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Does Sub-Lethal Exposure to Organophosphate Pesticide Affect Capture Rates in Free-Living Rodents?

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Live trapping has been advocated as a suitable technique for investigating the impacts of pesticides on wild small mammals (Edwards 1990). It has often been used in studies which have measured biomarkers of exposure, where samples were collected from captured animals (Hardy et al. 1993, Montz and Kirkpatrick 1985, Montz et al. 1983, Shore et al. 1997, Westlake et al. 1980). Live trapping and associated Capture-Mark-Release methods have been used to assess pesticide-mediated effects on populations, the general principle being to compare the numbers of individuals present after application with the numbers trapped either before treatment or in paired control areas. Several studies have used this approach and reported declines in populations following application of organophosphate (OP) and carbamate pesticides (Barrett 1988, Buckner and McLeod 1975, Edge et al. 1996, Johnson et al. 1991, Shore et al. 1997) although such impacts are not inevitable (Barrett and Darnell 1967, Jett et al. 1986, Tarrant et al. 1990).

Use of live trapping in field studies on pesticides relies upon the assumption that sub-lethally exposed animals enter traps with the same frequency as those which have not been exposed. However, the propensity of animals to enter traps is, in part, a function of their activity. It has been demonstrated that exposure to OPs can reduce activity in birds and mammals (Grue et al. 1991). Any sub-lethal effects of a toxicant on activity, or other behaviours, that prevent animals entering traps will bias capture data. When biomarkers (such as depression of brain acetylcholinesterase (AChE) activity following exposure to OPs) are being measured, failure to trap exposed animals will lead to an under-detection of biomarker responses, because non-exposed animals are disproportionately sampled. Such a bias may well lead to an under-estimate of exposure of the population as a whole. Conversely, in studies where population numbers are quantified, animals which survive exposure but do not enter traps post-application are assumed to be dead (or to have emigrated) whereas, in fact, they may be present but untrappable. This would result in an over-estimate of the toxicity of a pesticide to small mammals.

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The aim of the present study was to test the hypothesis that exposure to an OP affects the frequency with which free-living rodents are captured (trappability) and so might bias studies investigating the environmental impacts of pesticides. It was conducted in order to improve risk assessment procedures aimed at protecting the long-term viability of wild mammals in agro-ecosystems. Two live-trapping field experiments were carried out. Both involved dosing or sham-dosing animals with dimethoate and examining subsequent recapture rates. The first experiment was part of a wider project and was carried out in an arable system on wood mice *Apodemus sylvaticus*, the predominant species present in this habitat in the UK (Green 1979). The second study was specifically designed to examine the effects of exposure on trappability and capture rates were monitored more intensively than was possible in the first study. This was achieved by using bank voles *Clethrionomys glareolus* in a woodland habitat as the model system. Unlike the nocturnal wood mouse, bank voles are active day and night (Dell'Omo et al. 1998) and effects on recapture rates can be assessed frequently throughout each 24 hour period. This study had a further advantage in that the density of bank voles in the woodland was greater than that of wood mice in arable systems and so replication of the experiment was enhanced.

MATERIALS AND METHODS

In both studies, experimental treatment was an intraperitoneal (IP) injection of 50 mg kg^{-1} body weight dimethoate (*O,O*-dimethyl *S*-2-(methylamino)-2-oxoethyl phosphorodithioate, Cheminova, Lemvig, Denmark) diluted in saline (0.9% NaCl), or saline only (controls). The dose was chosen on the basis of previous laboratory studies which found that it caused substantial reductions in brain AChE activity which lasted for longer than 24 hours (Dell'Omo and Shore 1996a). The preferred route of administration would have been gavage. This is because it would have been more representative of the normal route of exposure, which is thought to be oral through ingestion of contaminated forage and by grooming of contaminated fur. However, gavage can be stressful to wood mice and accurate delivery of dose can also be problematic. For these reasons, IP injection was used.

