Bull. Environ. Contam. Toxicol. (2003) 70:138–145

DOI: 10.1007/s00128-002-0167-6

Bulletin of Environmental Contamination and Toxicology

Comparative Effects of Exposure to an Organophosphate Pesticide on Locomotor Activity of Laboratory Mice and Five Species of Wild Rodents

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Received: 11 April 2002/Accepted: 17 August 2002

Organophosphates (OPs) have received significant attention, both from pharmacologists and ecotoxicologists, since their initial use. Their main physiological effect is the inhibition of cholinesterase (ChE) activity and this is common to many vertebrates (Andersen *et al.* 1977, Murphy *et al.* 1968). The consequences of such inhibition vary depending upon its severity. Inhibition of brain ChE activity greater than 50-70% is usually, but not always, lethal (Rattner & Fairbrother 1991, Sheffield *et al.* 2001); less severe inhibition is associated with a range of physiological and neurobehaviourial effects that are dose-dependent (Bignami *et al.* 1975, Grue *et al.* 1991).

The variation between species in their sensitivity to OPs can be large (Johnson & Wallace 1987, Wang & Murphy 1982, Rattner & Fairbrother 1991, Sheffield *et al.* 2001). Consequently, it is difficult to directly extrapolate the results of studies on laboratory rodents either to other laboratory species or to wild rodents (Watkinson & Gordon 1993). There have been a number of experimental studies that have examined the toxicity of OPs to wild small mammals (Sheffield *et al.* 2001). These data, together with those for different laboratory species, provide an indication that variation between species in response to OPs does occur but comparisons between independent studies can be problematic. This is evident from different investigations on the same compound and species. For example, the acute oral LD_{50} for dimethoate in laboratory rats has been variously reported as 28-30 mg/kg (Kidd & James 1991) and 180-300 mg/kg (Gallo & Lawryk 1991). Such differences may reflect variation in sensitivity between different strains but are also likely to be due to variation between studies in physiological and environmental factors that affect the sensitivity of animals to OP insult. Furthermore, differences in the statistical methods used and in mathematical error in calculating the amount of cholinesterase inhibition (Marden *et al.*, 1994) may also be a cause of inconsistency between studies. Although there have been some studies in which multiple species have been studied simultaneously under identical conditions (Sheffield *et al.* 2001 for review of rodents), the number of species used in such comparisons has usually been limited and most studies have examined variation in either ChE depression or acute toxicity.

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Sub-lethal exposure to pesticides is likely to be a common occurrence in free-living small mammals inhabiting agricultural areas and managed forests. One important consequence of such exposure is a reduction in motor activity (Dell'Omo & Shore 1996a,b, Dell'Omo *et al.* 1997). Impairment of motor activity is an integrated, whole animal response to the neurological effects of ChE inhibition and is likely to be important ecologically because it will adversely affect foraging and territory defence, amongst other behaviours.

The extent to which this functionally important response varies between species, is unknown. The aim of the present study was to assess the inter-species variation in response to OP insult using locomotor activity as the measured end-point. This involved exposing five wild rodent species and laboratory mice to an OP pesticide and quantifying the resultant effects on their locomotor activity in an open-field test.

MATERIALS AND METHODS

The study was carried out in the field station "Chisti Lec" (Bubonizi, Toropez, Tver region, Russia). Wild rodents of both sexes were live-trapped in the forest and fields surrounding the station. They were primarily captured for studies on the hippocampus (Pleskacheva et al. 2000) but were first used in the present study. The species (following the nomenclature of Corbet and Hill 1991) were the bank vole (*Clethrionomys glareolus*), European pine vole (*Pitymys subterraneus*), root vole (*Microtus oeconomus*), pygmy wood mouse (*Apodemus microps*) and harvest mouse (*Micromys minutus*). House mice (*Mus musculus*) were also taken from the outdoor vegetated 20 x 20 m pens in which they are routinely maintained at the field station. These mice were the offspring of laboratory mice (hybrids between the C57 and CBA strains) introduced into the pens two years previously and that had since adapted to the semi-natural conditions.

