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Effects of Exposure to an Organophosphate on the Seed-Handling Efficiency of the House Sparrow

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Organophosphorus compounds (OPs) are widely used as pesticides and there have been many reported cases of the unintentional poisoning of wild birds (Grue et al. 1983). The toxicity of these compounds is mainly due to the inhibition of acetylcholinesterase (AChE), an enzyme which is essential for the normal functioning of both the central and peripheral nervous systems (Grue et al. 1983). Previous studies have shown that depression of normal brain AChE to 50% or less can bring about changes in bird behaviour (Grue and Shipley 1981; Grue 1982; Grue et al. 1982; White et al. 1983; Gallindo et al. 1985; Kreitzer and Fleming 1988). The relationship between bird behaviour and AChE in the range between 50% and 100% of normal AChE activity was investigated by Hart (1993). At low levels of exposure there were subtle effects on behaviour including changes in flying activity, singing behaviour and posture. Effects on posture, such as the time spent standing on one leg while resting, were found at relatively low levels of exposure (AChE activity 88% of normal), and may reflect impaired balance or coordination.

Temporary behavioral and physiological changes caused by sublethal exposure could have longer term consequences for survival or reproductive success. Exposure to OPs is known to disturb the feeding behavior of birds in ways which can bring about significant weight loss and ultimately death (Grue et al. 1991). At higher doses birds avoid food even when the source of exposure is not dietary (Pope and Ward 1972). At lower levels of exposure, when birds appear to be actively feeding, it is possible that impaired coordination may reduce their feeding efficiency. For example seed-eating birds have to remove husks from seeds by manipulating them in the beak and risk dropping them during handling (Greig-Smith 1984). If the food item cannot be retrieved, for example if the bird is feeding in a tree, this could be a significant cost to the bird. Birds made more clumsy by pesticide exposure may drop more seeds. Any disruption of the feeding efficiency of a small bird on a tight energy budget could depress its chances of survival. This study examined the relationship between brain AChE inhibition after exposure to an OP and the seed handling ability of the house sparrow (*Passer domesticus*).

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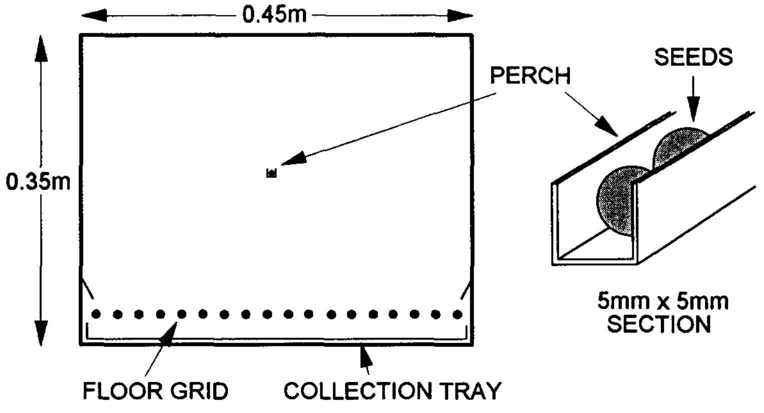

Figure 1. Front view of test cage (0.45 m deep) with enlarged view of a section through the test perch.

MATERIALS AND METHODS

Twelve adult female house sparrows were tested for their ability to feed on hemp seeds (*Cannabis sativa*) without dropping them during husking. House sparrows were used because they are typical of small seed-eating birds and are relatively easy to keep in captivity. Tests were carried out in individual indoor cages with specially-designed 'U' section perches into which the seeds were placed (Fig. 1). The design ensured that dropped seeds were very unlikely to fall back into the seed container. Birds had to stand on the perch to obtain seeds, and any that they dropped during handling fell through a metal floor grid into a collection tray out of the birds reach where they could be collected and counted. Tests were carried out in an indoor room at a mean temperature of 16°C and with a 12 hour light, 12 hour dark cycle.

A pilot study was carried out in order to determine the size range of seeds most suitable. We required the task to be reasonably difficult and aimed to find a seed size which was handled successfully on around 50% of attempts. The seed size was controlled by sieving, and the size range selected was 2.6 to 2.8 mm in diameter.