In the first study, mainly male wood mice were trapped in two separate fields of winter wheat near Cambridge, UK, during March and April 1995. A 5 x 10 grid of 50 Longworth traps baited with oats was established in each field, traps being spaced regularly at 24 m intervals (Tew et al. 1994). Traps were checked every morning. When first captured, animals were identified to species, sexed, weighed and given a unique fur clip. Only mice recaptured three-five times during an initial period were assigned to the subsequent phase of the study. In this way, non-resident animals were excluded from the experiment. On the third-fifth time they were trapped (generally six-nine days after they were initially caught), mice were weighed, injected with dimethoate or saline and immediately released. Captures of individual animals were then recorded on a daily basis during the following 4-18 days.

The second study was carried during August 1995 at the "Chisti Les" field station (Bubonizi, Toropez, Tver region, Russia) during a scientific exchange visit. In the surrounding mixed deciduous and conifer forest, a 0.6 ha grid of 72 Sherman traps, regularly spaced at 10 m intervals and baited with oiled bread, was established. Traps were checked every eight hr for eight days. Unlike the first study, trapping was not carried out prior to injection because maximum replication of treatment was desired. When voles were first captured, they were sexed, weighed and given a unique fur clip. Half were then injected with dimethoate using the same dose as in the first study, the rest were given saline only (controls). The voles were then released.

Statistical analysis of the effects of dimethoate on wood mice and bank voles was carried out using paired t-tests and General Linear Models (GLMs) respectively. In the GLMs, the effects of treatment (dimethoate or saline), sex and the interaction between them were examined. Difference values in the paired t-test and residuals in the GLMs were tested for non-normality using the Kolmogorov-Smirnov normaility test (Minitab v.12, Minitab Inc, PA, USA). The assumptions of normality were met using untransformed data except for the distance data for bank voles (Figure lb) which had to be log+1 transformed before analysis.

RESULTS AND DISCUSSION

In the first study, five resident wood mice (four males and one female) were injected with saline and seven (all male) were given dimethoate. There was no difference between the two groups in the time taken for animals to be recaptured after injection. Ail saline-injected mice and six of the dimethoate-treated mice were captured on the first day after injection and the remaining dimethoate-injected mouse was recaptured on the second day.

Table 1. Capture ratios, expressed as n_c/n_t (number of times captured/number of trap rounds after first capture or injection in which mouse could have been captured) and as % n_c/n_t, for wood mice before and after treatment with dimethoate or saline. *indicates mouse dead in trap, †indicates mouse was female.

dimethoate-treated mice						saline-treated mice					
mouse	n_c/n_t pre-exposure	% pre-exposure	n_c/n_t post-exposure	% post-exposure	diff (%)	mouse	n_c/n_t pre-exposure	% pre-exposure	n_c/n_t post-exposure	% post-exposure	diff (%)
1	4/4	100	4/10	40	60	1	3/4	75	4/6	67	8
2	4/4	100	8/11	73	27	2	3/4	75	2/18	11	64
3	4/6	67	6/6	100	-33	3	4/5	80	4/4	100	-20
4	4/4	100	4/5	80	20	4	4/7	57	5/5	100	-43
5	4/11	36	4/4	100	-64	5†	4/4	100	14/18	78	22
6	3/4	75	4/4	100	-25						
7	5/5	100	1/1*	100	0						
mean					-2.1	mean					6.2
(±SE)					15.8	(±SE)					18.3

