Executive summary (maximum 2 sides A4)

**Background and policy relevance**

EU Directive 94/414 requires that plant protection products have no unacceptable effects on the environment particularly non-target species. DEFRA Pesticides Safety Directorate (PSD) is responsible for assessing the environmental safety of pesticides in the UK. Solid formulations (seeds, pellets, baits and granules) present particular problems due to the high toxicity of some formulations and the possibility that they may be directly ingested by non-target vertebrates as food or grit. Also, vertebrates that feed on invertebrates may be at risk from pesticide residues taken up by the invertebrates or adhering to their exterior. At present there is little detailed information about the conditions which may lead to the poisoning of birds and mammals by these routes. This project addresses the need for more research into the determinants of exposure to solid formulations, both directly and indirectly (secondary poisoning). One part of the project also provides important information about residues in earthworms after spray application.

**Objectives**

1. Determine how to predict the daily intake of granules by birds and how this depends on the type and amount of natural grit present on the soil surface.
2. Develop a simple test which can be used in cases where high risk to birds is indicated for granular formulations.
3. Validate this approach for granular formulations by simulating actual use under captive conditions.
4. Determine the extent to which mammals and birds take pellets as food and how this depends on hunger and the availability of alternative foods.
5. Develop and test a method for measuring the avoidance response of small mammals to solid formulations (seeds, pellets and baits).
6. Improve estimates of pesticide residues in earthworms for use in risk assessments for worm-eating animals after pesticide applications.
7. Determine whether dead and dying earthworms and molluscs are more or less attractive to birds and mammals than unaffected prey.
8. Determine whether birds and mammals remove adhering granules or pellets before eating earthworms or molluscs.

**Findings**

**Risk to birds from ingesting granules**

1. Granules may be ingested by birds in mistake for grit. Other authors have assumed that the risk of this happening is highest for granules of similar size to the grit found in the gizzards of wild birds. However, we measured grit consumption in grey partridges and house sparrows directly, and found that the range of grit sizes actually taken by birds was broader than that found in the gizzards of wild birds.
2. For partridges, the range of grit sizes found in the gizzard is narrower than that actually eaten by the bird. Larger grit particles may get broken down in the gizzard and smaller particles may pass through it.
3. For partridges and sparrows grit deprivation increases the consumption of both grit and granules. This suggests that in environments where natural sources of grit are limited then birds may take granules as a substitute.
4. For partridges we showed that birds would consume granules in a simulated natural environment where grit and granules were presented at realistic worst-case densities. Consumption of granules was highest when suitable grit was absent but occurred even when grit was present in abundant amounts.
5. For partridges, but not for sparrows we also showed that increasing the hunger of the birds, increased the likelihood that they would take granules. Again, in naturalistic simulations, presenting seeds and granules together against a soil background, more granules were taken when birds were hungry but some granules were taken even when seeds were abundant.
6. For sparrows, the increase in consumption of granules under grit-deprived and food-deprived conditions was small. But given that for some pesticides two or three granules is often enough to kill a small bird, then any factor which encourages increased consumption of granules is important.
7. Our experiments suggest that birds may mistake granules for seeds as well as grit and that regulatory practice should take this into account. The design of a laboratory test to evaluate the risks of granular formulations to birds should ensure that
   - the test species is likely to consume grit and seeds of similar size to the granular formulation
   - birds should be made familiar in the laboratory with grit and seeds of similar size to the granular formulation.
   - birds should be deprived of grit and seeds before exposure to granules
   - seeds, grit and granules should be presented at realistic densities in a naturalistic context.
8. Future research on risks posed by granular formulations should investigate the effects of avoidance on consumption and mortality.

**Risks to birds and mammals from ingesting pellets and seeds**

1. Pellets used as the base material for slug pellets were shown to be attractive to both birds and mammals and should be considered as food items in risk assessment; strong evidence of avoidance may be necessary if they are to be considered safe.
2. Where tests of avoidance are conducted with small mammals, with either pellets or treated seeds, it would be advisable to test them under different conditions of availability of alternative food and hunger level to characterise the strength of the response. The presentation of the test material should be as realistic as possible.
3. Methiocarb treated slug pellets present a high risk to small mammals despite a strong avoidance response. Sampling of only one or two pellets before avoidance can lead to mortality and occurs even in the presence of alternative food.