After capture, all animals were allowed to acclimatise for 10-26 hr to the ambient conditions (14-20°C; natural daylength) of the room in which open-field tests were carried out. They were maintained separately in cages that contained a substratum of moss, plentiful drinking water and surplus food (standard laboratory mouse pellets (Provimi Kliba SA, Kaiseraugst, 4303, Switzerland) supplemented with bread, seeds and fresh vegetables). Animals were randomly assigned to either the control (sham-dosed) or experimental (pesticide-dosed) group although males and females were divided as equally as possible between the groups.

After the acclimation period, animals in the experimental group were given a 50 mg/kg body weight intraperitoneal injection of dimethoate (O,O-dimethyl S-2-(methylamino)-2-oxoethyl phosphorodithioate, technical grade (38.8% by weight), Cheminova, Lemvig, Denmark) diluted in saline (0.9% NaCl). This dose was lower than the LD_{50} values reported for laboratory mice by Sanderson & Edson (1968) and was intended to produce only a transient effect on locomotor behaviour. Previous experiments on other wild small mammal species had

demonstrated that animals given this dose recovered normal behavioural function within 6-12 hours (Dell'Omo & Shore 1996a, Dell'Omo *et al.* 1997). Animals in the control group were sham-injected with saline only, the volume injected being the same as in experimental animals. There was no mortality of treatment or control animals during the course of the experiment.

Treatments involved animals undergoing a series of repeated 10-min open field tests. These were carried out initially before animals were weighed and injected, then at 1.5, 3, 6 and 12 hr post-injection. The tests began at dusk (20:00) and were run through the night under ambient light conditions. At most, six animals were tested per night, the order in which individuals were tested being randomised by species and treatment. The open field arena consisted of a white opaque Plexiglas rectangular box (50 x 70 x 50 cm). Animals were gently placed in the centre of the arena and their trajectory paths were recorded by means of a video camera suspended above the open field. The X and Y co-ordinates of the position of the animal in the open field were sampled with a frequency of 4.2 Hz using an electronic image analyzing system (Wild-Leitz ASBA, Zurich). Data were automatically stored on disk and then processed off-line by WINTRACK, a spatial analysis software (http://www.unizh.ch/anatom/research/neuroanat/neuroanat.htm) originally developed by Wolfer and Lipp (1991). This program includes correction of recording artefacts and reconstruction of paths taken by the animal and produces a number of different variables that allow a detailed analysis of locomotor behaviour. However, we considered only activity scores for our purposes. This parameter represented the total (horizontal and vertical) locomotor activity of a subject with all inactivity events (in which the animal showed an actual speed of less than 5 cm/s) filtered out.

Data were analysed by analysis of variance (SuperAnova for MacIntosh) with Species and Treatment (dimethoate/saline) as factors and the activity counts in each five 10-min tests as Repeated Measures. Sex was not included as a factor and data for males and females were pooled. This was because sample sizes were not large enough and, in any case, sex differences in the open field behaviour of wild murid rodents are unlikely to occur (Frynta 1994). Post hoc comparisons were performed by Tukey's Honest Significant Difference (HSD) tests.

RESULTS AND DISCUSSION

The activity counts used to describe locomotor activity in the six species are shown in Fig. 1. Overall, there were significant ($F_{5,288}$=20.77, $p<0.01$) between-species differences in total activity, irrespective of treatment. This reflected intrinsic differences between species in their behaviour in the open-field arena, as was clearly evident from the pre-injection data (Table 1). Time (the Repeated Measure) was also a significant factor ($F_{4,288}$=48.8, $p<0.01$), activity decreasing most markedly immediately after the first (pre-injection) 10-minute open field test ($p<0.01$ in post-hoc tests). This indicated that, irrespective of treatment, all species habituated to some extent to the repeated testings in the open-field.

Table 1. Pre-injection activity scores as determined in a 10-min open field tests of laboratory mice and five species of wild rodents.

	n	mean	(+SE)		n	mean	(+SE)
Mus musculus	19	28.31	(±2.43)	*Microtus oeconomus*	6	23.62	(±2.42)
Pitymis subterraneus	10	14.36	(±1.86)	*Clethrionomys glareolus*	25	9.87	(±2.03)
Micromys minutus	6	52.25	(±8.97)	*Apodemus microps*	12	36.20	(±3.97)

Main effect of species $F_{5,77}=35.04$; $p<0.01$; $p< 0.01$ in comparisons of *Apodemus* and *Micromys* with all the other species and between them; $p<0.05$ in comparisons between *Microtus* and both *Mus* and *Clethrionomys*.