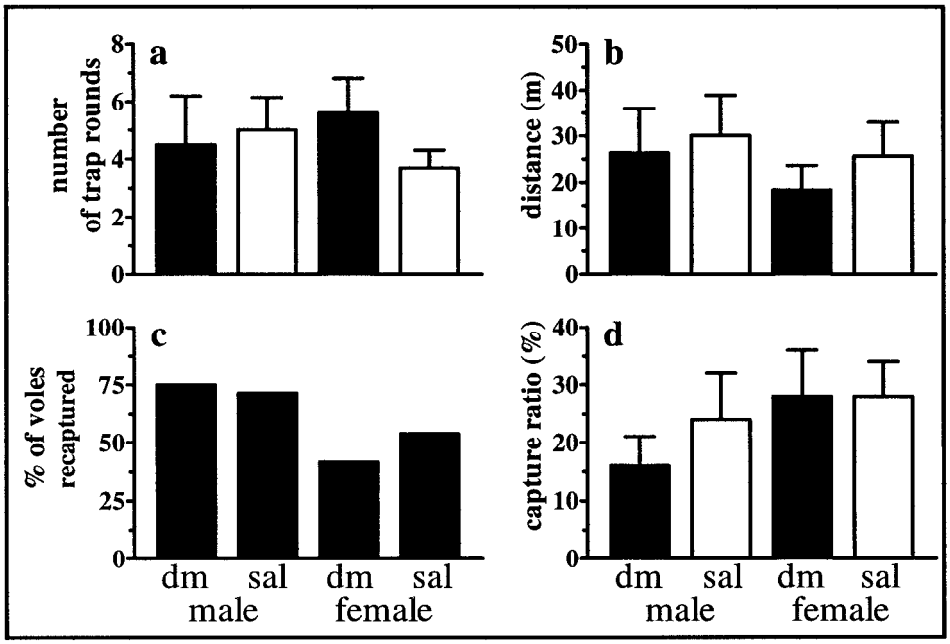

Figure 1. Recapture data for bank voles after a 50 mg/kg body weight IP injection of dimethoate (dm) or saline (sal). Data are: (a) number of trap rounds (8 hour intervals) before voles were first recaptured; (b) trap-revealed distance moved between injection and first recapture; (c) % of voles recaptured during the study; (d) capture ratio (see Table 1) expressed as a %. Where errors bars are shown (a,b,d), data are the mean ± SE. The numbers of animals (replicates) in each group are given in the text.

There was no evidence that dimethoate had a longer term effect on the trappability of wood mice. This was determined by comparing the capture histories of individuals before and after treatment. The mean difference in the capture ratio pre and post-treatment was close to zero both for wood mice injected with dimethoate and those given saline (Table 1). These differences were not statistically significant (dimethoate: paired $t_{(6)} = 0.134$, $P>0.05$; saline: paired $t_{(4)} = 0.346$ $P>0.05$) although, because of the small sample sizes in both groups, differences of less than 30-40% (2 x SE of the mean difference) may not have been detected.

In the second experiment, 15 male and 25 female bank voles were captured. Of these, eight males and 12 females were treated with dimethoate, the remainder were given saline. Short-term impacts were assessed by analysing the number of 8 hr trap rounds completed before dimethoate and saline-injected animals were first recaptured (Figure la) and the trap-revealed distance that the voles moved during this time (Figure lb). There were no statistically significant effect of dimethoate, sex or any significant interaction between dimethoate treatment and sex for either

of these measures ($F_{1,19} \leq 2.37$, $P>0.05$ in all cases). Thus, there was no evidence that dimethoate had a short-term effect on the trappability of bank voles.

Long-term effects of exposure were determined by examining the proportion of pesticide-treated and control voles recaptured throughout the study (Figure 1c) and the capture ratios of those animals (Figure 1d). The proportion of voles that were retrapped was almost identical for dimethoate and saline injected animals (Figure 1c). Statistical examination of capture ratio data indicated that the frequency with which voles were recaptured was not significantly affected by dimethoate treatment, sex or an interaction of these factors ($F \leq 1.24$, $P>0.05$ in all cases). Overall, therefore, there was no evidence of either a short or long-term effect of dimethoate on the trappability of bank voles.

