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Effects of Sublethal Exposure to an Organophosphate on the Flying Performance of Captive Starlings

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Organophosphorus compounds are widely used as pesticides and there have been many reported cases of the unintentional poisoning of wild birds (Grue et al. 1983). These compounds inhibit cholinesterases including 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 a fall in brain AChE activity to 50% of normal can bring about changes in bird behavior (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 behavior and AChE over 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 behavior including changes in flying activity, singing behavior and posture. For example, at relatively low levels of exposure (AChE activity reduced to 88% of normal), birds spent less time standing on one leg, perhaps because of impaired balance.

Such effects of sublethal exposure may damage birds' survival or reproductive success. Slight effects on coordination during flight may increase the risk of collisions and possibly make a bird more vulnerable to predation (Hunt et al. 1992). Less efficient flying may also reduce foraging success which could endanger the survival of adults and any nestlings they are feeding. This study tested whether flying performance of captive starlings (*Sturnus vulgaris*) was affected by low levels of the OP chlorfenvinphos (2-chloro-1-[2,4-dichlorophenyl]-vinyl diethyl phosphate). We measured birds' abilities to avoid collision with obstacles in the flight path between two food dispensers. Starlings were chosen as they have been the subject of previous studies on OP's and behavior (Grue and Shipley 1981; Grue 1982; Grue et al. 1982; Hart 1993). Chlorfenvinphos was used as we already have some understanding of the relationship between exposure to this compound and the behavior of starlings (Hart 1993).

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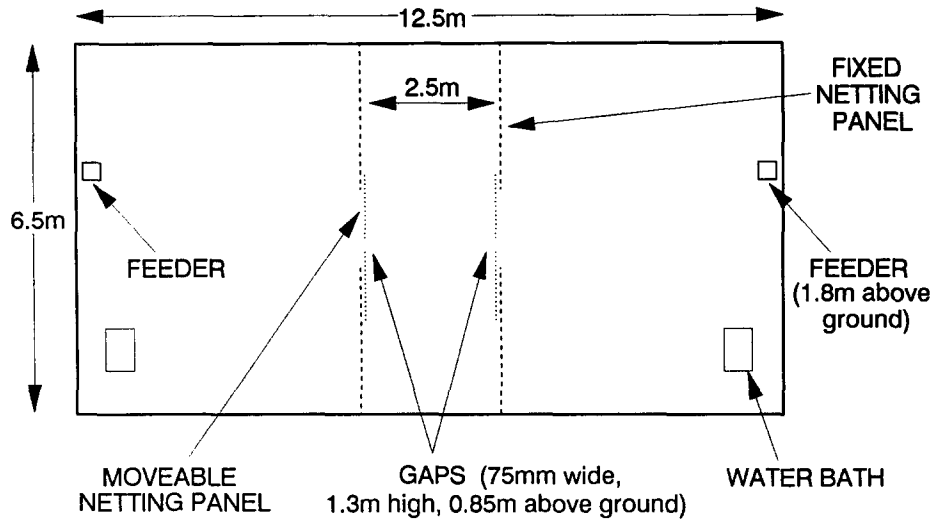

Figure 1 Aviary plan showing position of gaps and feeders.

MATERIALS AND METHODS

Wild-caught adult female birds were used from a batch caught six months before the first bird was tested. Birds were trained and tested between April and August 1992 in a large outdoor aviary (Fig. 1). The floor of the aviary was covered with a close-weave plastic mesh to prevent birds obtaining natural food. The only food sources were two electronically controlled feeders, one on each end wall of the aviary. These dispensed standard operant pellets (supplied by Camden Instruments) on the peck of a key. It had previously been demonstrated that birds could maintain themselves on this diet. Once food had been obtained from one feeder, that feeder became inactive and the other feeder was primed. The bird was therefore obliged to fly to the other end of the aviary to obtain more food. In flying from end to end, the birds had to negotiate two vertical gaps between netting covered panels. These panels were moveable so that the gap position and width could be altered during training. During the test period both slits were 75mm wide and positioned 0.9m to the same side of a line between the two feeders (Fig. 1).

Nine birds were tested sequentially (Table 1), three birds at each of three dose levels (1, 3 and 5mg/kg chlorfenvinphos). These doses were chosen to cover the range of exposure at which we would expect to see mild behavioral effects (Hart 1993). The dose levels were randomized within each run of three birds. After a period of training of up to two weeks, each bird was tested on two consecutive days. On the first day, the bird was dummy dosed with corn-oil (the carrier used for the chlorfenvinphos) at 0.1µl/g body weight. On the second day the bird was dosed with chlorfenvinphos. Dosing was by gavage using a micropipette. The control day with dummy dosing allowed us to eliminate the effects of the dosing

Table 1. Brain AChE activity (μmol substrate/min/g brain tissue) of each bird at the end of each test day. The date of testing is also shown.

