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Tissue Distribution of Mirex in Adult Crayfish (*Procambarus clarki*)

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Mirex has been used as a control for the imported fire ants Solenopsis invicta and Solenopsis richter since the early 1960's. Its effectiveness is purportedly due to the time lag between scavenging of the bait by the worker fire ants and the subsequent mortalities which occur within the mounds some time later. This delay in toxicity allows the distribution of the toxicant to all ants in the colony, including the queen (ALLEY 1973).

LUDKE et al. (1971) reported a similar delayed toxicity in juvenile crayfish, Procambarus blandingi, exposed to water contaminated with 1 ppb mirex, and water containing a number of grains of mirex bait. Tests conducted at our laboratory in which crayfish, Procambarus clarki , were exposed to mirex and mirex photoproducts supported their conclusions. In addition, it was observed that crayfish exposed to mirex exhibited a series of progressively more pronounced behavioral disfunctions when stimulated by the sharp rap of metal spatula on the rim of their container. The earliest consistently recognizable symptom of toxicosis observed in treated crayfish was an "eye twitch" involving a rapid forward jerk of the eyestalk. Next they exhibited a "body jerk" response characterized by a sudden upward flex of their walking legs. Occasionally, in heavily dosed animals, both responses occurred simultaneously. With continued exposure, the crayfish gradually lost control of their normal tail reflexes. At first, this was seen as a reduction in the speed with which the animals flipped their tails when disturbed. After long exposure to mirex, the only observed response to a stimulus was tail curling. At this stage, a disturbance often caused them to tumble over backwards into a "legs up" position since they could not flex their tails for balance. In the early stages of this "tail curl" response, the crayfish could usually right themselves if left undisturbed. Later, however, they lost the ability to right themselves and would often remain in the "legs up" position for several days before dying. It was also noted that those crayfish exhibiting early symptoms (body-jerk) generally survived when transferred to clean water, while those exhibiting late symptoms (legs up) always died.

Based on the above information, the present study was undertaken to determine if some insight into the mode of action of

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mirex and into the reasons for its typically delayed toxicity could be obtained. This was accomplished by examining the tissue distribution of labelled mirex relative to the onset of early and late symptoms of toxicosis following exposure to a low and high dose of ^{14}C mirex.

MATERIALS AND METHODS

Chemicals. ^{14}C-UL-Mirex (6.5 mCi/mMol, 98% radio-chemical purity) was obtained from California Bionuclear Corp. Soybean oil, once refined, was purchased from Riceland Foods Corp. The acetone used was reagent grade. Either distilled or non-chlorinated well water was used in the aquariums during the course of the experiments.

Animals. Adult American crayfish, P. clarki, were obtained from culture ponds at Louisiana State University.

Experimental Design. Twenty five adult crayfish weighing from 10 to 20 g, were placed individually into separate 1-gal glass jars which contained 500 mL of well water. To thirteen of the test jars (low dose group) was added 0.37 mL of a solution of ^{14}C-UL-mirex in acetone (10 µg/mL), thus giving a final concentration of 7.4 ng/mL (7.4 ppb). The water was stirred gently with a glass rod to effect mixing. To the remaining twelve test jars (high dose group) was added 0.37 mL of mirex solution in acetone (100 µg/mL), giving a final concentration of 74 ng/mL (74 ppb). The solubility of mirex in distilled water is about 2 ppb. It is not surprising, therefore, that it could be seen floating on the surface of the water and attached to the sides of the test containers. Thus free mirex probably acted as a reservoir which quickly replaced the water solubilized mirex as it was removed by the crayfish. The crayfish were not fed during the experiment.

When the "body jerk" response (early symptom) was observed in any of the individual test animals, they were removed, labelled as to dosing level (low dose; high dose), and frozen for later analysis. This process was continued until 7 low dose and 6 high dose animals had been accumulated. The remaining animals in each group were removed and frozen after the "legs up" response (late symptom) was observed, or occasionally, in the low dose group when the constant "tail curl" response was observed. The animals were frozen so that all analytical work could be completed in one day.

For the analysis of the radioactive content of the various tissues, the frozen crayfish were dissected as they thawed since rapid deterioration of the digestive gland occurred on thawing. The brain, an aliquot (approx. 500 mg) of the digestive gland, the green glands, the tail nerve cord, a portion of tail muscle, and a section of intestine were removed, blotted briefly on filter

paper and weighed. Each tissue sample was then homogenized in distilled water (0.5 mL) and placed in a scintillation vial. One mL of Chlorox[n] was added to each vial. The vials were then manually shaken and allowed to digest at room temperature for 12 h. Following digestion, 15 mL of a standard scintillation solution (0.005 g of 2,2-phenylene bis-5-phenyloxazole, 4 g of 2,5-diphenyloxazole, 500 mL of scintillation grade toluene and 5-0 mL of Triton[R] X-100 was added to each vial. After proper mixing, the samples, along with an appropriate blank, were counted and the dpm corrected to cpm/mg tissue. This value was then converted to ppb and recorded.

