Pesticides and Honey Bees: The Danger of Microencapsulated Formulations

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Microcapsules designed to improve the safety and persistence of pesticides are about the same size as pollen. Foraging bees collect pollen contaminated with such microcapsules and pack both together into hive combs. Sustained release formulations remain toxic in food stores of the colony. This combination of accumulation and persistence produces prolonged toxicity for bee colonies.

When microencapsulated methyl parathion was registered for agricultural use in 1974, we began inquiring whether the toxicity to bees of such formulations differs from that of conventional formulations. Our thesis is that it does.

Controlled release formulations are not considered to be different from conventional formulations under the pesticide regulations of the United States. The mode of action is still the same. However, the accumulation of lethal dosage becomes modified by encapsulation.

Our report concerns the effects of microencapsulated methyl parathion on honey bees, *Apis mellifera* L. Bees are indispensible to agriculture as manageable pollinators. For example, almonds in California need 300,000 colonies each spring for effective cross pollination. Bees are also rented to pollinate such crops as alfalfa, avocado, blueberry, cranberry, and melon. Perhaps a third of our food depends directly or indirectly upon insect-pollinated plants. Methyl parathion is used widely on crops, and, contrary to expectations arising from the results of some laboratory tests, encapsulated methyl parathion readily destroys or damages bee colonies where bees visit sprayed areas.

PENNCAP-M® insecticide, produced by the Pennwalt Corporation, was the first commercial microencapsulated pesticide [1]. This formulation of methyl parathion gives improved insect control along with increased safety to mammals [2]. The capsule walls are a cross-linked, nylon-type polymer produced from sebacoyl chloride, ethylenediamine, diethylenetriamine, and polymethylene-polyphenyl-isocyanate [3]. The walls slow the dissipation of methyl parathion. In order to apply the encapsulated methyl parathion with conventional sprayers and also to produce efficient dosages for insects, the capsules should not exceed 60 μm in diameter [4]. But at diameters less than 10 μm, capsule walls become too thin. Consequently, the particles in the commercial formulation are about 30 to 50 μm in diameter, the same size as entomophilous pollen.

Bees evolved branched hairs which fit and harvest pollen grains. These hairs also pick up similar sized microcapsules. Burgett and Fisher [5] traced PENNCAP-M from a deliberately sprayed plot of blooming trefoil, *Lotus* sp., to pellets of pollen collected by honey bees foraging the plot. Bees also collected powdered pollen which had 25 ppm *a. i.* (active ingredient) PENNCAP-M added [6]. They hovered above the mixture for several minutes while combing their body hairs and packing the combined materials into pollen baskets on their hind legs. These bees returned to their hive and entered uncontested by bees guarding the entrance.

PENNCAP-M capsule material adheres to bee hairs more readily than the standard dust diluents. Electrostatic charges may be involved [7]. When we sprayed dyed PENNCAP-M onto filter papers placed in the bottom of small cages of bees, particles collected on the legs of bees that moved.

Bees foraging on a treated crop are thus readily contaminated with the microcapsules which are then stored with pollen in the hive combs. We found 0.14 ppm of methyl parathion [8] in pellets of pollen collected by Atkins *et al.* [9] from the hind legs of bees foraging alfalfa that had been sprayed 48 hours earlier with the recommended rate (0.3 kg/ha *a. i.*) of PENNCAP-M. Atkins *et al.* [9] reported 0.1 to 2.4 ppm in pollen pellets.

Capsules are consumed by bees. The midguts of 74% of the live bees and 78% of the dead bees collected from a plot treated with dyed PENNCAP-M contained microcapsules [5]. Capsules were not found in the honey stomach (foregut) of bees [5] because the lips of the proventricular valve (honey stopper) rapidly rake both pollen and capsules from the honey stomach to form a bolus in the midgut [10]. We observed dyed capsules in such boluses within a minute after bees were fed PENNCAP-M in sugar syrup. Since the capsules have a low permeability to methyl parathion, such removal of contaminated pollen from the honey stomach should reduce the contamination of honey. We found only 0.06 ppm methyl parathion in honey from colonies killed by PENNCAP-M. Honey from colonies exposed to treated alfalfa plots [8] had less than 0.05 ppm [9, 11].

Johansen [7] obtained combs from bee colonies in Lewiston, ID and Yakima, WA that had been contaminated with PENNCAP-M 8 to 10 months earlier. Duplicate analyses [7, 11] showed 0.2 to 15 ppm methyl parathion in packed pollen. When he fed a 1:1 mixture of this pollen and sugar to caged worker bees, mortality was 32% at 24 hours and 51% at 48 hours.

