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Laboratory Evaluation of the Hazard to Wood Mice, *Apodemus sylvaticus*, from the Agricultural Use of Methiocarb Molluscicide Pellets

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Small mammals have previously been used to monitor the potential environmental effects resulting from the application of agricultural chemicals both under laboratory conditions and in the field at the recommended rates (Westlake et al., 1980; Westlake et al., 1983). The agricultural application of methiocarb, a carbamate molluscicide, is widespread in the UK but the extent of any hazard this presents to wood mouse (Apodemus sylvaticus) populations in the field situation is not known.

Laboratory studies have been carried out to determine the toxicity of methiocarb pellets to wild trapped wood mice in order to provide some background data prior to any further evaluation of hazard in the field. In this study, wood mice were exposed to dry and to dampened methiocarb pellets in order to reproduce field trial application conditions. Field observations of methiocarb pellets indicate that the physical character changes under dry and wet weather conditions. This may affect their relative attractiveness and potential toxicity to wood mice.

The laboratory assessment of exposed wood mice included measurement of brain esterase activities, methiocarb residues in selected mouse tissue, carcases, and histological evaluation of kidney, liver and lungs.

MATERIALS AND METHODS

Commerically available molluscicide pellets ('Draza') containing 4% methiocarb were used in the animal treatments. Initially two groups of wood mice were individually caged and fed ad libitum on whole wheat. The first group of eight mice were exposed to dry methiocarb pellets scattered over the floor of each cage to simulate a single field application. The second set of 10 mice were similarly caged but exposed to 20 methiocarb pellets dampened with 40% (v/w) distilled water and placed on glass petri dishes in the cage to prevent water being desorbed from the pellets into the sawdust floor litter of the cage. Cages were frequently inspected and any dead mice were post-mortemed as soon as possible. Surviving mice in the first set were killed using ether after 27 days total exposure and in the second set after seven days from initially placing the methiocarb pellets in the cage. Dampened methiocarb pellets caused mouse deaths and a third set of eight mice were exposed to dampened

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pellets for seven days. Any dead mice were then used to provide whole organs (no histological sampling) for residue analysis.

At death mice were weighed and post-mortemed, with whole liver weights also being recorded. Samples of kidney, liver and lung from each mouse were placed in buffered 10% formalin and routinely processed to paraffin blocks; sectioned at 6 μ and then stained using Ehrlichs haematoxylin and eosin (H&E). Brain tissue was also sampled and brain acetylcholinesterase (AchE) activity was determined by the method of Ellman et al. (1961) as modified for brain tissue by Westlake et al. (1980).

Methiocarb residue analysis was carried out on diethyl ether extracts of skinned headless carcasses, liver samples and whole stomachs from mice that had been found dead. Tissue extracts were cleaned up using silica columns (Sep-Pak) and analysed using a gas-liquid chromatograph fitted with a nitrogen-phosphorus ionization detector.

RESULTS AND DISCUSSION

From the first set of eight mice (Table 1) exposed to dry methiocarb pellets, two mice died at 12 hours and one mouse died after six days. The remaining five mice survived 27 days exposure to dry pellets. Dry methiocarb pellets were attractive to the mice and were frequently collected by the mice and placed in the hay nesting material. This behaviour caused the pelt, tail and limbs to be stained blue from the dye in the pellets. Most mice in this group also showed blue colouration in their snout and oral cavity. At post-mortem mice showed pronounced blue colouration in their body fat deposits and in most cases the bladder contained blue coloured urine, also confirming ingestion of the pellets or pellet dust while grooming. External and internal blue colouration increased with time of exposure to the pellets. Two out of three mice (numbers 1 and 5) which died showed blue colouration of their stomach contents. Residue analysis confirmed that methiocarb was present in both stomachs and 5.1 $mgkg^{-1}$ methiocarb was present in the stomach contents of mouse 1. Methiocarb residues were also confirmed in the livers and carcases of mice 1 and 5.

Histological examination (Table 1) showed that there was severe kidney damage with inflammatory changes in the glomeruli, tubules and pre-papilla areas. Kivers showed variation in nuclear size with slight hepatocyte enlargement together with a diffuse lobular spread of mainly mononuclear leucocytes and kupffer cell enlargement. Three mice livers exhibited hepatitis. Lungs showed slight interstitial thickening but generally were lesion free. In surviving mice the brain AchE activity was not depressed below control activities but two out of three mice (1 and 5) which died showed a marked decrease in AchE activity.

