide exposure (Schricker and Stephen, 1970; Smirle *et al.*, 1984), and increased activity (Lindauer, 1953; Free and Spencer-Booth, 1959), and neural function has been shown to be adversely affected by some of these (Stephen and Schricker, 1970; Beckmann, 1974). As the 3 insecticides used in this study are all nervous system poisons, neural impairment may be involved in both the lethal and sublethal effects found.

This work suggests that an insecticide has both lethal and sublethal effects, which do not necessarily have the same relationship for each chemical. In rating pesticide hazards to the honey bee, a bioassay involving sublethal effects should be considered in addition to acute toxicities studies in the laboratory and the field. Longevity and foraging appear to be potential measures to use in the development of such a bioassay, although more work remains to be done to refine this technique. Evaluation of a wider range of pesticides, including herbicides and fungicides, should be made, and the relationship between acute and sublethal pesticide effects on the individual and the colony studied.

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