After training to feed on hemp seeds and acclimatization to the test cages and perches birds were tested on three days (Table 1). On each day normal food (turkey starter crumbs) was removed 1.5 hours before testing. Birds were then given four perches, one after the other. Each perch contained exactly 100 seeds and was presented for a 1.5 hour period before being exchanged for a fresh one. This allowed us to gain some measure of the time course of any effects. Tests were carried out at the same time each day (0930 to 1530) and birds were weighed before testing on days 2 and 3 to determine the dose volume. After testing on day 1 and day 2 the birds were returned to their normal diet. After testing on day 3,

Table 1. Treatments on each test day.

Day	Dosing	Reason for test
1	No dosing	To measure normal dropping rates for comparison with dosing trials.
2	Corn-oil	To test for any effects of dosing procedure alone.
3	Chlorfenvinphos (corn-oil for controls)	Test day

six hours after dosing, the birds were reweighed and humanely killed with CO_2. They were then stored frozen below -20°C for up to three weeks before determination of brain AChE activity using a method based on Ellman et al. (1961) adapted for use on a microtitre plate. The assay was carried out at 37°C with 0.5mM acetylthiocholine iodide, and AChE activity was expressed as micromoles substrate/min/g brain tissue.

We used four dose levels of 0 (control), 2, 6 and 10 mg/kg which were chosen to cover the range of exposure at which we would expect to see mild behavioural effects. Technical grade chlorfenvinphos (donated by Shell Research PLC, Sittingbourne, Kent, UK) was prepared in acetone and mixed with corn-oil to produce solutions that could be administered at a rate of 0.2 μL/g body weight. We placed the solution directly into the birds' crops using a micropipette. Three birds were tested at each dose. Dummy dosing on day 2 was carried out in an identical manner with the appropriate volume of corn-oil.

For each perch presented, we counted the number of seeds remaining in the perch and the number of whole seeds dropped on to the floor. Feeding inefficiency was expressed as the percentage of seeds removed from the perch that were dropped.

RESULTS AND DISCUSSION

The mean % dropped was 49.6 on day 1 (SD 13.0) and 50.2 on day 2 (SD 13.4). These values were close to our intended mean success rate of 50% and meant that we were well placed to detect any subtle effects on the ability of the birds to handle the seeds. There was a good correlation between brain AChE activity at the end of the third day and dose ($r = -0.84$, $p < 0.0005$, regression equation AChE = 31.2 - 1.46[dose]). Mean AChE activities for the 0, 2, 6 and 10mg/kg groups were 33.7, 26.4, 20.0 and 18.4μmol/min/g respectively (SDs 4.8, 3.1, 2.9 and 3.3).

Table 2. Number of seeds left in perches on day 3.

		Seeds remaining (out of 100)			
Bird	Dose(mg/kg)	Perch 1	Perch 2	Perch 3	Perch 4
11	0	28	0	0	0
13	0	1	0	0	21
16	0	0	0	0	0
1*	2	0	0	0	0
2	2	0	0	0	1
18	2	23	1	0	9
9	6	3	0	0	0
10	6	0	0	0	0
15	6	0	0	0	0
3	10	0	0	0	0
8	10	0	0	0	0
12	10	14	0	0	0

(* this bird left 2 seeds in perch 2 on day 2).

AChE activity levels of exposed birds ranged between 85.2% and 48.4% of the mean value for control birds and the estimated I50 dose was 9.8mg/kg, very close to the highest dose level used. This provided a good range of inhibition values against which to correlate dropping rate.

All seeds were removed from perches on the first two days apart from one perch on the second day where only two seeds were left by one bird. Some seeds were left in perches on day 3 with most left in the first perch (Table 2).

Animals made to feel ill often appear to attribute it to something they ate and avoid that particular food thereafter (Garcia et al. 1985). In previous studies some birds have responded to oral exposure by refusing uncontaminated food even when there was no alternative (Grue 1982; Hart 1993). However most seeds were left by birds in the 0 and 2mg/kg groups and there was no correlation with dose or AChE activity. As only three birds left significant numbers of seeds in the first perch while seven birds left none, it seems unlikely that birds were treating the food as the source of any ill effects. If birds were suspicious of the food it is unlikely that the most exposed birds would remove seeds from the perch in the same numbers as on previous days.

