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Palatability of Dead Earthworms and Slugs to the Wood Mouse (*Apodemus sylvaticus*) and the Potential for Secondary Poisoning

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After exposure to a pesticide, dead or poisoned invertebrates may be more available to predators than unexposed ones. For example, after exposure, normally deep-burrowing earthworms such as *Lumbricus terrestris* may remain on the surface in a paralyzed state until death (e.g. Bunyan et al. 1981). They are therefore more available and easier to catch by predators such as earthworm-eating birds and mammals, increasing the risk of secondary poisoning. The potential risk from the ingestion of poisoned earthworms is well known (Cooke et al. 1992) and it would also seem possible that poisoned molluscs such as slugs would present a similar risk. However, it is sometimes suggested that predators may in fact avoid these dying, dead and decaying invertebrates in favour of normal unaffected prey which are likely to contain lower levels of pesticide. This study aimed to provide more information about the responses of small mammals to these prey to further refine the assessment of risk.

Live and dead invertebrate prey (earthworms and slugs) were presented to wood mice (*Apodemus sylvaticus*) under choice and no-choice conditions to determine relative preferences. Wood mice were used as they are known to predate on earthworms (Gorman and Zubaid 1993; Green 1979; Pelz 1989), and slugs have been found in the stomach contents of wood mice collected on arable land (Johnson et al. 1992). The results of these tests were then used to draw conclusions about the likelihood of ingestion of these items for use in risk assessment.

MATERIALS AND METHODS

Twelve wood mice were used in this study, seven males and five females with a mean weight of 26.3g (range 17.5-38.8g) before the test. All mice were captive bred at this laboratory and were adult at the start of the test. Wood mice were maintained in standard individual mouse cages until transfer to the test room. They were then placed in larger cages (49 cm x 37 cm x 19 cm high) for the test. These cages were used to provide sufficient floor area for the presentation of test containers containing the prey. These were maintained under reversed daylight conditions with a 12-hour night period with red light. During the light 'day'

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period the lights were dimmed for the first and last 30 minutes to represent dawn and dusk. The temperature of the room was 20-21°C during the course of each test period.

All tests were conducted during the red light ('night') period when mice were most active. During the acclimatization period the wood mice were offered fresh live earthworms to confirm that they would feed on this material under test conditions. They appeared to prefer the invertebrates over their normal diet (standard rodent pellets) and so it was unnecessary to remove food during tests to encourage feeding.

Earthworms and slugs were presented in plastic containers (approximately 19cm x 13cm x 4cm high) with a hole cut in the lid so as to produce a horizontal rim 2.5cm wide around the top of each container to inhibit the escape of live prey. Before testing the mice were accustomed to obtaining food from these containers.

All earthworms used in the tests were adult *Lumbricus terrestris* obtained from a commercial supplier. All earthworms presented were either alive (as collected from the soil), fresh dead (killed shortly before presentation), or decaying (killed and placed on a moist soil surface in a room with a temperature of 15°C and a relative humidity of 70% for 24h $\pm$ 2h before presentation). During pilot work it was established that the best method for killing the earthworms while retaining their integrity was to immerse them in warm water (38-41°C) for 10 minutes and removing excess water by blotting them on filter paper before weighing. An attempt was made to produce 'sick' earthworms (to represent dying or sublethally exposed prey) by subjecting the earthworms to carbon dioxide or nitrogen gas in a plastic container. However, while these methods appeared successful in the laboratory when the earthworms were held in small containers, most of the earthworms quickly recovered when placed outside in test containers making them indistinguishable from live earthworms.

All slugs used were grey field slugs (*Deroceras reticulatum*) and were collected from the wild from a site where pesticides were not being used. Slugs were either presented alive, fresh dead (placed in a freezer for 30 minutes or water at 38-41°C for five minutes), or decaying (killed as before and placed on a moist soil surface for 24h in a room with a temperature of 15°C and a relative humidity of 70%).

Wood mice were offered live or fresh dead earthworms in both choice and no-choice tests and consumption was measured. In the choice test the mice were offered two containers, one containing two live earthworms and one containing two freshly killed earthworms for two 4h periods on consecutive days. Mean fresh weight of the earthworms was 2.1g. The position of each type of earthworm was selected at random for each mouse on the first test day and reversed on the second day to compensate for any strong position preference. At the end of the

trial the remaining earthworms were collected and weighed and consumption calculated allowing for dehydration losses measured in control samples.