Bird	Dose (mg/kg)	AChE activity	Date of test
1	3	37.4	8 May
2	1	38.4	22 May
3	5	38.5	12 June
4	3	31.7	24 June
5	5	24.4	16 July
6	1	39.6	28 July
7	1	27.9	6 August
8	3	26.9	14 August
9	5	10.2	27 August

alone as a cause of behavioral change and allowed each bird to be used as its own control. Dosing was carried out at around 0930 on each day and birds had no access to food after 1700 on the previous day.

On each day, direct observations of behavior were made for six hours after dosing. These included recordings of position and movements around the aviary, along with all successful and unsuccessful feeding attempts at each feeder. Behavior was recorded using the Observer 2.0 package on Tandy 102 portable microcomputers. Each flight through the gaps was also videotaped using video cameras positioned under each gap. The timer used in video recording was synchronized with that on the behavior logger. By combining behavioral observations with the synchronized video recordings we could determine which flights through the gaps were from feeder to feeder and assess the quality of each flight. Clear flights were defined as flights during which there was no contact with the netting as the bird flew through both gaps. Only flights from feeder to feeder were considered so that the task facing the bird on each flight was the same. The effect of dose on each behaviour was determined using a one-way ANOVA. Light levels and air movement in the aviary were also measured.

At the end of the second day, six hours after dosing, the birds were humanely killed with CO_2. They were then stored frozen below -20°C for up to eight weeks before determination of brain AChE activity using a method based on Ellman et al. (1961) adapted for use on a microtitre plate.

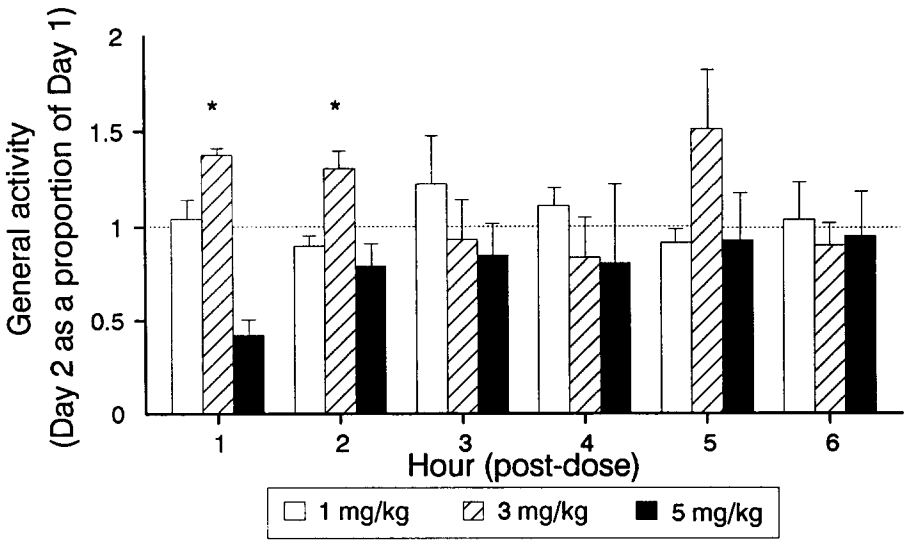

Figure 2. General activity on the test day as a proportion of the control day (* significant difference between groups; bars represent standard errors).

RESULTS AND DISCUSSION

During trials, the birds tended to fly between the feeders in bouts of several flights separated by periods during which they either rested on one of the feeders, or spent time on the ground drinking, bathing or attempting to forage. There were no gross effects on flying ability on the test day although birds did occasionally miss the gap completely and fly into the netting. However, this was also seen on control days. A more subtle measure of flying ability compared the number of flights during which birds made no contact with the netting, with those where the netting was clipped with the wing tip. The flying ability for each bird was calculated as the percentage of total flights from feeder to feeder that were clear. On the control day the mean proportion of flights in which birds managed to fly through the gaps without making contact with the sides was only 52%. The task was thus not trivial and any impairment of birds' abilities to perform it should have been easily detectable.

The difference between control and test days in the number of flights, the proportion of clear flights or flight speed was not significantly correlated with the daily differences in wind speed or light levels ($p > 0.05$) suggesting that neither wind speed or light level had a strong influence on flying behavior. These were therefore excluded from further analysis.