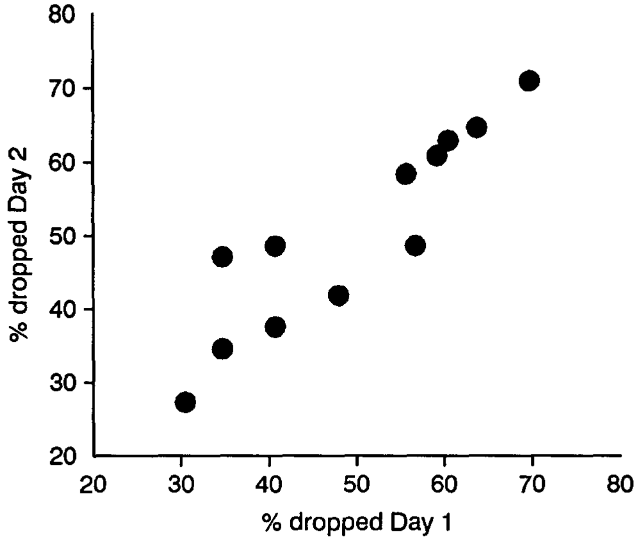

Figure 2. % dropped on Day 1 (control day) plotted against Day 2 (dummy-dosing day).

There was no significant difference between mean % dropped on the first two days (paired t-test, $t = 0.35$, $p = 0.73$), and the correlation within individuals from one day to the next was highly significant ($r = 0.91$, $p < 0.0005$) as shown in Fig. 2. This indicated that the behaviour was consistent from day to day and there was little effect of the dummy oral dosing procedure on dropping rate. Mean drop rates on day 2 for the 0, 2, 6 and 10 mg/kg groups were 37.7%, 51.3%, 53.9% and 57.7% respectively. Birds were assigned randomly to the test groups and the mean drop rates for the dosed groups were similar, but that of the control group was slightly lower. However, measuring the change in seed-handling efficiency of each individual before and after exposure, rather than ability on the day of exposure only, removed any effects of individual differences. The consistency of this behaviour from day to day meant that any change in ability of an individual on day 3 was almost certainly due to the effects of exposure to the OP.

To control for individual differences in feeding inefficiency the difference in the measure of drop rate between the test day and the dummy dosing day (Day 3 - Day 2) was used to determine the effect. The differences in % dropped for each perch plotted against brain AChE activity at the end of the test are shown in Fig. 3. As the drop rate was expressed as a percentage, the data for each perch on each day was subjected to the arcsine square-root transformation before analysis. Differences were then calculated from the transformed data for each perch. These differences were then correlated with brain AChE activity. There was a significant increase in the proportion of seeds dropped with decreasing brain AChE activity for the first perch only ($r = -0.674$, $p = 0.016$). The main effects occurred where