The no-choice tests were conducted similarly except that only one container was presented containing either two live or two fresh dead earthworms. On the first day each mouse was presented with one type of earthworm, with the alternative offered on the second day. The order of presentation was randomized. Mean earthworm weight in the no-choice tests was 2.4g.

To test the palatability of earthworms that had been dead on the surface for some time the mice were then offered live and decaying earthworms in a two-day choice test as above. Due to the low level of consumption of decaying earthworms, the relative palatability of fresh dead and decaying earthworms was also tested in a one-day choice test. Mean fresh weight of earthworms used in these experiments was 2.3g.

To determine the palatability of slugs to wood mice they were offered live and fresh dead slugs under choice test conditions and the number of prey of each type taken was recorded. The slugs used had a mean weight of 0.43g and eight slugs of each type were offered to each mouse. Initially, freeze killed slugs were offered and the mice appeared to prefer these to live slugs. To confirm that this was not due to the killing method alone, mice were then given the same test using slugs killed by immersion in warm water.

The test was repeated using live and decaying slugs. The slugs used had a mean fresh live weight of 0.30g. Unlike the previous experiment, freeze killed slugs were rinsed before presentation to remove excessive soil that had adhered while they were left to decay.

To confirm the apparent low palatability of live slugs the mice were given a further no-choice test with live slugs only. Eight slugs (mean weight 0.30g) were offered to each mouse under the same conditions as the previous tests.

RESULTS AND DISCUSSION

The mean consumption of live and fresh dead earthworms in choice and no-choice tests is shown in Figure 1. Under choice test conditions, live earthworms appeared to be slightly preferred over dead ones and this difference was significant (Wilcoxon signed rank test, $p = 0.045$). The same trend was observed under no-choice conditions but the difference was less marked and not significant (Wilcoxon signed rank test, $p = 0.126$).

The mean consumption of decaying earthworms when offered in choice test with live or fresh dead earthworms is shown in Figure 2. Both live and fresh dead

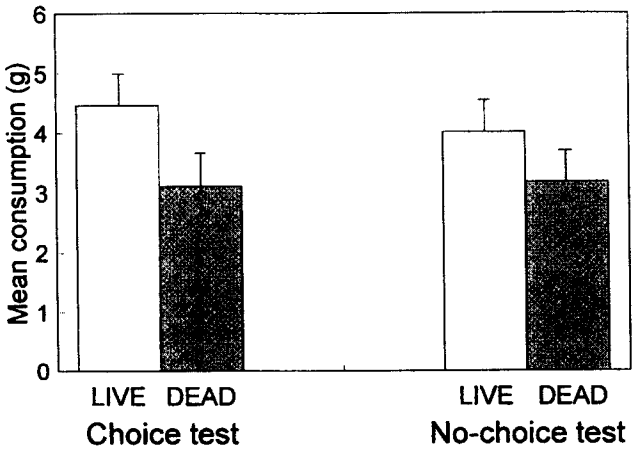

Figure 1. Mean consumption of live and fresh dead earthworms in choice and no-choice tests. For choice tests the data are mean total consumption over two days for each type of earthworm. For no-choice test the data represent mean consumption in one day (n = 12 in both cases, bars represent standard errors).

earthworms were significantly preferred over decaying ones (Wilcoxon signed rank tests, p = 0.004 and 0.003 respectively).

The mean numbers of live and fresh dead slugs taken in choice tests are shown in Figure 3. The results for each killing method are shown. Both 'freeze killed' and 'water killed' slugs were significantly preferred over live ones (Wilcoxon signed rank tests, p = 0.008 and 0.003 respectively) although consumption of slugs killed with warm water appeared even higher.

One possible explanation for the difference in palatability between live and dead slugs is that only live slugs would be actively secreting mucous. If the mucous were unpalatable this would reduce their attractiveness to the mice. This may also at least partly explain the apparent differences between the consumption of freeze killed and water killed slugs. Where slugs were killed with water, any mucous would be washed from the surface of the slug and could potentially make them more palatable than dead ones found in the field. However, the freeze killed slugs were not washed so these results provide firmer evidence that dead slugs would be preferred in the field.

