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Biochemical and Histological Effects of Diclofop-methyl in Mice and Voles Under Laboratory Conditions

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The assessment of the environmental hazards presented to wildlife by new pesticides or new uses of established pesticides has previously made use of biochemical measurements supported by residue analysis (Westlake et al 1980, 1982 a, b). The use of the wood mouse in these studies has demonstrated the value of small mammals as indicator species for assessing the effects of the organophosphate seed treatments, carbophenothion and chlorfenvinphos on wildlife. To obtain further knowledge of the type of compound that could be monitored and the usefulness of biochemical and histological approaches, a field trial was later undertaken using small mammals as indicator species to monitor the effects of the foliar herbicide, diclofop-methyl. In the present study, wild trapped wood mice (Apodemus sylvaticus) and bank voles (Clethrionomys glareolus) were fed in the laboratory on wheat treated with diclofop-methyl to establish reliable background data to assist in the interpretation of the field trial.

The laboratory treatments indicate that biological effects in wild-trapped small mammals are detectable using both biochemical and histological approaches. Although the functional changes observed are likely to be reversible on removal from exposure to diclofop-methyl, these effects are likely to reduce an animal's resistance to further environmental stress or chemical insult.

MATERIALS AND METHODS

Wood mice and bank voles were wild trapped in Surrey woodland away from known agricultural chemical use and caged in pairs of the same sex in a room maintained at 19.5 $\pm$1.5^{o}C and a relative humidity range of 55 to 62% with a 12 hour photoperiod and a 24 hour cycle. The animals were maintained on untreated wheat and water ad libitum until placed on treated diets. Groups of 6 wood mice were fed on wheatgrain treated with technical grade diclofop-methyl at levels of 20,200, 500 and 1000 ppm for 1 and 2 weeks and at 20 and 200 ppm for 4 weeks. The highest treatment level of 1000 ppm relates to approximately two and a half times the field

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application rate of 1.1 to 1.26 kg ai/ha. Groups of 5 bank voles were fed on wheat treated with technical diclofop-methyl at levels of 200 and 1000 ppm for 2 weeks. Further groups of control wood mice and bank voles were fed on untreated wheat for 1, 2 and 4 weeks. Immediately after each treatment period small mammals were anaesthetised with ether and a blood sample (1 ml) was taken by cardiac puncture with a heparinised syringe. Plasma, prepared immediately by centrifuging the heparinised whole blood at 2000g for 10 minutes, was stored at -20°C to await analysis. Livers were removed and 4 mm slices were taken from both major lobes of each liver and placed in buffered neutral 10% formalin for histopathological evaluation. After fixation the tissue slices were routinely embedded in paraffin wax, sectioned 6u thick using a base sledge mictotome and stained with Ehrlichs Haematoxylin and Eosin. Liver samples for cytochrome P_{450} estimation were prepared (Stanley et al, 1978) to give 20% w/v homogenates. The microsomal suspension was prepared as previously described (Bunyan et al 1972) and cytochrome P_{450} estimated by the method of Omura and Sato (1964). Remaining liver portions were homogenised in 3 volumes of distilled water and centriguged at 16,000 g for 45 minutes at 10°C. Plasma and liver nitro phenyl acetate esterase (NPAE) were determined by the method of Mendoza and Wales (1970) modified as previously described by Westlake et al (1980). Glutamate oxaloacetate transaminase (GOT) was determined by the method of Bergmeyer and Bernt (1963) modified as previously described by Westlake et al (1980).

RESULTS AND DISCUSSION

Exposure of wood mice to 20 ppm diclofop-methyl in the diet resulted in no significant biochemical changes, however treatment of the diet at 200 ppm resulted in increases in relative liver weight, liver cytochrome P_{450} and plasma NPAE after 1, 2 and 4 weeks (Table 1). The lower concentrations in the diet also caused a mild increase in hepatocyte size (hypertrophy) (Table 2). Increases in hepatocyte binucleation reflected the increased DNA content (Barka and Popper, 1967). The severity of these treatments on nuclear function was observed as eosinophilic intranuclear inclusion bodies. This was further supported by the increased frequency of anuclear hepatocytes which shows that nuclear breakdown was also occurring, eventually leading to necrosis of hepatocytes in the higher treatment groups.

At higher concentrations of 500 and 1000 ppm in the diet for 1 and 2 weeks, similar significant changes in relative liver weight, liver cytochrome P_{450} and plasma NPAE were observed in the wood mice with increased liver NPAE only after 2 weeks. These effects accompanied a severe increase in hepatocyte size with single cell necrosis and loss of cytoplasmic protein, the histological findings being consistent with chronic hepatitis.

Table 1. Relative liver weight, liver cytochrome P_{450} and NPAE activity and plasma NPAE and GOT activities in Wood mice and Bank voles after feeding diclofop-methyl treated diets at 20, 200, 500 and 1000 ppm levels for 1, 2 or 4 weeks.