**Risks to birds and mammals from ingesting exposed invertebrate prey**

1. Pesticide residues in earthworms can be affected by factors such as soil OM content, rainfall, time of exposure and properties of the active substance and may vary greatly from the level predicted by current methods. The best predictor seems to be the logKow of the substance. There is therefore a need to consider the properties of the active substance along with the conditions under which exposure may take place and to adapt predictions accordingly.

2. After a granular application, measurement of residues in unwashed worms with adhering soil and washed worms indicated that washing reduced residues significantly. It is recommended that the potential for adhered soil to increase the pesticide loading of earthworms collected by predators be considered when estimating risk.

3. Palatability tests have shown that wood mice will readily take dead invertebrate prey even when live prey and normal food are available. At this stage it must be recommended that dead and dying prey should be considered to be palatable to wood mice unless there is strong evidence (for example from a specialised avoidance study) that this is affected by the palatability of the pesticide itself.

4. Palatability tests have shown that when dead or decaying earthworms only are presented that they are readily taken by blackbirds although if there is a choice live ones are preferred, even though they are apparently more difficult to detect. In tests with slugs there was greater variation between birds but those birds that did take slugs appeared to readily take live, dead and decaying prey. It must therefore be recommended that dead and dying prey also be considered as a risk to blackbirds and possibly other worm eating birds unless other factors such as palatability of the formulation affect this.

5. Some treated pesticide granules appear to adhere more firmly to earthworms than untreated granules of the same material. This may have implications for the likelihood that birds and mammals will be able to remove them from exposed prey before ingestion. Where the potential removal of adhered material is considered important when assessing risk it would be best to use actual formulation and monitor the behaviour of the species at risk towards the prey.

6. Captive blackbirds did not appear to be affected by adhered material such as soil or silica when feeding on earthworms and made no overt attempt to remove it. This suggests, at least for this species, that any adhered material is likely to be ingested along with the prey.

**Technology transfer**

The results of this project assist PSD and industry by providing better estimates of exposure, guidance on data requirements, and suitable designs for specialised tests where necessary. This will improve risk assessment procedures for these formulations and hence reduce the risk to wildlife from their use. The results with pellets and small mammals have already been used in the design of recent regulatory studies. The main results will be submitted for publication in scientific journals.
INTRODUCTION

EU Directive 94/414 requires that plant protection products have no unacceptable effects on the environment particularly non-target species. DEFRA Pesticides Safety Directorate (PSD) is responsible for assessing the environmental safety of pesticides in the UK. Solid formulations (seeds, pellets, baits and granules) present particular problems due to the high toxicity of some formulations and the possibility that they may be directly ingested by non-target vertebrates as food or grit. Also, vertebrates which feed on invertebrates may be at risk from pesticide residues taken up by the invertebrates or adhering to their exterior. At the start of the project there was little detailed information about the conditions which may lead to the poisoning of birds and mammals by these routes. There was therefore a need for more research into the determinants of exposure to solid formulations, both directly and indirectly (secondary poisoning). The results will assist PSD by enabling better estimates of exposure, guidance on data requirements, and suitable designs for specialised tests where necessary. These will lead to improved risk assessment procedures for these formulations and hence reduce the risk to wildlife from their use.

This report summarises the results obtained for all seven objectives of the project. More detailed accounts are provided in separate milestone reports to DEFRA.

SCIENTIFIC OBJECTIVE 1

“Determine how to predict the daily intake of granules by birds and how this depends on type and amount of natural grit on the soil surface”

Many birds, especially seed-eaters need grit to help them break down and digest their food. Pesticides applied as granules resemble grit. Unfortunately they are often highly toxic and birds mistaking them for grit might easily consume a lethal dose. Less than 10 typical insecticide granules would normally be enough to kill a sparrow (de Leeuw et al. 1995).

The closer that granules resemble the natural grit of a given bird species, the greater, presumably, is the danger that they will be consumed. Different birds have different grit preferences. Gizzards of bigger birds, for example, tend to contain bigger grit particles (Best 1992, Luttik & de Snoo 2002).

Most of the work carried out on potential risk to birds from pesticide granules has focused on the possibility that birds might mistake them for grit. Granules have no food value and do not smell or taste like food. However, birds often select food by sight and granules can look like small seeds. Hungry birds may mistake granules for food. Early spring, when insecticide granules are used on potatoes and sugar beet, is a time when food is particularly scarce and birds may therefore be more likely at this time to pick up granules. Therefore we have investigated the effects of hunger, as well as other factors, on birds’ tendencies to eat granules.

Size preference for grit.

