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Biochemical and Histological Effects of the Aphicide Demeton-S-Methyl on House Sparrows (*Passer domesticus*) under Field Conditions

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Field trials with extensive biochemical measurements supported by histological studies have proved valuable in the assessment of the environmental hazard of pesticides to non-target species (Hardy *et al.* 1987; Westlake and Tarrant 1988). This integrated approach is particularly sensitive for detecting sub-lethal or transient physiological changes as opposed to direct mortality. Such studies allow for a wider interpretation of the toxicological effects of a specific pesticide application on a population rather than individuals. An opportunity to further investigate this approach was provided by a seven year field study (The Boxworth Project) set up by the Ministry of Agriculture, Fisheries and Food, U.K., to examine the potentially harmful environmental effects that might occur due to sustained use of pesticides on winter wheat (Greig-Smith 1989).

The inhibition of brain acetylcholinesterase (AChE) activity is the primary toxic lesion caused by organophosphorus compounds. This inhibition has been widely used in monitoring exposure to organophosphorus pesticides and in the diagnosis of death due to these compounds (Ludke *et al.* 1975; Hardy 1987). However, serum butyrylcholinesterase (BChE) is a more sensitive monitor of exposure than brain AChE (Thompson *et al.* 1988) although its physiological function is unknown. Unlike some avian species house sparrow serum does not contain high levels of AChE activity (unpublished data).

Glutamate oxaloacetate transaminase (GOT) is present in the matrix of mitochondria in a wide variety of tissues including cardiac and skeletal muscle in addition to the liver. Although not tissue specific elevation of serum GOT is one of the most frequently measured indicators of liver malfunction. Damage to hepatocytes for example by pesticides (Anthony *et al.* 1986), may result in membrane leakage leading to release of GOT into the blood and hence elevation in plasma GOT activity. Therefore, GOT provides a biochemical indicator of physiological damage which can be further confirmed and investigated at the cellular level. This initial effect of pesticide exposure, predominantly exhibited as

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mitochondrial damage leading to the release of the enzyme GOT represents a direct pattern of hepatic injury which can be confirmed by histopathological studies. An immunological pattern of hepatic injury may also be observed. It is characterised by damage to cell membranes and involves necrosis of peripheral (zone 1) hepatocytes with mononuclear cell infiltration. This expands the cellular composition of portal areas and possibly bile duct areas. This inflammatory infiltrate may extend into the liver lobule and include piecemeal necrosis of hepatocytes which can persist to produce a prolonged chronic active hepatitis. These mechanisms of liver degeneration can produce metabolic disturbances of the hepatocytes themselves or of intercellular substances.

Changes in liver morphology can be observed in stained liver sections under light microscopy. These morphological changes which are due to the sub-acute effects of a pesticide may be minimal and easily reversed. They are more readily quantified using image analysis techniques (Tarrant 1988).

One of the pesticides in regular use in the Boxworth Project was a summer aphicide containing the organophosphate demeton-S-methyl (S-2-ethyl-thioethyl 0,0-dimethyl phosphorothioate). Observations showed that house sparrows (*Passer domesticus*) fed on the crop in large numbers where they would be exposed to the demeton-S-methyl spray and could therefore be used as a non-target indicator species for the effects of pesticides on birds. This present study was undertaken to investigate exposure to a field application of demeton-S-methyl measurable as a change in activities of serum enzymes and brain acetylcholinesterase and its possible effects on liver histology of house sparrows.

MATERIALS AND METHODS

Demeton-S-methyl was applied as a spray to wheat fields at Boxworth Experimental Husbandry Farm, Cambridgeshire, U.K., at the application rate of 0.485 L.a.i/ha. The formulation was an emulsifiable concentrate in a xylene solvent base and had previously been given M.A.F.F. clearance and pesticide approval for use as a summer aphicide on wheat. (product registration number 01331). House sparrows were mist netted on three consecutive days between the 19th and 21st of July 1988 following application of the aphicide. Control serum and tissue samples were obtained from birds trapped before the aphicide was applied or from other untreated areas of the farm. After capture the birds were placed into cotton drawstring bags and transported immediately to a mobile laboratory at the field site. Blood samples were collected from the birds by puncture of the brachial vein and serum was separated by centrifugation and assayed immediately for cholinesterase activity. The birds were then humanely killed, body weights were measured, the brains and livers were removed, and the birds sex recorded.

