Residues of the Insecticide Mirex Following Aerial Treatment of Cat Island

by GEORGE P. MARKIN, H. L. COLLINS, and J. H. SPENCE
U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Gulfport, Miss. 39501

Throughout the Southeast the insecticide mirex incorporated into an oil-based bait is the predominant method of control for the red imported fire ant, *Solenopsis invicta* Buren, (MARKIN et al. 1972). When aerially applied at the rate of 1.7 g of mirex in 565 g of inert carrier per acre, 90 to 99% control of this ant can be obtained (LOFGREN et al. 1964). Multiple applications of the bait have been considered as a method of totally eradicating the ant from limited areas (LOFGREN et al. 1972).

In 1968, applications of a mirex bait were made to Cat Island in an attempt to eradicate this ant from a limited and isolated area. At that time no information existed concerning the residual levels of mirex which would occur following treatment with this pesticide. Correspondingly, a limited monitoring program was set up in association with the first treatment of the series. When this study indicated that detectable mirex residues were occurring in non-target organisms, a more extensive study covering 20 individual animal groups, plus soil, water, and sediment was initiated and continued for 3 years after the final treatment. This paper presents the monitoring data from this study.

METHODS AND MATERIALS

Collection Site and Sampling Techniques:

Cat Island is located 6 miles off the coast of Gulfport, Mississippi. It is privately owned, and contains a variety of habitats; pine and oak forests, open pastures, sand dunes, saltwater marshes, and freshwater ponds. In the experiment, the entire island was treated, including 2,760 acres of dry land, an estimated 1,000 acres of marshes and coastal waters, and a 280-acre bay on the south side of the island. The three bait applications were aerially applied using a Piper Pawnee spray plane in June 1968, September 1968, and June 1969. Each application required approximately 5,000 lbs. of bait or 15 lbs. of the actual toxicant, mirex. The entire island and its surrounding waters and bays in the 12-month period, therefore, received 45 lbs. of the insecticide mirex.

The bait used in this study was not the standard 4X mirex bait normally used for fire ant control (MARKIN et al. 1972) but an experimental bait in which the corncob grit carrier of the standard bait had been replaced with Fur-Ag ^{R}, a treated form of corncob

Bulletin of Environmental Contamination & Toxicology,
Vol. 12, No. 2 © 1974 by Springer-Verlag New York Inc.

which was softer and more absorbant. While containing slightly more soybean oil (20%), the amount of mirex (.3%), remained the same as in the standard 4X mirex bait (BANKS et al. 1970).

In open pastures or forested parts of the island, nine 1-acre collecting sites were set up for biological and soil samples. Three 1-acre sections of freshwater ponds were used for aquatic, water, and sediment samples. Marine samples were collected from the inner end of the 280-acre south bay as well as two small land-locked saltwater ponds (the ponds were actually connected to the bay during periods of extreme high tide), each approximately 2 acres in size.

Marine samples were collected with a 30-ft minnow seine, aquatic samples with a 14-inch sweep net, and terrestrial arthropods and toads with pitfall traps. Birds and lizards were collected generally over the entire island with either a shotgun or small caliber pistol. At each collection interval, a minimum of five specimens were collected for all of the larger animals (crabs and vertebrates) and at least 10 to 100 individuals of the smaller animals and combined together into composit samples. Soil was sampled at 10 randomly chosen sites, each 2 cm deep and .1 m square, in each of the plots, combined, mixed and a 1-kg subsample removed for analysis. Sediment samples were collected with a hand-thrown grab sampler from 10 locations in each freshwater or saltwater sampling site, combined, and a 1-kg subsample removed for analysis. Water was collected from each site in a 1-gal prewashed glass jug with teflon-lined top.

In August 1969, approximately 2 months after the final application of bait, a hurricane struck the gulf coast and high tides flooded part of the island. As a result, some larger animals such as cattle and deer were drowned, but of the smaller animals being used as indicators, only toads could not be found immediately following the hurricane or for the duration of the visits to the island. All other indicator animals were present immediately following the hurricane and seemed not to have been affected.

Sample Processing:

Samples collected in the field were stored on ice in either glass vials or wrapped in aluminum foil. In the laboratory, samples were weighed and washed in tap water to remove mud, algae, and dirt. The entire sample was then ground and mixed in a Waring blender, if large, or in a motar and pestal with 5 g of Na_2SO_4, if less than 10 g. A 5- to 50-g subsample was removed and mixed with .200 ml hexane and 100 ml isopropyl alcohol and rotated for 2 hrs. The extract was filtered through prewashed glass wool and washed three times in distilled water, filtered through anhydrous Na_2SO_4 and glass wool, and distilled through a Snyder column to 15 ml. Further

concentration, if needed, was by heating in a water bath with an air stream passed through a drierite filter.

In 1970, a contaminant with an identical retention time peak as mirex, the PCB Archlor 1260, was identified. Therefore, any of the 1971 and 1972 samples suspected of containing PCB's were further processed using a silicic acid column (ARMOUR and BURKE 1970; GAUL and CRUZ-LAGRANGE 1971).