There was a poor correlation between dose and brain AChE activity at the end of the test day (Table 1). This appeared to be at least partly due to a seasonal variation in control levels. The experiment extended over a period of five months and levels of AChE activity in control birds that were undosed appeared to show a

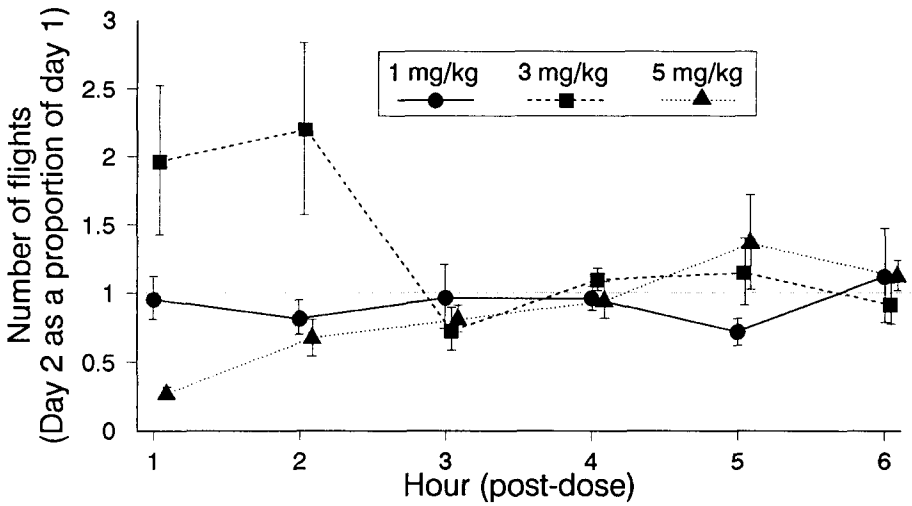

Figure 3. Number of flights between feeders during each hour on the test day expressed as a proportion of the control day (bars represent standard errors).

seasonal shift with levels increasing slightly during the first half of the test period and falling significantly during the second. Even when this shift was taken into account there was still a large variation in calculated AChE activity for each dose suggesting a large individual variation in starting levels of activity or individual differences in response to the doses. All behavior was therefore related to dose rather than AChE activity.

Bird activity while at the ends of the aviary, expressed as the number of changes of position (feeder to ground, ground to aviary wall, etc.) excluding flights through the gaps, is shown in Fig. 2. There was a significant difference in activity between the treatment groups in the first ($F=46.0$, $p=0.0002$) and second ($F=10.2$, $p=0.012$) hour. While the 1mg/kg group was little affected, there was an increase in activity in the 3mg/kg group and a reduction in the 5mg/kg group compared to the previous day.

Mean number of flights between feeders expressed as a proportion of the previous day is shown in Fig. 3. Again the main effects occurred in the first two hours with a significant difference between groups in the first hour ($F=6.7$, $p=0.029$). Birds in the 3mg/kg group appeared to be stimulated during the first two hours flying around twice as much as normal. The 5mg/kg group showed a depressed level of flying activity compared to the previous day for the first two hours followed by a recovery to normal levels.

The time taken to fly between the gaps for each flight for the first two hours of each day was also determined from the video-recordings and used to calculate

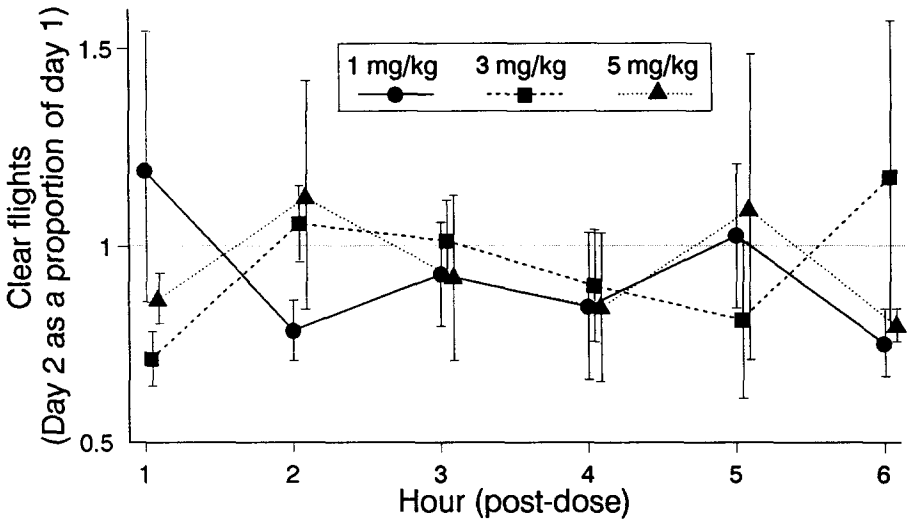

Figure 4. Number of clear flights (no contact with the netting) on the test day expressed as a proportion of the control day (bars represent standard errors).

flight speed. Birds that were most affected may have been expected to fly more slowly through the gaps to reduce the risk of error and collision but there was no significant effect of dose ($p > 0.05$).