The mean number of live and decaying slugs taken in choice tests is shown in Figure 4. As before, the results of tests using each killing method are shown. Again both 'freeze killed' and 'water killed' decaying slugs were significantly preferred over live ones (Wilcoxon signed rank tests, p = 0.003 and 0.015 respectively). Interestingly in this case the freeze killed slugs appear to be even

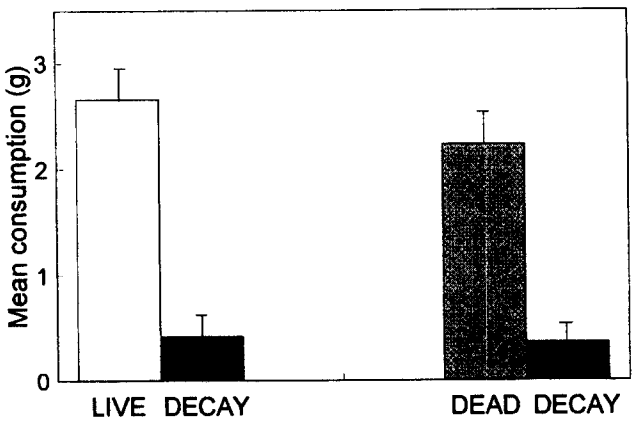

Figure 2. Mean daily consumption of live, fresh dead and decaying earthworms in choice tests (n = 12 in both cases, bars represent standard errors).

more palatable than the water killed slugs which may result from freezer killed slugs having been washed before presentation in this experiment.

During the final test when the mice were offered live slugs only, the mean number of slugs taken was 2.08 (n = 12, s.d. = 1.88), slightly higher than observed in the choice tests but still far lower than the consumption of dead slugs in the previous tests.

The data obtained for wood mice suggest that they will readily take fresh dead invertebrate prey, even when live prey and normal food are available. While there appeared to be a slight preference for live earthworms in choice tests, the consumption of fresh dead prey under these conditions was such that the preference would do little to reduce risk from contaminated prey in the wild. Despite the apparent attractiveness of dead invertebrates to wood mice, they seemed to find decaying earthworms and live slugs less palatable. Given the rapid breakdown of the decaying earthworms and the strong odour they produce, this is perhaps not surprising despite the apparent palatability of decaying slugs. The reason for the apparent low palatability of live slugs is not known but it is possible that secretions of live animals may be repellent. If this was also the case in the wild then this could increase risk if poisoned prey were selected over live ones.

It is likely that surface feeding earthworms that are exposed and become paralyzed on the surface at night would be encountered in a relatively fresh condition by nocturnal feeders (such as small mammals like wood mice), but may be in poorer condition when encountered by diurnal feeders (such as birds).

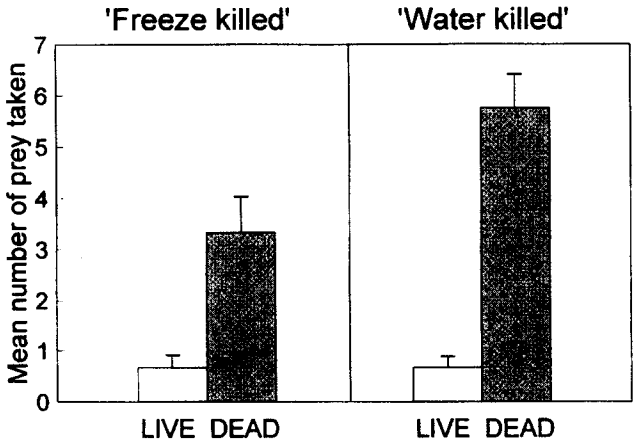

Figure 3. Mean consumption of live and fresh dead slugs in one-day choice tests using each killing method (n = 12 in both cases, bars represent standard errors).

The results are summarized in Table 1 with an indication of the likely response to dying or sublethally exposed prey based on the results for live and fresh dead prey.

Table 1. Summary of results indicating whether each condition of each type of prey would be expected to be palatable to each species tested (✓ = palatable, ? = uncertain, x = relatively unpalatable but may be consumed at low levels).

	CONDITION			
PREY	Live	Dying	Dead	Decay
Earthworms	✓	✓*	✓	x
Slugs	x	?*	✓	✓

* based on results for live and dead prey

It was not possible to reliably produce earthworms that mimicked prey that had been poisoned but were not yet dead. However, in most cases it should be possible to predict the likely response to prey in this condition based on the existing data.