In the second set of 10 mice (Table 2) exposed to dampened methiocarb pellets, five mice died after three days. As a result of this rapid lethal effect, the survivors were killed after seven days so that data could be more comparable between poisoned and surviving mice. The dampened pellets did not initially seem so attractive to the wood mice but some pellets were removed by the mice and stored under the petri dish container with a few showing signs of being partially eaten. Dampened pellets were not moved around the cage or collected together as much as the dry pellets. The mice which died and those which survived to seven days did not show

Table 1. Days of exposure, brain esterase and histological evaluation of tissues from wood mice exposed to dry methiocarb pellets

Animal No.	Days Exposure to Methiocarb pellets (dry)	Brain Acetylcholinsterase % of control[a]	Histopathology (graded observed changes)		
			Kidney	Liver	Lung
1*	0.5	56	++	+	0
2	27.0	113	++	+	+
3	27.0	129	+++	++	+
4	27.0	120	+++	++	++
5*	0.5	41	+++	++	NS
6*	6.0	111	+++	+	NS
7	27.0	107	++	+	+
8	27.0	122	++	+	+

[a]Control wood mouse brain AchE activity 10.08 ± 0.49 I.U. (Westlake et al., 1983)

* Mouse died while on test

\+ Minimal change

++ Slight change

+++ Severe change

NS Not sampled

Table 2. Days of exposure, brain esterase level and histological evaluation of tissues from wood mice exposed to dampened methiocarb pellets

Animal No.	Days Exposure to methiocarb pellets (wet)	Brain AchE % of control activity[a]	Histopathology (graded observed changes)			Body/Liver Wt[b]
			Kidney	Liver	Lung	
9	7	117	+++	+	+++	4.4
10	7	105	+++	++	++	3.6
11	7	96	++	++	+	6.0
12	7	95	++	+++	+	6.1
13*	3	132	++	++	++	4.9
14*	3	107	++	++	+++	3.7
15*	3	88	+++	++	+++	4.8
16*	3	105	++	++	+++	6.6
17	7	89	+++	+++	++	7.7
18*	3	128	+++	++	++	4.4

a Control wood mouse brain AchE activity 10.08 ± 0.49 I.U. (Westlake et al., 1983)
b Control mouse mean Body/Liver Wt ratio 4.3 (n = 6)
* Mouse died while on test
\+ Minimal change
++ Slight change
+++ Severe change

any external or internal blue colouration and their bladders, although distended with urine, were not blue in colouration as observed in the mice on dry pellets. This lack of blue colouration may result either from reduced contact with the pellets or the fact that less dust was released. All of the mouse stomachs were found to be empty with no blue colouration. Because of this, methiocarb residue analysis was only made on carcases from three of the dead mice but no methiocarb residues were detected (limit of detection 0.04 mg/kg). Only two mice (15 and 17) showed a small decrease in brain AchE activities. Histologically (Table 2), the kidneys showed severe inflammatory changes, especially in the pre-papilla collecting duct areas and interstitially, but less severe changes were associated with the glomeruli and tubules. Liver damage was exhibited as hepatocyte hypertrophy with pronounced cytoplasmic and nuclear degeneration with focal necrosis being observed in nine out of ten mice. All the livers showed a marked inflammatory response (hepatitis) which generally consisted of a diffuse leucocyte inflammation and also in five of the mice included polynuclear leucocytes. Lung changes were more severe in the dead than in the surviving mice. In the third set of eight mice, one mouse died on day 2 and a second mouse died on day 3 with six mice surviving to day 7. The two dead mice both had blue colouration to stomach contents. Both mice had methiocarb residues in live and stomach. Brain AchE was reduced in poisoned mice but AchE levels in the six surviving mice were within control levels. Residue analysis on three carcases selected from the six surviving mice did not detect any measurable methiocarb levels.

Histological changes were greater in the wood mice exposed to dampened pellets than the dry pellets. Wood mice appear to be less susceptible to inhibition of brain AchE levels than avian species (Westlake et al., 1981) with only two of the mice which died (Table 1) having appreciable brain AchE inhibition. This variability of brain AchE inhibition is often observed in fatalities due to carbamate compounds (Martin et al., 1981) and is explained by the spontaneous reactivation after death of the carbamate inhibited esterase activity. The body weight to liver weight ratios of surviving mice (Table 2) were larger when compared to control values, with the dead mice having similar or slightly lower values. Surviving mice showed greater histological changes and this may have arisen because of their longer exposure time to the methiocarb pellets. The histological changes observed in mouse kidneys may partly have been due to infection but these were more pronounced in surviving mice. This increased exposure to methiocarb may have enhanced an underlying chronic kidney infection which could be expected to be present in wild trapped mice. The observed bladder distension may have arisen because of the kidneys decreased powers of urine reabsorption.

The study shows that wood mice are susceptible to poisoning by methiocarb pellets. Methiocarb residues were detected in stomachs (range 43 to 5067 $mgkg^{-1}$) and livers (range 1.2 to 41.4 $mgkg^{-1}$) from dead wood mice. Stomach methiocarb residues were below the LD_{50} of 100 $mgkg^{-1}$ (Pesticide Manual 1977) for rats but above levels known to cause death in birds (Tharwat et al., 1985). The recommended field application rate in the UK for methiocarb pellets is 5.5 Kg/ha (equivalent to 22 mg ai/m^2). Surface sampling of methiocarb pellets after such an application gave a range of 19.6 to 243.0 mg weight of pellets/m^2 (average 110.0 mg/m^2 equivalent to 4.4 mg ai/m^2). Previous work by this laboratory involving the monitoring of wild small mammal populations has shown that wood mice are capable

of travelling large distances over fields and could thus be exposed to appreciable numbers of methiocarb pellets. Because of the formulation on grain meal, it would appear to be attractive to wood mice. This study has shown that methiocarb pellets used to control molluscs represents a potential hazard to wild populations of wood mice.

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