Pesticide granules may be accidentally eaten by birds in mistake for similar sized grit and seeds. The grit found in birds’ is related to the size of the bird and for granivorous birds often overlaps the size range of pesticide granules. The majority of work on grit preferences is based on the grit found in the gizzard. However, it is not certain whether gizzard grit found mirrors the grit actually consumed by birds. When Grey partridges were offered a wide range of grit we found that their choice was rather wider than that suggested by studies found in the gizzards of wild partridges (Figure 1.).
Figure 1 Grit consumption by partridges in 4-choice and no-choice experiments in aviaries vs. grit found in wild partridge gizzards by Lutik et al (2002)

We offered different sizes of grit to birds under two different conditions: 4-choice where birds were allowed simultaneous access to 4 sizes of grit and no-choice where birds were given only one particular grit size at a time. We found that birds differed markedly from each other in their tendency to take grit. Birds were consistent in their preferences between 4-choice and no-choice tests, but ate significantly more of the less-preferred grit size when it was offered on its own without any alternative (Figure 2). This suggests that in environments where grit is limited, birds will take what is available.

We found that the amount of grit taken may change with season and sex. In particular we found in spring, laying females took much larger quantities of grit than normal and appeared to prefer smaller sizes than birds tested in winter time.

Figure 2 Box and whisker plot showing consumption of grit from 4 size categories. (Boxes indicate interquartile range, line within box is the median value, whiskers indicate nearest datum to 1.5 box lengths)
Grit consumed compared with grit retained in the gizzard

The above results suggest that grey partridges, depending on the individual bird, its sex, the time of year and the range of grit available to it, will take a wide range of grit sizes. This is in contrast to the grit recovered from gizzards of wild birds which showed a peak within the range 1.7-2.3mm (Figure 1). A possible explanation for this difference, is that birds in the wild also consume a wide range of grit sizes but that the gizzard selectively retains the medium-sized particles. We tested this hypothesis by offering birds a free choice between the four grit sizes, monitoring their consumption, and then examining gizzard contents.

We compared the proportions of the four grit sizes actually consumed in choice tests with the proportions found in the gizzard and found a highly significant difference (p<0.001). In relation to the grit actually consumed by the birds, the gizzard was significantly more likely to retain the medium sized particles. It can be seen from Figure 3 that the distribution of grit found in gizzards is similar to that found in wild bird gizzards with a tendency to avoid the smallest and largest sizes. It would seem that the difference between grit size distributions of wild bird gizzards and actual consumption in the laboratory is more likely to be explained by selective retention by the gizzard than by restricted availability of grit in the wild.

These results suggest that the grit found in partridge gizzards may not be a good guide to birds preferences for grit actually consumed.

Figure 3. Proportions of grit sizes consumed in 4-choice trials compared with grit found in gizzard at end of trial

Effects of grit deprivation on grit consumption

Given that birds will deliberately seek out grit to eat and given that suitable grit is unlikely to be evenly distributed across different landscapes, it is likely that birds inhabiting some areas may be chronically deprived of grit or may need to travel long distances to obtain it (Beintma, Baarspul & de Krijger 1997). If birds have a need for grit, that is not well satisfied by the soil available to them, they may be more likely to consume granules as an acceptable substitute. We therefore deprived birds of grit for 23 hours in a day and monitored their intake during the 1 hour’s access that we allowed them.

We found that grit-deprived birds increased their grit intake relative to non-deprived birds. The difference was significant for house sparrows (Figure 4) but not for partridges.
Effects of food deprivation on grit consumption

Hungry birds might also be expected to eat more grit, either by mistaking it for food, or because grit increases the efficiency with which birds digest their restricted food intake. Partridges ate significantly more grit when they were deprived of food than when food was freely available (Paired t-test, $t=4.43$, df=7, $p=0.003$) (Figure 5). Sparrows also showed an increase in grit consumption when hungry but the difference was not significant.
Effect of grit deprivation and food deprivation on consumption of granules.

The previous experiments suggested that hungry birds and grit-deprived birds are likely to increase their grit consumption. For the purposes of pesticide risk assessment, it is important to know whether grit and food deprivation affect the consumption of pesticide granules.

We conducted a 2 factorial experiment in which birds’ consumption of blank “Temik” granules (containing only the black gypsum carrier material) was investigated under four conditions, grit deprived and food deprived (G-F-), Grit deprived, food available (G-F+), Grit available, Food deprived (G+F-) and Grit available, Food available (G+F+).

