

Distortion of Mirex Residues in Insects Owing to Use of Isopropyl Alcohol as a Collection Solvent¹

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Biological specimens collected in the field are often preserved in solvents (1), and 70% isopropyl alcohol is commonly used for this purpose (2). One of the standard devices used to collect insects in the field is the pitfall trap, which usually consists of a funnel placed over a small container of 70% isopropyl alcohol (IPA) into which insects and other small organisms fall and drown (3). Collectors may regard this discolored alcohol as used, discard it, and bottle the collection with fresh alcohol.

This study was undertaken to determine the effects on residues caused by the use of 70% IPA as a collection solvent for insects containing mirex.

Materials and Methods

The insects used in the test were wild and laboratory-reared imported fire ants, *Solenopsis invicta* (Buren) and laboratory reared German cockroaches, *Blattella germanica* (L.). Laboratory ants were treated with mirex by allowing them to eat fire ant bait containing soybean oil and 0.3% dissolved mirex one day before collection. Wild ants were collected by hand in a treated area in the field and submerged in 70% IPA. (Mirex and IPA were used as commercially available.) The specimens were obtained either alive or submerged in 50 ml of 70% IPA. All specimens were washed with IPA-acetone (1:1) to remove external deposits, then separated and dried overnight at room temperature. The samples were then weighed, ground with anhydrous sodium sulfate, and extracted in a Soxhlet apparatus for two hours with hexane that had been washed with concentrated sulfuric acid and water before being distilled from metallic sodium. The IPA baths and washings from each sample were combined and reduced in volume on a rotary evaporator, the aqueous residue was extracted 3 times with a total of 250 ml of hexane, and the extract was dried with sodium sulfate. Each extract was concentrated to about 5 ml with a 3-ball Snyder column and eluted through a column containing 18 g of activated Florisil (100-200 mesh, PR grade) with 250 ml of hexane. The samples were concentrated to 10 ml and analyzed on an

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F and M 810 gas chromatograph with a Ni^{63} electron capture detector and a 1.3 m X 4 mm ID glass column packed with 3% DC-200 on 80-100 mesh Gas Chrom Q. The retention time of mirex was 7.0 min with argon-10% methane carrier gas at 30 ml per min and the column oven at 240°C, injector at 280°C and detector at 290°C. Some samples were analyzed on a Varian 2100 gas chromatograph with dual tritium electron capture detectors and two 2 m X 2 mm ID glass columns packed with either 3% OV-17 or 3% OV-210 on 100-120 mesh Varaport 30. The retention times of mirex were 5.8 and 4.6 min, respectively, with nitrogen carrier gas at 30 ml per min and the column oven at 220°C, injectors at 240°C and detectors at 250°C.

In addition, some samples were shaken with concentrated sulfuric acid and re-analyzed to insure that mirex, which is not affected by this treatment, was not confused with another material with the same retention time. No contaminants causing major interference were observed.

Results and Discussion

1. Weight Loss. Weight loss of the samples averaged 50% when ants were killed by freezing and dried to constant weight at room temperature (Table 1). Ants dried to constant weight in

TABLE 1

Weight Loss of Laboratory Ants Caused by Drying

Treatment of ants ^{a/}	No. replicates	Weight of specimen (g)	Average % wt loss
1. Live vs. Frozen 24 hr and dried 48 hr/20°C	3	1.0013 .5046	 50.0
2. Live vs. b/ Soaked 24 hr and dried 24 hr/20°C	1	.5692 .2414	 57.6
3. Live vs. b/ Soaked 72 hr and dried 24 hr/20°C	2	.6850 .2693	 60.6
4. Live vs. Dried 24 hr/110°C	2	.8685 .4175	 52.0

a/ Major and minor worker imported fire ants.
b/ Soaked in 70% isopropyl alcohol at 20°C.

an oven at 110°C lost an average of 52% of their weight. However, when ants were soaked in 70% IPA for 24 or 72 hours and dried to constant weight, the weight loss averaged 57% and 60%, respectively. This increased weight loss is believed to result from the extraction of body fat from the insects by IPA and may be related to the proportion of fat body initially present in the insects. Large variations in fat loss between insect species or between metamorphic forms of a species may therefore be expected.

2. Extraction of Insecticides. Chlorinated hydrocarbon insecticides are recognized as lipophilic and are known to be stored in the fat bodies of insects. Although crystalline mirex is not soluble in 70% IPA, this solvent appears to extract some body fat from an insect, and some stored mirex may come with it. The isopropyl alcohol bath thus becomes laden with fat and mirex. Analysis showed that ants collected in the field and killed in IPA lost from 27 to 88% of their stored mirex to the alcohol bath while awaiting analysis (Table 2).