Species	Period on diet (weeks)	Treatment level (ppm)	Relative liver weight[a]	Liver		Plasma	
				Cytochrome P_{450}[a]	NPAE[a]	NPAE[a]	GOT[a]
Woodmouse	1	20	114	59	157*	101	116
	1	200	173*	129	269	228**	120
	1	500	224**	254**	101	215*	219
	1	1000	250**	226**	122	274*	329
	2	20	112	NM	88	113	110
	2	200	225**	NM	148	135*	144
	2	500	242**	193*	234*	206**	125
	2	1000	243**	224*	208**	196**	249
	4	20	113	125	96	161*	82
	4	200	215**	179*	133	258**	147
Bank vole	2	200	219**	104	21**	146	NM
	2	1000	265**	151	35**	181	NM

a. mean value expressed as % of control mean
Significant difference from individual control values *p 0.05, ** p 0.01
NM not measured

Mean plasma GOT activities generally increased in all the treated wood mice indicating that considerable tissue damage and leakage from cells was occurring. This rise parallels the plasma NPAE levels which are also considered to reflect membrane changes during hepatocyte proliferation (hyperplasia). These plasma enzyme changes are analogous to those occurring following the reaction of the liver to organochlorine compounds (Westlake et al, 1979). The increase in relative liver weight could not be explained by any significant increase when liver or plasma lipids were measured. The observation of hydropic rather than lipid degeneration with the Sudan black staining of liver sections may explain this increase in relative liver weight. The microvesicular lipid droplets (Baptista and Bianchi, 1981) identified possibly indicate greater hepatic disruption than the macrovesicular (Baptista and Bianchi, 1981) lipid droplets which characterise the increased relative liver weight resulting from chlorinated hydrocarbon compounds such as the DDT metabolite DDMU (Tarrant et al, 1983).

The biochemical changes observed in the bank voles after 2 weeks treatment with 200 or 1000 ppm diclofop-methyl were not as significant as occurred for the wood mice with only the relative liver weight increasing significantly and liver NPAE activities decreasing. However the 200 ppm treatment resulted in greater histological changes in the vole livers compared to the mice

Table 2. Summary of histological findings per group in the livers of wood mice and bank voles after feeding diclofop-methyl treated diets in laboratory feeding studies

Animal	Time (wk)	Dose	Hyper plasia	Hyper trophy	Loss of eosino-philia	Nuclear change		Nuclear inclusion bodies	Inflammatory changes	Necrosis
						Binu-cleated	Size			
Mouse	1	500 ppm	+	++	++	+	+	+	++	++
Mouse	1	1000 ppm	none	++	++	none	+	none	++	+++
Mouse	2	500 ppm	+	+	+	+	+	+	++	+
Mouse	2	1000 ppm	none	+++	+++	none	+	none	+++	+++
Mouse	4	20 ppm	+	+	+	+	+	+	+	none
Mouse	4	200 ppm	+	+	+	+	+	+	++	+
Vole	2	200 ppm	-	++	++	-	+	none	++	++
Vole	2	1000 ppm	-	++	+	-	+	none	+	+

+ to +++ = increasing severity of tissue changes when compared to slides from control animals.

livers. The inflammatory response was greater at 200 than 1000 ppm, while at 1000 ppm, regenerative replacement of necrotic hepatocytes, greater lipid degeneration and a decrease in cytoplasmic protein was observed.

Further chronic treatments with diclofop-methyl were made to Japanese quail in the laboratory at levels up to 1000 ppm for 2 weeks and at 20 ppm for up to 8 weeks, however no effects were observed on tissue esterases, tissue derived plasma enzymes or relative liver weight although hepatic cytochrome P_{450} significantly decreased (p 0.05) at most treatment levels. These results are not presented here in further detail. There would not appear to be any potential hazard to avian species from the field application of diclofop-methyl.

The mainly hydropic enlargement leading to increased relative liver weights and diffuse hepatocyte necrosis represent reversible effects in mouse and vole livers. Any further pathological or physiological insult to the affected livers or environmental stress could be expected to produce liver failure once the regenerative reserve capacity had been exceeded. This damage while limited and reversible may explain the increased incidence of inflammatory and hepatocyte degenerative changes observed in the vole livers containing tape worm cysts but not in the mice livers. The presence of tape worms may explain the hepatic degenerative changes (Cook et al, 1981) due to diclofop-methyl in the voles. Their absence in mice may account for reduced liver damage potentially giving them greater hepatic resistance to stress as demonstrated by chronic exposure to diclofop-methyl.

Chronic laboratory exposure of small mammals to diclofop-methyl resulted in biochemical and histological changes which were used as background information to aid interpretation of field trial data. The field use of diclofop-methyl resulted in limited biochemical and histological changes in a wild population of small mammals (Westlake et al, unpublished). It would appear that this herbicide would not pose a hazard to small mammals when applied at the maximum recommended field rate. This comparative assessment of the sub-lethal effects of a given agricultural chemical emphasises the value of using wild trapped small mammals as indicator species and the varying biological affects which may occur under laboratory and field conditions.

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