Brain samples were homogenised whole in 0.1% Triton X-100 in 25 mM tris-HCl (pH 7.6) and assayed immediately for AChE activity. Serum

cholinesterase activity towards butyrylthiocholine was measured by the method of Ellman *et al.* (1961) as adapted by Westlake *et al.* (1980). Serum glutamate oxaloacetate transaminase (GOT) activity was assayed by the method of Bergmeyer and Bernt (1963). Brain acetylcholinesterase activity towards acetylthiocholine was assayed according to the method of Ellman *et al.* (1961). The significance of the biochemical results were evaluated using non-parametric Mann-Whitney test.

After removal livers were weighed and 4 mm slices were taken from each lobe and placed into buffered neutral 10% formalin for histological evaluation. After fixation the tissue slices were routinely embedded in paraffin wax (58°C), 6μm sections were taken using a base sledge microtome and stained with Ehrlichs Haematoxylin and Eosin. The stained liver sections were then evaluated using both visual observations and quantified using image analysis. Image analysis was performed on a direct visual image from the microscope displayed, via a video camera, onto a monitor screen attached to a personal computer. Parameters measured were hepatocyte bi-nucleation, hepatocyte size and counts of inflammatory foci observed as aggregations of predominantly monocytes in liver tissue or adjacent blood vessels and bile ducts. The significance of the histological results were evaluation using non-parametric Mann-Whitney and Kruskal-Wallis tests.

RESULTS AND DISCUSSION

Exposure of house sparrows to demeton-S-methyl was indicated by inhibition of serum BChE activity and also resulted in slight inhibition of brain AChE and raised serum GOT levels in some birds (Table 1). This was associated with effects measurable as changes in liver cell parameters including hepatocyte size, bi-nucleation (Table 1) and the formation of granulomatous foci (Fig. 1). Inhibition of serum BChE, to a mean of 64% of control values by the third day after spraying, was not associated with a similar decrease in brain AChE activity which illustrates the greater sensitivity of serum BChE to inhibition by demeton-S-methyl and its potential usefulness in non-destructive monitoring of pesticide exposure (Thompson *et al.* 1988).

Elevated serum GOT levels indicated that exposure may have resulted in cellular damage within the liver after spraying. This evidence for liver damage is supported by the histological evaluation of the Haematoxylin and Eosin stained liver sections. On the first day after spraying there was a decrease in hepatocyte size and a significant increase in hepatocyte bi-nucleation (Mann-Whitney test $p < 0.05$) when compared to control values. This indicates that the response of house sparrow livers was an induction of replication to increase liver cell numbers in order to overcome the toxicological response to demeton-S-methyl. This response has also been demonstrated in regenerating livers in laboratory rats dosed with chloroform (Beams and King 1942) and in wood mice exposed to a herbicide (Westlake *et al.* 1988). However, this induction did not result in an increase in the total liver weights, expressed as

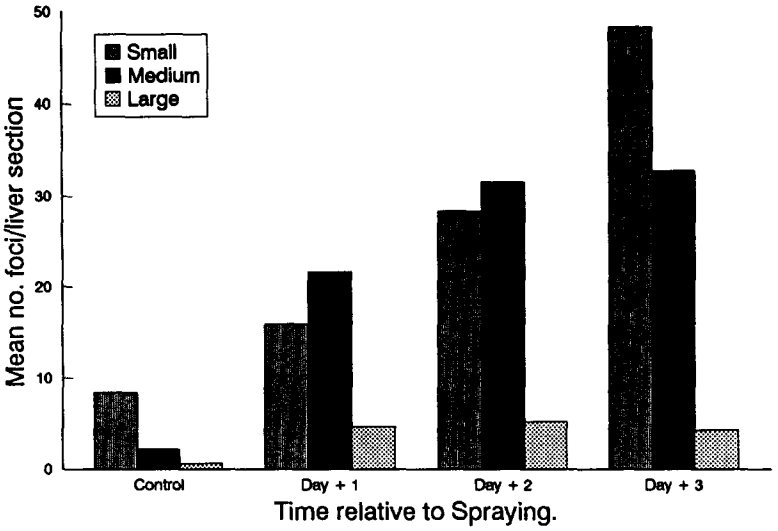

Figure 1. Counts of inflammatory foci for whole liver sections from house sparrows in control birds compared to days 1, 2 and 3 post application of demeton-S-methyl (group means).