An Idaho beekeeper supplied us with 3 combs that contained pollen. We were told that these combs were from colonies that had been killed 19 months earlier when PENNCAP-M was sprayed. We found 7 to 41 ppm methyl parathion in the packed pollen (Table I). We confined worker bees and a queen on each comb and supplied them with water and sugar syrup. All the combs poisoned the adult bees (Table I). Wilson [13] obtained other combs from the same apiary 14 months after the colonies were killed and added 1 contaminated comb to 3 uncontaminated combs in small outdoor colonies. Even with 3/4 of the combs uncontaminated, the bees were poisoned by the 14 month old contaminated combs.

We found that pollen from a colony killed when PENNCAP-M was sprayed on alfalfa in Arizona contained 54 ppm methyl parathion one week after treatment. Another dead colony had 27 ppm in stored pollen a month after the fields were sprayed. One comb with pollen from a colony deliberately exposed to blooming rape sprayed with PENNCAP-M had 0.65 ppm methyl parathion in the stored pollen [11]. This pollen killed 63% of exposed bees [7]. Another comb had 1.17 ppm in the stored

Table I. Persistence of methyl parathion in 3 combs stored [a] 19 months after colonies were killed.

Combs	Methyl parathion in pollen [ppm] [b]	ChE activity in heads [c] [μM/min/head]	Mortality of exposed bees [%] [d]	Sealed brood produced [No. cells]
Contaminated	7–17	0.123	57	0
	7–41	0.114	72	0
	14–26	0.114	61	0
Control	0	0.166	12	10
	0	0.166	9	14
	0	0.157	20	18

[a] Storage temperature ranged from −10 to +35 °C. Colonies were killed at Lewiston, ID and shipped to us 16 months later so we did not protect the integrity of the samples during the entire storage period.

[b] Ranges of 3 samples of 0.5 g taken from each side of each comb and analyzed by methods sensitive to 0.1 ng.

[c] Heads of surviving bees after 3 weeks exposure to combs. We used Ellman's method [12] to measure cholinesterase inhibition in bee heads as an index of organophosphate poisoning. We prefer to state cholinesterase activity per head because heads have more activity than thoraces and because changes in moisture and in glandular contents are not variables if the unit is per head instead of by weight.

[d] 200 workers and a queen were added to each comb and held at 30 °C in a 0.5 m³ cage for 3 weeks.

pollen after 7 months, and, after 10 months, this pollen killed 94% of the exposed bees [7].

When Atkins et al. [14] dusted encapsulated methyl parathion on worker bees in a laboratory dust tower, the LD$_{50}$ was 0.241 μg/bee compared to 0.111 μg/bee for emulsions. When we fed encapsulated methyl parathion in syrup (Table II), it was less toxic than an emulsion. We found the safety factor given by encapsulation of oral dosages to be the same magnitude for bees that others found for rats [15]. Had we provided combs for the storage of syrup, the bees might have amassed more

Table II. Toxicity of methyl parathion.

Fed 2 h in syrup [ppm]	Methyl parathion consumed ± SD [ng/bee]	Mortality ± SD [a] 2 h [%]	24 h [%]
Emulsified			
2	58 ± 12	60 ± 12	85 ± 9
4	116 ± 44	86 ± 7	98 ± 3
6	150 ± 29	95 ± 5	98 ± 2
Encapsulated			
2	92 ± 18	0	12 ± 9
4	165 ± 17	9 ± 8	31 ± 15
6	276 ± 24	8 ± 6	44 ± 10

[a] 3 cages of ca. 30 bees each per dose, fed 50% sucrose.

of the toxic capsules because they rake them from the syrup into midgut boluses before the syrup is deposited in comb cells.

Residue levels of methyl parathion found in dead bees are highly variable, from zero to far in excess of the lethal dosage. In thoraces of dead worker bees from Atkins' alfalfa [9], we found 2.4 ppm in those collected at 24 hours, 0.57 at 48 hours, 0.25 at 72 hours and 0.88 at 96 hours when 0.3 kg/ha (*a. i.*) was applied. No residue was found in bees from his untreated plot. After application of 0.6 kg/ha, 0.39 ppm was found in bees at 24 hours. Carlson [11] analyzed whole bees from the same samples [9] using the method of the Association of Official Analytical Chemists, and, despite differences in our methods, the residues in thoraces and in whole bees were similar.