This study is the first to assess the effects of a sub-lethal exposure to an OP on the trappability of free-living small mammals. There appeared to be no detectable impact of dimethoate on the capture frequency of wood mice or bank voles. Dimethoate was used as a model OP, rather than being of interest *per se*, and published studies suggest that different OPs have largely similar effects in mammals (Bignami et al. 1975). Thus, the conclusions drawn from the present study are likely to be applicable to a range of OPs. Furthermore, the experimental protocol that was used might be considered a worst-case scenario for sub-lethal effects. Injection of wood mice with 50 mg kg^{-1} dimethoate results in a maximum inhibition of brain AChE of 75% (Dell'Omo and Shore 1996a). Generally, inhibition greater than 50% indicates lethal poisoning (Greig-Smith 1991). Although this is obviously not true for wood mice, it doubtful whether this species or bank voles can survive brain AChE inhibition much greater than 75%.

The results presented here are important because they indicate that animals with pronounced biomarker response to OP exposure do enter traps. Brain AChE activity in wood mice following injection of 50 mg kg^{-1} dimethoate is inhibited by 60% and 40% 12 and 24 hr after exposure (Dell'Omo and Shore 1996a). In the present study, brain AChE activity in wood mice captured the night after they were injected with dimethoate would have been inhibited to the same extent. If it is assumed that bank voles are affected by dimethoate in a similar way to wood mice, voles captured in the first three trap rounds after injection with dimethoate would also have had depressed brain AChE activities. Hence, it can be concluded that live traps sample both exposed and non-exposed individuals. Measurement of biomarkers in live-trapped animals should, therefore, indicate the proportion of the population that has been sub-lethally exposed.

Analysis of the time interval between treatment and first recapture for both wood mice and bank voles did not indicate any effect of dimethoate. Severely, but sub-lethally, exposed animals were similar to controls in that they mostly re-entered traps within 24-48 hours. Given that OPs do impair locomotion (Grue et al. 1991), it was surprising that no effect of dimethoate on trappability was detected

at all. In wood mice, this may be because locomotor function recovers more rapidly than brain AChE activity, at least under laboratory conditions (Dell'Omo and Shore 1996a). Furthermore, radio-tracking studies on a small number of free-living wood mice given 50 mg kg^{-1} dimethoate recorded impairment of locomotor activity on the night of injection but not the following night (Dell'Omo and Shore 1996b). In the present study, mice which were injected in the morning and then spent the day in their burrows presumably had recovered their locomotor function when they emerged in the evening, some 10-12 hours later, and so were as likely to be captured as non-exposed individuals. In contrast, frequent trap rounds were carried out in the bank vole study specifically to detect transient effects but they failed to do so. Laboratory studies have indicated that locomotion is severely depressed in bank voles following injection of 50 mg kg^{-1} dimethoate (Dell'Omo *&* Shore *unpub. data*) and so the lack of detectable effect in bank voles was not because they are relatively insensitive to OPs. However, on average, bank voles were only recaptured between four and six trap rounds (32-48 hours) after they were injected (Figure la), irrespective of whether they were given dimethoate or saline. Hence, the natural trap behaviour of voles in re-entering traps only one or two days after first being captured would have obscured any short-term effect of dimethoate on trappability. Thus, post-application trapping campaigns, which would normally only carry out trap rounds once or twice every 24 hours, are likely to be insensitive to, and so unbiased by, transient effects on locomotion.

In conclusion, the results of this study indicate that live-trapping campaigns lasting more than one-two days after pesticide application should not be biased by any failure of surviving animals to enter traps. Trapping data would be expected to give a realistic picture of the toxic effect of a pesticide on small mammals in terms of both effects on population numbers and biomarker responses in individuals. However, it should be recognised that the normal exposure pattern in free-living rodents is likely to be one of multiple but declining doses as animals repeatedly forage in areas where pesticide residues are degrading. If repeated but declining exposure resulted in a cumulative inhibition of brain AChE activity, fatalities might take several days to occur. These deaths would be missed by short trapping campaigns carried out immediately after pesticide application. Whether such cumulative toxicity occurs remains to be determined but post-application trapping studies may need to extend beyond two-three days to ensure that potential toxic effects in the field are identified.

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