In the case of earthworms, it is possible to make predictions about the likely response of wood mice to dying prey. However, it is less certain how wood mice would respond to dying slugs as it would depend on whether they were treated more like live slugs (unpalatable) or fresh dead slugs (palatable). This in itself may depend on how the slugs react to pesticide exposure which may be product

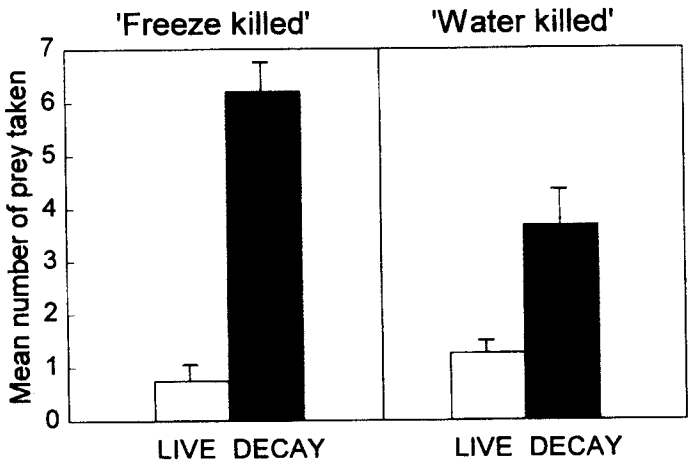

Figure 4. Mean consumption of live and decaying slugs in one-day choice tests using each killing method (n = 12 in both cases, bars represent standard errors).

specific. Given these uncertainties it would be safer to assume that dying slugs would be palatable unless there is evidence to the contrary from a specific avoidance study.

It is concluded from Table 1 that most types of dead and dying invertebrates should be considered potentially palatable to wood mice, albeit to differing extents. While it is possible that other species may respond differently, the range of prey taken means it would be prudent to assume similar behavior unless there is evidence to the contrary. The palatability of the invertebrates may also be affected by the presence of the pesticide itself, or coformulants. It is also possible that predators may learn to avoid certain invertebrates after feeding on contaminated prey. For example, rats have been shown to develop conditioned taste aversions following exposure to organophosphate pesticides (Roney et al. 1986). In another study, free ranging red-winged blackbirds (*Agelaius phoeniceus*) developed illness-induced conditioned taste aversions to insects after exposure to prey contaminated with parathion (Lee 1999). Where such avoidance responses are considered likely based on existing palatability data, it would be necessary to conduct specialized tests with exposed prey and appropriate predators to determine whether consumption was reduced below expected levels. However, the outcome of such a study is not easy to predict even for a strongly repellent compound such as methiocarb. Johnson et al. (1992) conducted a study where wood mice were offered slugs (*Deroceras reticulatum*) that had been killed by exposing them to methiocarb pellets for 24h. An average of 5.9g of material was consumed (range 1.3-10.4g). No mice died but the results showed that they would consume substantial quantities of dead and moribund slugs that had been killed using an otherwise repellent formulation.

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REFERENCES

Bunyan PJ, Van den Heuvel MJ, Stanley PI, Wright EW (1981) An intensive field trial and a multi-site surveillance exercise on the use of aldicarb to investigate methods for the assessment of possible environmental hazards presented by new pesticides. Agro Ecosyst 7:239-262

Cooke AS, Greig-Smith PW, Jones SA (1992) Consequences for vertebrate wildlife of toxic residues in earthworm prey. In: Greig-Smith PW, Becker H, Edwards PJ, Heimbach F (eds). Ecotoxicology of Earthworms. Intercept, Andover, UK, p 139

Gorman ML, Zubaid A (1993) A comparative study of the ecology of woodmice *Apodemus sylvaticus* in two contrasting habitats: deciduous woodland and maritime sand-dunes. J Zool London 229:385-396

Green R (1979) The ecology of wood mice (*Apodemus sylvaticus*) on arable land. J Zool London 188:357-377

Johnson IP, Flowerdew JR, Hare R (1992) Populations and diet of small rodents and shrews in relation to pesticide usage. In: Greig-Smith P, Frampton G, Hardy A (eds) Pesticides Cereal Farming and the Environment. The Boxworth Project., HMSO, London, p 144

Lee H (1999) Effects of organophosphate insecticide application to the conditioned taste aversion of red-winged blackbirds, *Agelaius phoeniceus*, Icteridae. Korean J Biol Sci 3:41-46

Pelz HJ (1989) Ecological aspects of damage to sugar beet seeds by *Apodemus sylvaticus*. In: Putman RJ (ed) Mammals as Pests, Chapman and Hall, London, p 34

Roney PL, Costa LG, Murphy SD (1986) Conditioned taste aversion induced by organophosphate compounds in rats. Pharmacol Biochem Behav 24:737-742