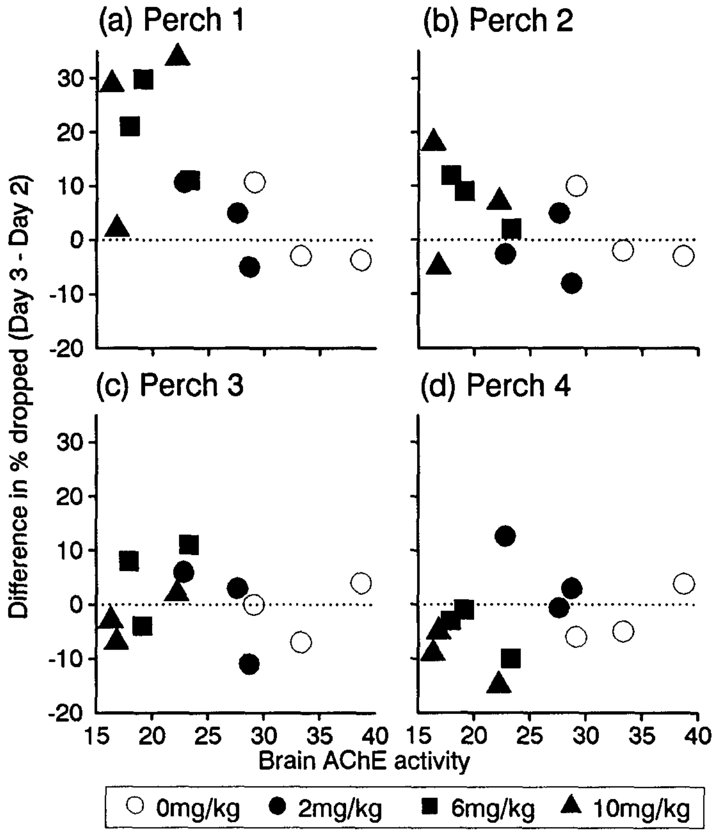

Figure 3. Difference in % dropped between dosing and dummy-dosing days plotted against brain AChE activity (μmol/min/g) at the end of the dosing day.

brain AChE activity was inhibited to less than 70% of control levels (mean AChE activity of 0 mg/kg birds). The trend was not significant for the second perch (Fig. 3b) and had all but gone by the third, 3 to 4.5 hours after dosing (Fig. 3c). By the fourth perch (Fig. 3d) the birds in the 10mg/kg group appeared to handle seeds more successfully than on the previous day suggesting a compensatory mechanism where hungry birds may take more care handling seeds. However the small sample size prevented us from testing this statistically.

The possible ecological significance of this increase in drop rate can be seen when body weight change (weight at end of day - weight at dosing) on the test day is plotted against dropping rate (Fig. 4). There was a significant negative correlation ($r = -0.841$, $p < 0.0005$) showing that birds which dropped most seeds lost more weight. It is therefore clear that even under these conditions where food is plentiful, birds can only maintain weight by handling seeds adeptly. The results indicate that sparrows exposed to an OP become clumsy and less able to husk

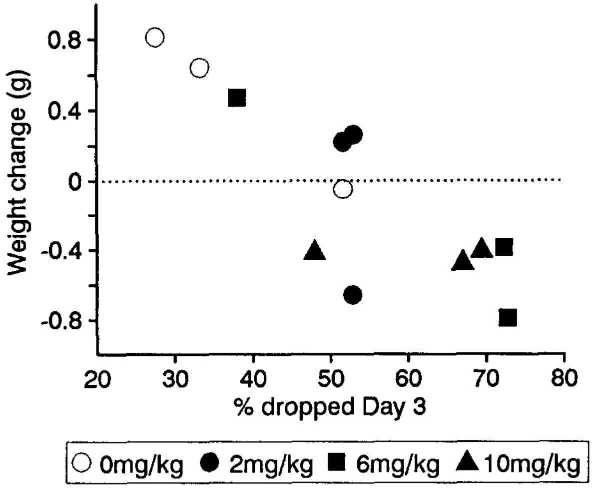

Figure 4. Body weight change (weight at end of trial - weight at dosing) on dose day plotted against % dropped.

awkward seeds without dropping them. The effects become measurable when brain AChE activity is inhibited to less than around 70% of control levels. The effect was short-lived (less than three hours) and this agrees with the results of other studies where effects of low exposure were also brief (Hart 1993). The consistency of this behavior for an individual bird from day to day combined with the ease of detection of the effect means that this method would be suitable for development as a behavioural bioassay. While measuring the change in performance of each bird removes any effects of individual differences, the method may be slightly improved by using a stratified random method to assign birds to treatment groups so that they contain a similar range of seed-handling abilities.

The implications for survival chances of birds in the wild will depend on environmental stressors (temperature, daylength, etc.), the amount of coordination required to obtain food and the type and distribution of food in the environment. Following exposure birds can become less active (Grue and Shipley 1981; Holmes and Boag 1990; Hart 1993) possibly due, at least in part, to the same disturbance of coordination which impairs seed-handling efficiency. While there may be enough food in the environment for the bird to maintain itself, albeit inefficiently, a patchy distribution may put this food out of reach of an exposed bird which is less able to move freely between patches. In this experiment birds did not have to move appreciably within their environment to search for food and there was only one type of food available which was difficult to handle. The effects in the wild could therefore be more or less severe depending on the patchiness of the food supply and the availability of easily-handled food items, even if these were less profitable.

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