Sample Analysis:

Primary analysis was done using a Microtek Model 220 gas chromatograph equipped with an electron capture detector. The column (6' x 1/4") was packed with 1.5% OV-17 and 1.96 QF-1 on Gas Chrom Q. Retention time was 30 minutes with 80 ml gas flow (5% Argon-95% Methane) with injector, oven, and detector temperatures of 250°, 200°, and 210° C respectively. Confirmation of all samples was done on Hewlett Packard Model 402 using a column (6' x 1/4") packed with 3% DC-200 on Gas Chrom Q. With a flow rate of 100 ml/min (5% Argon-95% Methane) and the injector, oven, detector temperatures of 245°, 175°, and 205° C respectively, retention time was 36 minutes. Recovery of mirex averaged 75-80% in 1968 but by 1972 had been increased to 97% when fortified samples were taken through the complete process. For a more detailed description of the equipment, sample processing, sample analysis, and method of confirmation, see MARKIN et al. 1972. Level of detection for biologicals and soil samples in 1968, 1969, and 1970 was .01 ppm. By 1972 accuracy of the procedure had been improved until the lowest level of sensitivity was .001 ppm. Lowest level of sensitivity for both freshwater and saltwater originally was .00001, though by 1972 it had been raised to .000001 ppm.

RESULTS AND DISCUSSION

A series of pretreatment samples were collected before the first bait application in June 1968. Additional samples were collected at intervals after treatment for 3 months. Analysis of these samples indicated that mirex was reaching non-target organisms (Table 1). With the evidence that mirex was reaching non-target organisms, a more extensive sampling program utilizing 19 indicator species or groups of animals was started before the third application of bait and continued for 3 years after the third and last application (Table 2).

High residues, 5.68 to 58.21 ppm, found in ants, crickets, and cockroaches immediately following the first bait application indicated that these insects were feeding directly on the bait. Similarly, the sudden occurrence of high residues of mirex in marine organisms such as catfish and mullet indicated that they also fed directly upon the bait. However, the rapid loss of mirex in the fish indicated that it was probably in their gut at the time the

TABLE 1.

Mirex residues found following the first treatment of Cat Island. All biological samples are the average of at least 5 individuals. N.S. means no sample collected.

Samples	pre-treatment	Mirex residues in PPM			
		48 hrs	1 wk	1 month	3 months
Mosquite Fish (*Gambusia affinis*)	<0.01	<0.01	.13	.69	.21
Toads (*Bufus* sp.)	<0.01	.16	.28	8.45	1.10
Red Imported Fire Ant (*Solenopsis invicta*)	0.01	58.21	3.21	NS	NS
Circkets (*Gryllus* sp.)	0.187	5.68	.51	.41	.62
Cockroaches (Blattidae)	<0.01	30.60	24.00	1.24	.84
Spiders (Araneida)	<0.01	<0.01	3.60	2.81	1.98
Fiddler Crabs (*Uca pugilator*)	<0.01	.96	1.54	.63	.74
Ground Beetles (Carabidae)	<0.01	.53	2.56	.51	.08
Soil	<0.01	<0.01	<0.01	<0.01	<0.01
Sediment	<0.01	<0.01	<0.01	<0.01	<0.01
Water	<0.00001	<0.00001	<0.0001	<0.00001	<0.00001

Sample	1 month pre 3rd treatment	Mirex residues - PPM - after 3rd bait treatment				
		72 hours	3 weeks	1 year	2 years	3 years
Aquatic						
Mosquite Fish (*Gambusia affinis*)	.09	.98	.74	.03	.011	Neg
Grass Shrimp (*Palaemonetes pugio*)	.07	NS	.31	.01	.003	Neg
Marine						
Mullet (*Mugil cephalus*)	.47	1.26	.21	.01	.009	Neg
Catfish (*Siluroidue*)	.11	2.59	.31	.08	.019	.001
Pinfish (*Lagodon rhomboides*)	.09	.66	.65	.16	.019	.002
Blue Crabs-Bay (*Callinectes sapidus*)	.23	.32	.35	.03	.003	.002
Blue Crabs-Pond (*Callinectes sapidus*)	.40	1.26	.71	NS	NS	NS
Shrimp-Bay (*Penaeus aztecus*)	.07	.23	.35	.01	.008	.003
Shrimp-Pond (*Penaeus aztecus*)	.31	1.15	NS	NS	NS	NS
Hermit Crabs (Paguridae)	.14	.03	.70	.02	.005	.008
Fiddler Crabs (*Uca pugilator*)	.21	.01	1.25	.03	.043	Neg
Oysters (*Ostreidae*)	.14	.50	.63	.01	.006	.001
Whelks (Buccinidae)	.12	.14	.03	<.01	<.001	Neg
Physical Samples						
Soil	<.01	NS	.016	.001	.001	.0021
Sediment - fresh water ponds	.021	NS	.06	.002	.001	.0019
Sediment - salt water ponds	-	NS	1.95	NS	NS	NS
Water (fresh)	<.00001	NS	<.00001	<.00001	<.00001	<.00000
Water (salt)	<.00001	NS	<.00001	<.00001	<.00001	<.00000

TABLE 2.