Analyses of log-transformed data indicated that grit deprivation and food deprivation significantly increased the consumption of granules. (Figure 6)

Figure 6 Mean consumption of blank Temik granules under food and grit deprivation (Log-transformed data with 95% confidence intervals). G+F+ grit available and food available, G-F+ grit deprived & food available, G+F- grit available & food deprived, G-F- grit deprived & food deprived.

(Data are back transformed means of log-transformed data together with 95% confidence intervals).

When partridges had both food and grit available to them (G+F+), they ate few granules. Most granules were eaten when birds had neither food nor grit (G-F-). Both grit deprivation (p = 0.01) and food deprivation (p <0.02) significantly increased granule consumption. The effects of grit and food deprivation appear to be additive (there is no interaction between the factors).

A similar experiment carried out with house sparrows showed that grit deprivation led to significantly increased granule consumption but food deprivation did not.
Based on the experiments with grey partridges and house sparrows summarised above, we would recommend that a robust laboratory test of risk to birds from granular pesticide should:

1) **Select a species that is likely to consume grit of about the same size as the granular formulation.** It is usual (e.g. Best & Gionfriddo 1991a, Luttik & de Snoo 2002), to assume that the grit a bird chooses to eat is well-represented by the grit found in its gizzard. Furthermore, the size of grit in the gizzard is related to the body mass of the bird. However, our work above suggests that it might be unwise to exclude commonly occurring bird species on the grounds that the granules to be tested do not fall within the size range of grit found in the gizzard.
   a. Our experiments suggest that the range of grit actually eaten by birds may be broader than that found in their gizzards. Small particles may pass more quickly through the digestive tract, while large particles may be broken down by mechanical and chemical attack to form smaller particles in the gizzard (Vance 1971).
   b. Our experiments suggest that although birds may prefer grit within a given size range, if that size range is absent, then they will settle for what is available.
   c. Size preferences may vary with season or sex. We found that, laying females in spring had a greatly increased intake of small grit, perhaps searching for a source of calcium, rather than looking for an aid to digestion.

It may be necessary to establish empirically the grit size preferences of the candidate species.

2) **Select a species that is likely to eat seeds in a similar size range as the granular formulation.** Species chosen will need to reflect those present in the environments where the granules are likely to be used, but given this, grit is more likely to be used by granivores and granules are more likely to be mistaken for seeds than for insects. Therefore the test species should normally be a seed-eater.

3) **Birds should be deprived of grit.** If the laboratory test is intended to simulate naturally occurring “worst-case” conditions, then it is reasonable to simulate circumstances where little natural grit is available.

   In the current experiments we established the birds appeared to have a need for grit in that they consumed more of it after a period of deprivation. However, the fact that birds grit intakes here were very variable and the fact that birds deprived of grit do not appear to suffer by it (Gionfriddo & Best, 1999) suggests that the need is not a daily physiological necessity like the need for food. In our experiments we found that for birds used to having a supply of grit, depriving them of it for two or three days was enough to cause them to increase intake on the next encounter.

4) **Birds should be familiar with grit.** Before depriving birds of grit it is important to establish that they are familiar with it and accustomed to taking it. If birds have been kept for lengthy periods in a laboratory cage or aviary without grit or on a standard laboratory diet where grit is incorporated in the pellets or crumbs, then they may ignore grit on its first (and later) encounters. Birds chronically deprived of grit may fail to recognise it when offered it.

5) **Birds should be deprived of food.** If the laboratory test is intended to simulate naturally occurring “worst-case” conditions, then it is reasonable to simulate circumstances where birds are relatively hungry. We showed that partridges were more likely to eat granules when they had been deprived of food for 16 hours. This is a relatively mild privation, often experienced in the wild, during a long winter night for example. In our laboratory experiments, birds were well fed, *ad libitum* at all other times. In reality many birds may be chronically hungry and it may be appropriate to simulate this in a laboratory test by maintaining birds below their free feeding weight. (In the UK this would require licensing by the Home Office and would need to be carefully carried out and closely monitored to avoid the risk of starvation.)

6) **Birds should be familiar with small seeds.** Pesticide granules currently available vary in size but typically, lie within a size range of 0.5-2mm. A range of wild seeds commonly taken by farmland birds also fall within this size range (eg. Chickweed *Stellaria media*, Annual meadow grass *Poa annua*, Groundsel *Senecio vulgaris* and Charlock *Sinapis arvensis*). Birds fed a standard laboratory diet of crumbs or pellets or cereal grains may not recognise small seeds as food and may be less likely to mistake granules for food when presented. Therefore it is important that birds have been routinely offered small seeds, as similar as possible to granules in colour and size.