Treatment with dimethoate did significantly affect behaviour, causing a reduction in activity compared with control animals ($F_{1,288}=6.72$, $p<0.01$). The interaction term between Treatment and Repeated Measures was also significant ($F_{4,288} = 4.66$, $p<0.01$), because there was an initial and very marked depression in activity at 1.5 hr after injection followed by a period of recovery. The time course of this depression and subsequent recovery differed between species (Treatment x Species x Repeated Measures interaction: $F_{20,288}=2.69$, $p<0.01$; $p<0.05$ or less in post hoc tests). Dimethoate-treated *M. minutus* and *A. microps* had activity counts that were similar to those of control animals by 3 hr after injection (Fig.1). In the other species, the activity counts in treated animals were still lower than in control animals at this time ($p<0.05$ or less in post-hoc tests except for *P. subterraneus* for which $p<0.10$), although there was a slight recovery in the activity of both *P. subterraneus* and *M. oeconomus* (Fig. 1). Only the dimethoate-treated laboratory mice had significantly depressed activity counts compared with controls by 6 hr after treatment ($p<0.01$ in post-hoc tests), whereas the activity of all the other species had apparently completely recovered to pre-injection levels. In laboratory mice, recovery of activity was achieved 12 hours after exposure.

In general, sub-lethal exposure to dimethoate produced a depression in the activity of all the species tested in the open-field arena. Similar effects have been observed in laboratory rodents exposed to other anticholinergic compounds (Bignami *et al.* 1985). The onset of this depression was always rapid, occurring within 30 min from injection, and was characterised by an almost complete depression of locomotor activity, animals lying in a corner of the open-field. Some of the laboratory mice and voles also performed stereotypic gnawing and face-washing behaviour. Although the type of OP-induced effect was consistent across all species tested, the severity and time course of the effects were not. Differences between species were most obvious in the recovery phase and the microtine voles appeared to recover less rapidly than the two wild species of mice. However, differences between species in their response were not simply a factor of how closely or distantly related they were taxonomically; the slowest and fastest species to recover their activity were both mice whereas voles showed an intermediate response.