Mirex residues (PPM) from specimans collected from Cat Island, Mississippi, following the 3 applications of a mirex bait (Fur-Ag^{R}). Level of sensitivity for detection of mirex increased during the 4 years the experiment was underway, therefore, <.001 means none was detected at that particular level of sensitivity, at that time.

Sample	1 month pre 3rd treatment	Mirex residues - PPM - after 3rd bait treatment				
		72 hours	3 weeks	1 year	2 years	3 years
Terrestrial Vertebrates						
Snowy Egrets (*Leucophoyx thule*)						
juvenile	2.83	NS	1.44	2.21	.043	.014
adults	NS	NS	.32	.44	.035	.026
eggs	NS	NS	12.81	NS	.182	.023
Sand Pipers (*Ereunetes pusillus*)						
juvenile	.64	NS	.78	.55	NS	.411
adults	.17	NS	NS	1.32	.107	.291
Lizards (*Eumenges* sp)	.34	.53	5.46	.32	.033	.009
Toads (*Bufus* sp)	.60	1.10	11.52	NS	NS	NS
Terrestrial Arthropods						
Crickets (*Gryllus* sp)	.13	2.82	.77	.01	.017	Neg
Cockroaches (Blattidae)	.02	16.31	1.04	.01	.008	NS
Ground Beetles (Carabidae)	.38	NS	4.21	.06	.015	Neg
Ground Spiders (Araneida)	.74	.53	1.57	.04	.051	.016

animals were first collected and later most of it was eliminated without being incorporated into their bodies. Other organisms such as oysters and fiddler crabs showed a gradual increase for several weeks after application, indicating that they were either concentrating the mirex from the seawater or receiving it as an early step in the food chain. Predators such as lizards, toads, and spiders showed a more gradual buildup over the entire period of treatment.

While pretreatment population levels of the 19 indicator organisms were not determined, it was noticed that following each treatment, and for 3 years after the last treatment, all organisms being monitored, except toads, could still be found and appeared to be abundant. The exception was one of the marine sites being studied, a shallow 2-acre saltwater pond in the open sand-duned part of the island with no direct connection to the bay except during extreme high tides. Residues in shrimp and crabs from this pond before the third application were .31 to .40 ppm. Following the third application of bait the residue levels in the animals had increased to 1.15 to 1.26 ppm and corresponded with a sudden decrease in population. Within 17 days after the test, shrimp could not be found in the pond and at least 50% of the crabs collected were either paralyzed or dead. At the remaining two marine collecting sites, the open bay, and the other landlocked pond, no similar increase in residues in the animals was observed during the remainder of the summer and no population decline was observed. Fiddler crabs living in the salt marsh around the island contained residues as high as 1.5 ppm. However, their population remained high (est. at 2 to 10/m^2) following the third treatment and for the reaminder of the period of observation. In view of the lack of mortality or high mirex residue at other collecting sites, and the massive residues in both crabs and shrimp from the ponds where mortality occurred, it was felt that this particular collecting site was atypical from the rest of the island. However, it did indicate that mirex was potentially dangerous to some crustaceans at high enough concentrations.

The only positive evidence of an effect on an overall animal population was observed in ants. Before treatment, 1,106 mounds of the imported fire ant were found in 100 manhours of survey. Five weeks after the first treatment, only one live mound of fire ant could be found. Following the second and third application of mirex, no colonies of fire ants were found. In 1970, new dealeated queens were found on the island. By 1971, eight new colonies were found and in 1972 over 40 small to medium size colonies were found. The evidence was interpreted as showing that the imported fire ant was eradicated from the island. However, it was soon reinfested by newly mated queens flying the 6 miles from the coast (MARKIN et al. 1971).

A similar reduction of the Florida harvester ant *Pogonomyrmex badius* was observed. The ants were fairly extensive before the first treatment but after the third treatment could not be found. It was noticed that even 3 years after the final treatment no new colonies of this ant had been discovered on the island. Five other species of ants, *Conomyrma insana* (Buckley), *Iridomyrmex pruinosus* (Roger), *Crematogaster clara* (Mayr), and two species of *Campenotus*, could be found on the island following the third application and appeared not to have been affected.

REFERENCES CITED

AMOUR, J. A., and J. A. BURKE: JAOAC 53, 761 (1970).

BANKS, W. A., C. E. STRINGER, C. S. LOFGREN, N. W. PIERCE, and F. J. BARTLETT: J. Econ. Entomol. 63, 1990 (1970).

GAUL, J., and P. CRUZ-LAGRANGE: Unpublished, Laboratory Information Bulletin, U.S. Food and Drug Admin., New Orleans District (1971).

LOFGREN, C. S., and D. E. WEIDHASS: Bull. Entomol. Soc. Amer. 18, 17 (1972).

MARKIN, G. P., J. H. FORD, J. C. HAWTHORNE, J. H. SPENCE, J. DAVIS, H. L. COLLINS, and C. D. LOFTIS. USDA, APHIS, 81-3 series, p 22 November (1972).