7) **Seeds, grit and granules should be presented at realistic densities and in a naturalistic context.** If the laboratory test is to simulate natural worst-case conditions, then the seeds grit and granules, should be presented on a soil surface at densities typical of worst case conditions in the field. In the experiments:
   a. In the pre-trial phase, seeds and grit should be offered in abundance on the soil surface, so that birds become used to foraging for grit and seeds.
b. In the experimental phase, granules should either be offered alone, simulating a worst case where the field contains no grit and no food; or together with grit and seeds at realistic densities. Ideally both sorts of experiment should be conducted. Presenting granules on their own might be considered too extreme a worst case and it is possible that birds recognising the absence of familiar food and grit may decide not to forage at all. If granules are presented along with familiar food and grit then birds may be stimulated to forage and take granules in mistake when searching for seeds and grit. In this case we might expect the probability of taking a granule to be related to the density of natural grit or seeds available.

There are other issues in the design of a standard laboratory test that have not been addressed by our work so far.

1) In a laboratory test of risk posed by granules to wildlife, the key outcome to be assessed is the mortality of the wildlife exposed. In the experiments described above, only blank granules were used, birds were housed singly and the key variable was consumption of granules rather than mortality of individuals. Using individual consumption of granules enabled us to achieve a more graded measure of risk rather than the all-or-none measure of mortality. Using blank granules enabled us to use the same bird again under different conditions of hunger and grit deprivation. Our work on granules and grit consumption showed that it was very variable within and especially between individuals. Repeated use of the same individual and measuring the graded consumption of granules enabled us to detect subtle effects of hunger and grit deprivation using relatively few individuals.

Because re-use of birds is not an option when presenting toxic materials, it is likely that the numbers of individuals necessary will be larger than the 16 or so typically used in our experiments. The simplest way to increase the number of individuals would be to have several birds per aviary. This has the advantage of giving a value for mortality between 0 and \( n \) rather than 0 and 1 and for many species of birds probably represents a more realistic foraging environment where competition may be an important factor.

2) Because we used blank granules in these experiments, we did not investigate the repellent or sub-lethal effects that real granules containing pesticide may have on consumption. For example it is possible that the consumption of a single granule may induce nausea in the foraging bird, causing it to reject further granules when it meets them. Because granules are relatively thinly spread and difficult to eat quickly in large numbers, this may be an important mechanism preventing poisoning.
Effects of Grit and Food Deprivation on Granule consumption in a naturalistic setting

In the foregoing experiments partridges were offered grit, food and granules in separate food hoppers. In the arable environment, it is unlikely that birds would come across these items in such a concentrated, distinct piles. Rather, they would meet them thinly spread across the soil surface. Under these circumstances it may be more difficult to distinguish between them and it may be more difficult to consume large quantities of one of them. For example, seeds on the soil surface may only be picked up one at a time, whereas seeds in a food hopper may be eaten by the billful. Therefore we mimicked the normal agricultural habitat by placing granules along with, grit and seeds, in realistic densities against a soil-like background.

The difficulty in attempting to simulate a naturalistic setting with seeds, grit and granules on soil is being able to measure accurately, the consumption of each and soil. For a soil background we used sand sieved to remove all particles above 0.4mm, which might be used as grit. For seeds, we used rape seeds which are similar in colour and shape to blank Temik granules but larger (2mm for rape compared to 1mm for Temik) Grit was sieved to include only the medium and larger sized particles (1.7-3.3mm) which the birds generally preferred. In this way we were able to recover and count or weigh all grit, granules and seeds offered to birds during the trials.

The densities of blank granules were based on the maximum approved application rate (33.6 kg/ha for potatoes) and assumed that 95% were incorporated in the soil with 5% available to birds on the surface. We estimated a single Temik granule to weigh an average of 0.86mg. Therefore we might expect as a worst case to find 195 Temik granules per square metre of soil surface. In these experiments we offered birds granules in trays measuring 60x60cm and therefore we offered birds 70 granules in each trial.

Unpublished work by CSL and the Game Conservancy Trust has estimated the density of weed seeds on arable land soil surface is approximately 300mg m⁻². Rape seeds in our experiments weighed an average of 3.67mg. Therefore we sprinkled them on our trays at density of 80 seeds m⁻² or 30 per tray.