The mean percentage of clear flights for each hour on the test day expressed as a proportion of the control day is shown in Fig. 4. Again the main differences were seen in the 3mg/kg and 5mg/kg groups in the first hour after dosing. In both cases there appeared to be a reduction in the proportion of flights that were clear in the first hour. While the 3mg/kg group showed the highest level of flying activity it also showed the most impaired flight. Birds in the 5mg/kg group appear to have been slightly less affected, but the proportions are based on a small number of flights. However, there was no statistically significant difference between groups.

The range of behavioral effects observed confirmed that we had covered the full range of exposures that we had set out to investigate. These ranged from negligible effect (1mg/kg) to stimulation of activity at slightly higher levels (3mg/kg) and reduced activity at higher exposures (5mg/kg). The cause of the stimulation in general activity, and flying seen in the 3mg/kg group is unclear, but has been seen in previous experiments with birds at similar levels of exposure (Hart 1993). The reduction in activity at higher doses is also consistent with results of other studies and may be associated with nausea and a reduced desire to feed (Hart 1993). However, it is also possible that effects on coordination led to an increase in the perceived risk of flying. In a study by Cuthill and Guilford (1990) an increase in perceived risk of collision was shown to affect the foraging

decisions of birds. Starlings faced with the choice of flying through a wide gap to obtain a small food reward or a narrow gap to receive a larger reward, avoided the narrow gap unless they were hungry.

Closer examination of the behavior data indicated that the birds in the 5mg/kg group, which were less active in the first two hours, spent more time on the ground than birds in the other groups. During this time they were observed searching the ground for food indicating that a general lack of appetite is unlikely to be the cause of the reduction in flying. Previous studies have shown that birds exposed to pesticides by oral dosing appear to associate their malaise with something they ate. Subsequently they may avoid unadulterated food, even when there is no alternative (Grue 1982; Hart 1993). In another study, exposure to dicrotophos caused starlings to avoid their laboratory diet but to increase the amount of time spent searching the ground for natural food (Grue and Shipley 1981). Mean consumption of pellets in the first hour by birds in the 5mg/kg group was reduced to 30.7% of the amount taken on the control day, and returned to normal levels after the first two hours. Two birds continued to feed on pellets at a reduced but steady rate through the first hour while one avoided pellets almost completely after the first four minutes. Birds in the 3mg/kg group showed no such avoidance of the test food. Two birds greatly increased consumption at the start of the test day, while the other fed normally.

To test whether effects on flying activity are at least partly responsible for the observed reduction in flying activity at 5mg/kg we would need to force birds dosed at this level to fly. However, the act of flushing birds in order to determine flying ability may not have the desired effect. In a study involving quail dosed with parathion, Galindo et al. (1985) found that there was less difference in behavior between exposed and control birds when they were confronted with a predator.

It is possible that subtle effects on flying may make an individual more likely to be caught by avian predators (Hunt et al. 1992). The stimulation of flying in the 3mg/kg group, if it occurred in the wild, might make birds more obvious to a predator. The reduction in flying activity seen in the 5mg/kg group may reduce a bird's ability to escape avian predators, particularly if they flush easily but fly badly. However, the behavioral changes observed in this experiment were short-lived and any consequent increase in predation risk to individual birds is therefore unlikely to be of ecological significance.

The performance of a difficult flying task by starlings was not substantially affected by exposure to levels of OPs below those which cause general reductions in behavioral activity. Although the ecological consequences of exposure on the flying ability of active birds appear to be small, effects on other types of behavior may be more significant. For example, at similar low levels of exposure, the seed-handling ability of captive house sparrows has been shown to be impaired in such a way that birds drop more seeds during dehusking (Fryday et al. 1994). This

effect, although short-lived, led to weight loss even under laboratory conditions where food was easily available. Such effects on the feeding efficiency of a small bird in the wild could have more severe effects on the chances of survival.

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