Table 1. Results of histological and biochemical investigations of house sparrows trapped 1-3 days after demeton-S-methyl application.

Days	0	1	2	3
Number of birds	5	7	6	6
Liver Wt/Body Wt	.23	.20	.21	.22
Binucleation[1]	1.9 (0.7-3.0)	5.0[a] (1.5-12.8)	3.3[b] (1.7-5.3)	4.0[b] (2.6-5.6)
Cell Count[1]	15.7 (11.1-17.7)	18.3[a] (13.3-20.0)	16.4 (13.5-18.8)	17.1 (16.4-17.7)
Brain AChE Activity[2]	100 (80-129)	104 (82-121)	82[b] (71-130)	90 (71-104)
Serum BChE Activity[2]	100 (57-219)	72 (49-80)	73 (26-103)	64 (43-83)
Serum GOT Activity[2]	100 (93-123)	85 (60-120)	87 (58-108)	136 (45-223)

[1] mean of 10 fields per liver.
[2] mean % control activity (range).
[a] Mann-Whitney test $p < 0.05$.
[b] Mann-Whitney $p < 0.1$.

liver weight to body weight ratios. After spraying they remained lower than control values (Table 1).

Evidence for hepatocyte damage is further supported by an increase in inflammatory cell foci observed as granulomatous areas. These consisted mainly of monocytes, diffusely spread throughout the liver section. Although similar inflammatory foci were found in livers from control birds, both their frequency and size increased post-spray (Figure 1) with small foci predominating. There were significantly more monocyte foci of all three sizes amongst the birds sampled post-spray (Kruskal-Wallis test $p < 0.05$ small and large foci, $p < 0.01$ for medium foci). These granulomatous foci occurred more generally at the portal triad areas of the liver lobule with partial cuffing of veins by monocytes. There was also an increase in small aggregations of monocytes amongst the hepatocytes suggesting cell damage and this further supports the liver damage inferred by the raised serum GOT levels post-spray. A majority of the post-spray house sparrow livers also showed infiltration of predominantly monocytes into the sinusoid spaces together with a slight kupffer cell enlargement, which is indicative of a mild hepatitis.

Diffuse hepatocyte lipid vacuolation, mainly observed as medium to small vacuoles with no displacement of the nucleus, was present in a number of livers from both pre and post-spray birds and was generally present in livers containing many inflammatory foci. Accumulations of small lipid vacuoles are often indicative of hepatocyte damage and arise due to metabolic dysfunction (Anthony *et al.* 1986).

The observed liver changes were minimal in nature and were consistent with a low key response to a xenobiotic challenge. Even in birds with high foci counts, liver lobule architecture was generally normal with no fibrous scarring or large areas of necrosis. Similar liver foci have previously been observed in livers from house sparrows exposed to a carbamate in an orchard field trial (Hardy *et al.* 1987) and in mice dosed with a pyrethroid (Okuno *et al.* 1986).

The biochemical results from this study demonstrated that the house sparrows had been exposed to demeton-S-methyl. The birds showed a wide variation in their exposure to demeton-S-methyl as shown by their biochemical measurements. Further support for this evidence of exposure was the presence of developing wheat grains in the bird crops showing that the birds had been feeding in the sprayed fields. As the birds were free ranging the time individuals spent on the wheat field would ultimately determine their degree of exposure. The difference between pre and post-spray birds in biochemical and histological evaluations suggests that exposure to demeton-S-methyl resulted in effects at the cellular level and may also affect the immunological status of the birds to produce a chronic persistent hepatitis in some individuals.

All birds captured post-spray were in good physical condition with no gross pathological lesions even though they showed significant

changes in liver histology and serum enzyme values. This resilience when challenged with a pesticide, despite significant physiological and tissue changes, has previously been noted in rats (Anthony *et al.* 1986) and mice (Bhatnagar and Jain 1986). Although of unknown long term significance at the population level, in the short term individual birds may become more susceptible to endemic infection or show adverse behavioural changes leading to an increased vulnerability to predation and nest desertion.

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