TABLE 2
Extraction of Mirex From Imported Fire Ants^{a/} Soaked in
70% Isopropyl Alcohol

Test no.	Weight of ants	Mirex (ug) in		ug mirex/ g ant	% loss of stored mirex
		Ants	Alcohol		
1	.1482	20.7	18.0	139.6	46
2	.1959	20.0	23.8	102.1	54
3	.3681	1.76	13.0	4.78	88
4	.3216	31.8	31.8	98.9	50
5	.1934	8.4	24.6	43.4	75
6	.9511	75.0	127.0	78.8	70
7	.9586	35.9	13.2	36.9	27

a/ Samples of major and minor workers collected in the field, held at 20°C in isopropyl alcohol until analysis and dried 24 hr/20°C.

3. Extraction of Mirex from Ants and Migration of Mirex into Cockroaches. In the first test, mirex-fed laboratory-reared worker ants and 10 previously uncontaminated laboratory-reared German cockroaches were held together in 50 ml of 70% IPA. Five cockroaches were removed after 24 hours; the other specimens were removed after 48 hours. All specimens were washed with IPA-acetone (1:1) and treated as described previously; the washings were added to the 70% IPA bath. Analysis showed the ants retained only 46.7% of the total mirex found; 40.0% was found in alcohol solution and 13.3% in the cockroaches (Table 3).

TABLE 3

Extraction of Mirex From Treated Imported Fire Ants and Its
Translocation into Cockroaches When Soaked Together in
70% Isopropyl Alcohol

Sample	Soak time (hrs)	Sample wt ^{b/} (g)	Contents as ug mirex (% of total)	ug mirex/g initial wt	ug mirex/ g final wt ^{d/}
1. Ants	48	.5003	4.19 (46.7)	8.38	
5 cockroaches	24	.4217 ^{c/}	.30 (3.4)		.72
5 cockroaches	48	.3071 ^{c/}	.89 (9.9)		2.89
alcohol	48		3.58 (40.0)		
2. Ants	24	.9998	121.17 (92.1)	121.19	257.09
5 cockroaches	24	.3106	1.24 (.94)	4.01	10.70
alcohol	24		9.13 (6.94)		
3. Ants	24	1.0326	137.23 (96.18)	132.91	276.95
5 cockroaches	24	.3190	1.36 (.95)	4.27	11.70
alcohol	24		4.09 (2.87)		
4. Ants	24	1.0329	128.47 (86.97)	124.38	262.02
5 cockroaches	24	.2707	1.57 (1.06)	5.81	18.30
alcohol	24		17.69 (11.97)		
5. Ants	24	1.0904	169.34 (91.66)	155.30	330.23
5 cockroaches	24	.2850	1.16 (.63)	4.07	11.18
alcohol	24		14.24 (7.71)		
6. Ants	48	1.1063	110.95 (78.59)	100.29	209.46
5 cockroaches	48	.3589	1.28 (.91)	3.56	10.17
alcohol	48		28.94 (20.50)		
7. Ants	48	1.0093	110.95 (84.77)	109.93	227.63
5 cockroaches	48	.2980	2.48 (1.89)	8.34)	24.53
alcohol	48		17.45 (13.33)		
8. Ants	48	1.0376	125.55 (82.28)	121.01	249.80
5 cockroaches	48	.2290	.66 (.43)	2.89	9.44
alcohol	48		26.38 (17.29)		

a/ All samples soaked in 50 ml 70% isopropyl alcohol at 18°C.

b/ Initial weight of insects killed by freezing.

c/ Final weight.

d/ ug/g on final weight basis - after extraction and drying at 20°C for 24 hrs.

Seven more tests were made with frozen, uncontaminated cockroaches and laboratory-reared mirex-fed worker ants which were held together in alcohol for 24 or 48 hours. Analysis showed that the ants retained an average of about 87% of the total mirex found, 12% was found in the alcohol solution, and 1% was found in the cockroaches. An average of 4.7 µg/g was found in the cockroaches soaked for 24 or 48 hours with one high value of 24.5 µg/g.

The use of a 0.5% soap solution in water as a substitute for IPA in pitfall traps cannot be recommended, since cockroaches disintegrated after a short immersion. Technical Dursban or dichlorvos (as pieces of Vapona[®] Strip) are presently in use at this laboratory as more suitable toxicants in pitfall traps because neither should interfere with mirex analyses.

Summary

Collection or storage of insects in 70% IPA was found to cause loss of body weight, major loss of nonpolar insecticides from submerged specimens, and transfer of insecticide to previously uncontaminated specimens. These effects may by no means be limited to mirex, since any chlorinated hydrocarbon is relatively lipophilic and may behave similarly. Residues reported for treated insects that have been collected or stored in alcohol may have been reduced to 12% of the original value. Moreover, small untreated vertebrates and invertebrates trapped in alcohol with insecticide-laden insects are likely to absorb significant amounts of insecticide from the solution.

The changes in residue levels owing to the 70% IPA used, though variable, were found to be as large as an order of magnitude, and the use of a correction factor would be badly misleading.

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