We have analyzed 66 samples of dead bees from fields sprayed with emulsified methyl parathion and 63 samples from fields sprayed with PENNCAP-M. The residues from thoraces averaged 3 ppm for sprayed emulsions and 0.6 ppm for PENNCAP-M. The samples were not from comparable plots, but these levels may serve as a guide identifying the cause of bee kills.

When fields are sprayed with methyl parathion emulsion, the bees become highly irritable, the hives become sticky with regurgitate, and dying bees accumulate around the hive entrance. When fields are treated with PENNCAP-M, the response is delayed and less dramatic. In that case, we find pupae and larvae at the hive entrance indicating the destruction of brood. Brood from Atkins' colonies [9] had as much as 1.2 ppm residue. We found 0.02 to 0.2 ppm methyl parathion in larval cadavers 19 months after the Idaho colonies were killed with PENNCAP-M. Live brood from colonies damaged in Arizona had 0.06 ppm after almost all the worker bees had been killed. Although contaminated pollen is packed in combs near the brood, we cannot be certain whether brood is poisoned or whether disoriented adult bees fail to feed or even kill the larvae.

Johansen [7] found methyl parathion in beeswax from contaminated combs only when the wax contained some pollen. The methyl parathion found in wax was less than 10% of that found in the packed pollen.

Almost all the bee kill problems have been attributed to the contamination of blooming weeds in field edges or in orchard cover crops rather than intentional treatment of crops in bloom. However, Johansen [7] reported about 2500 colonies damaged or killed in the Lewiston, ID area in a single misuse of PENNCAP-M in 1976. He also found that residues of PENNCAP on sprayed alfalfa remained highly toxic to honey bees for 3 to 5 days while emulsions lost most of their toxicity in 0.5 to 1 day. Because bees forage blossoms, one might not expect insecticide residues to kill bees for longer than sprayed flowers stay open; new blossoms should be free of contamination by non-systemic insecticides. However, bees forage leaves for extrafloral nectar and for aphid honeydew, and PENN-CAP-M is recommended for aphid control.

Some states have obtained regulations designed to prohibit application of microencapsulated insecticides in areas where bees may be killed. Potential remedies include modifying the release of methyl parathion from capsules, adding stickers or antistatics to the formulation, changing capsule sizes to reduce pick-up, adding pheromones to change foraging patterns, temporarily incapacitating bees to stop foraging, decontaminating combs, stimulating housecleaning and guarding by bees, and diluting toxins with supplemental feedings. In addition to a vigorous research program, the manufacturer of PENN-CAP-M is using education, extension recommendations, and emphatic label instructions to protect the product from misue. Nevertheless, beekeepers still have no effective countermeasures.

[1] Mention of a commercial product does not constitute an endorsement by the U. S. Department of Agriculture.

[2] N. Cardarelli, Controlled Release Pesticide Formulations, pp. 210, CRC Press, Cleveland 1976.

[3] J. R. Lowell, Jr., W. H. Culver, and C. B. DeSavigny, Controlled Release Pesticides, (H. B. Sher, ed.), p. 145, American Chemical Society, Washington, D. C., 1977.

[4] A 30 μm capsulate contains about 10 ng of methyl parathion. When we fed worker bees 50 ng per day in syrup, survival was reduced. Because most target insects are smaller than bees, the use of capsules larger than 30 μm would be like shooting quail with cannon.

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[6] A. Stoner, P. E. Sonnett, W. T. Wilson, and H. A. Rhodes, Amer. Bee J. **118**, 154 (1978).

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[8] We extract residues with benzene, remove the solvent on a rotary evaporator, then redissolve the residue for injection into a gas-liquid chromatograph fitted with a phosphorus-sensitive flame photometric detector.
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[16] We thank Phyllis and Joe Wright and Joe Jensen, beekeepers of California; Joseph O. Moffett and Art Wardecker, USDA Bee Lab, Tucson, AZ; E. L. Atkins, Univ. California-Riverside; Edward J. Bowles and James R. Lowell, Jr., Pennwalt Corp., Fresno, CA; and especially Carl A. Johansen, Washington State Univ., Pullman, WA; Adair Stoner and W. T. Wilson, USDA Bee Lab, Laramie, WY; R. E. Carlson, Pennwalt Corp., Tacoma, WA; and C. Duncan, Central Washington Univ., Ellenburg, WA, who shared samples and data with us.