RESULTS

The average distribution of ^{14}C-mirex in various tissues of crayfish exposed to ^{14}C-mirex are given in tables 1 (high dose) and 2 (low dose).

TABLE 1

Tissue distribution of ^{14}C-mirex in crayfish exposed to a high dose (74 ppb) and sampled following the onset of early and late symptoms.

Tissues	ppb mirex/gram tissue	
	early symptoms[1]	late symptoms[1]
Muscle	0.05 (± 0.02)	0.6 (± 0.2)
Brain	0.06 (± 0.03)	5.9 (± 2.9)
Nerve Cord	0.04 (± 0.01)	6.2 (± 3.2)[2]
Green Gland	0.2 (± 0.6)	5.6 (± 1.4)
Gill	1.1 (± 0.1)	1.7 (± 0.3)
Digestive Gland	1.4 (± 0.9)	7.8 (± 2.0)
Intestine	11.2 (± 5)	3.2 (± 0.8)

[1] N = 6
[2] N = 5; one sample contaminated

In general, the early symptoms of toxicosis occurred during the first 2-4 days in the high-dosed animals and during the first 4 to 7 days in the low-dosed animals. The late symptoms usually occurred between days 7 and 14 for the high-dosed animals, and between days 10 and 21 for the low-dosed animals. A few of the low-dosed animals had to be removed prior to exhibiting a permanent "legs up" behavior.

TABLE 2

Tissue distribution of ^{14}C-mirex in crayfish exposed to a low dose (7.4 ppb) and sampled following onset of early and late symptoms of toxicosis.

Tissues	ppm mirex/gram tissue	
	early symptoms[1]	late symptoms[2]
Muscle	0.04 (± 0.01)	0.6 (± 0.1)
Brain	0.05 (± 0.03)	0.4 (± 0.8)
Nerve Cord	0.04 (± 0.02)	0.4 (± 1.0)
Green Gland	0.18 (± 0.04)	1.8 (± 0.4)
Gill	0.7 (± 0.2)	0.8 (± 0.1)
Digestive Gland	1.7 (± 0.6)	4.6 (± 1.7)
Intestine	7.8 (± 3.2)	1.9 (± 0.7)

[1] N = 7
[2] N = 6

DISCUSSION

The symptoms of poisoning observed in crayfish during exposure to ^{14}C-mirex suggest that the compound was acting as a neurotoxin. LOWE et al. (1972) reported similar effects of mirex on juvenile grass shrimp (Paleomonectes pugio). In their experiments, the symptoms observed were irritability, uncoordinated movement, loss of equilibrium and paralysis. In addition, they reported that mirex caused paralysis in juvenile blue crabs (Callinectes sapidus) and fiddler crabs (Uca pugilator). Again, the primary debilitating effect appeared to have been on the neural tissues.

Further evidence for neural involvement can be seen in tables 1 and 2. Two points are of particular interest. First of all, at the time the earliest symptoms were observed, the level of labelled mirex in the neural tissues of both the high and low-dosed groups was very low. In fact, the concentrations present were near the lower limits of accurate detection. By the time the late symptoms of poisoning were observed, the levels in the neural tissues had increased 5-14 fold in the low-dosed group and 100-300 fold in the high-dosed group. Secondly, when the average tissue burdens in each test group were totaled, it was found that the total for the 7 tissues in the high-dosed early-symptom group in which the crayfish were exhibiting mild effects was higher than the total (on a per gram of tissue basis) in the low-dosed late-symptom group in which the crayfish were exhibiting severe symptoms and were, in fact, near death (14.0 vs 10.3 ppm). Likewise, the totals for the

tissues from the low-dosed early-symptom group and from the low-dosed late-symptom group were identical (10.5 vs 10.3 ppm). The most significant difference in the distributions of labelled mirex appears to be the increased levels in the neural tissues of the crayfish from the late-symptom low-dosed group. The levels in the neural tissues of the high-dosed late-symptom group were even higher, possibly accounting for the earlier onset of late symptoms in this group.

It seems obvious that after a few days (depending upon the level of exposure) the crayfish are capable of accumulating enough mirex to kill them. The question then is why the delay in toxicity? The data in tables 1 and 2 suggest that the early concentration of mirex in the tissues of the intestine and hepatopancreas removes enough mirex from circulation to delay its buildup in the neural tissues to debilitating levels. However, with time and continued exposure the mirex levels in the green gland and neural tissues approach the levels in the intestine and hepatopancreas possibly indicating increased circulatory levels caused by the saturation and/or dysfunction of the hepatopancreas.

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