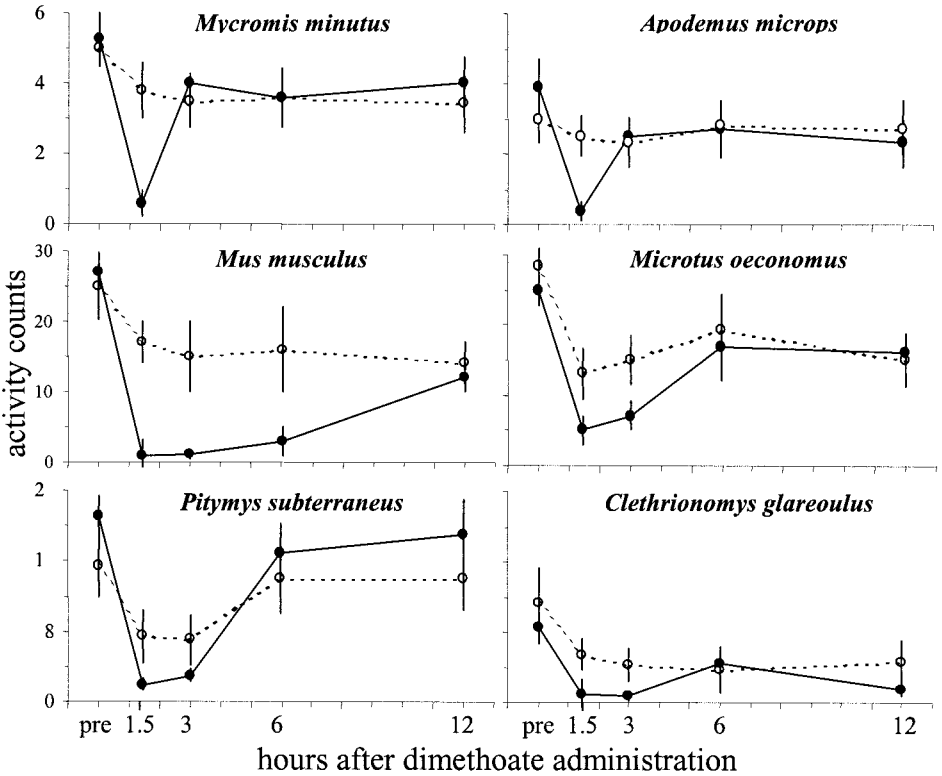

Figure 1. Time course profile of the activity counts measured during repeated 10-min open field tests in laboratory mice and five species of wild rodents. Tests were carried out before the administration of 50 mg/kg dimethoate (pre) and at various intervals thereafter. Data are means and SE for saline-injected control animals (----o----) and dimethoate-treated subjects (—•—).

The observed differences between species are difficult to explain on the basis of body size or metabolism. The dose of dimethoate given was identical in terms of amount of active ingredient administered per kilogram body weight. Although the smallest species, the pygmy field mouse and harvest mouse (body weight 4-8g and 12-20 g, respectively), recovered faster than the larger microtine voles (adult body weight ranging from 12-21 g, 13-26 g, and 23-58 g for the European pine vole, the bank vole and the root vole, respectively), the voles all recovered more rapidly than the laboratory mice (body weight of 17-25 g). Given that the body weight of the heaviest root voles was twice that of the laboratory mice, it would appear that rate of recovery is not directly proportional to body weight. Furthermore, in a previous study (Dell'Omo *et al.,* 1997), common shrews (*Sorex araneus*) that weighed only 7-10 g, took 3-6 hr to recover normal activity after being given a 50 mg/kg intraperitoneal dose of dimethoate. This was as long as the larger voles in the present study. Differences in normal ChE activity or its affinity for dimethoate

might explain some of the variation between species in their time to recovery. It would have been of interest to compare natural levels of ChE activity and the extent of the inhibition caused by the dose of dimethoate, but it was not possible to measure ChE activity with the facilities available at the field station in Russia. However, the slow recovery in locomotor activity in laboratory mice in our study is consistent with the findings of Roberts *et al.* (1988) who observed that brain AChE activity recovered more slowly in laboratory rodents than in two feral rodent species. It is also possible that differences in the normal diurnal pattern of motor activity between species might have influenced the rates of recovery in different species (Lenmer & Berger 1978). Animals were dosed at around dusk and so inhibition and subsequent recovery would have occurred at time when harvest mice and *Apodemus* are highly active whereas the activity patterns of *Clethrionomys* and *Microtus* species are less exclusively nocturnal (Dell'Omo *et al.* 1998).

In conclusion, the present study has demonstrated that inter-species variation in the integrated, functional neurobehavioral response to OP insult does occur. However, the similarities in the pattern and time-span of effects in the species tested in the present study, and in other rodent and insectivore species that have been tested in the same way (Dell'Omo *et al.* 1996a, 1997), are more striking than the differences. It is evident that these acute sub-lethal effects of dimethoate on locomotor activity are basically similar across a range of small mammal species; in fact, exposure to dimethoate was immediately followed in all species by a dramatic reduction of activity and it was only during the recovery phase that differences in sensitivity were apparent. Thus, although LD50 and other acute toxicity data suggests that sensitivity to acute lethal doses of OPs can vary markedly between species, the integrated neurobehavioral response of small mammals to sub-lethal OP intoxication appear to be remarkably uniform. Furthermore, our observations, together with those of Roberts et al. (1988), suggest that laboratory rodents may be more sensitive than their wild counterparts to the sub-lethal effects of OPs and so may prove a useful conservative model with which to predict the likely sub-lethal effects of anticholinergic compounds on wild species.

Acknowledgements. This work was supported by the European Environmental Research Organisation (EERO) grant to G Dell'Omo and by grant NF 31.57139.99 to H-P Lipp.

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