The amount of naturally grit varies greatly according to soil type. Sandy, gravelly soils will contain a super-abundance, peaty soils may contain very little. For the purpose of this experiment we represented a super-abundance by adding 5g of grit, comprising equal weights of 1.7-2.3mm and 2.3-3.3mm sizes to each tray. This was equivalent to the number found in a typical sandy soil. (Luttick & de Snoo, 2002)

As in the previous experiment, each bird experienced all four experimental conditions (G-F-, G+G-, G-F+ and G+F+) in a latin square design. The experiment was conducted in January and, grit deprived birds had grit withdrawn for two days before being offered. Granules were offered between 9am and 3pm. Food deprived birds (F-) had food withdrawn only for the 6 hour duration of the trial. In addition to the 30 rape seeds sprinkled on the test tray, non-food deprived birds had a 20g of seed mixture available in a separate food hopper.

At the end of the experimental trial, the tray contents were sieved into their individual constituents of sand, seeds, grit and granules. Counts of granules eaten were expressed as proportions and arcsine transformed.

Results

Analysis of variance showed a significant effect of grit deprivation and food deprivation on the consumption of granules (Table 1).

Table 1 ANOVA of granules eaten by 16 partridges under conditions of food and grit deprivation

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From Figure 7 it can be seen that as in the earlier experiment carried out for Objective 1, more granules were eaten when birds were hungry and grit deprived than when they were not deprived and food and grit were present in abundance.

In this situation where the 70 granules sprinkled on surface were depleted without replacement, the more granules that are consumed, the harder the remainder are to find. The probability of randomly consuming a granule hidden among sand particles becomes ever smaller as more granules are eaten. Surprisingly on 10 out of the 64 trials birds ate more than 50 of the 70 granules presented. Birds consuming large numbers of granules were clearly intent on doing so. Even when birds had food and grit available they still managed to eat an average of 12 granules in 6 hours.

**Estimating exposure for risk assessment**

The preceding experiments suggest that birds may eat granules in mistake for grit and/or in mistake for food. Models of the potential exposure of wildlife have tended to concentrate on the former. In our work we have therefore estimated exposure assuming that birds are simultaneously foraging for grit and seeds on a surface that contains grit, seeds and granules. The probability of eating a granule under these circumstances is given by

\[
P(EatGran) = \frac{N_{Gran} * GranGritPref}{N_{Gran} * GranGritPref + N_{Grit} + N_{Seed} * SeedGritPref} + \frac{N_{Gran} * GranSeedPref}{N_{Gran} * GranSeedPref + N_{Seed} + N_{Grit} * GritSeedPref}
\]

Where

- \( N_{Gran} \) = Number of granules per m\(^2\) that overlap size range of preferred grit
- \( N_{Grit} \) = Number of grit particles per m\(^2\) that overlap size range of preferred seeds
- \( N_{Seed} \) = Number suitable seeds available per m\(^2\)
- \( N_{Grit} \) = Number of suitable grit particles per m\(^2\)
- \( GranGritPref = \) preference ratio of granules compared to grit. (>1 indicates granules preferred, <1 indicates grit preferred)
- \( GranSeedPref = \) preference ratio of granules compared to seeds. (>1 indicates granules preferred, <1 indicates seeds preferred)
- \( SeedGritPref = \) preference ratio of seeds compared to grit when bird is foraging for grit (>1 indicates seeds preferred, <1 indicates grit preferred)
- \( GritSeedPref = \) preference ratio of grit compared to seeds when bird is foraging for seeds (>1 indicates grit preferred, <1 indicates seeds preferred)

The number of granules potentially available to birds can be calculated fairly simply from knowledge about the size of grit particles and seeds normally taken by the species concerned and by information on application rate and incorporation rate of granules in the soil. The number of suitable grit particles and seeds available can be determined empirically for a given soil. Less easy to establish are the preference ratios of a species for granules, grit and seeds. In models looking at granules mistaken for grit particles it has typically...
been assumed that birds show no preference between granules and similarly sized grit particles. Both are consumed randomly in proportion to their availability. For typical granular formulations it can be shown (eg Figure 8) that unless the incorporation rate exceeds 95% the risk to small birds is high, with the Toxicity Exposure Ratios below 10.

**Figure 8. Example of how Toxicity Exposure Ratio varies with granule incorporation rate and with density of grit (n per m²)**

(These data assume a house sparrow needing 83 grit particles daily on a grit-rich (50,000 particles m⁻²) soil and a grit-poor (5000 particles m⁻²) soil.

When the possibility of mistaking granules for seeds is also included in the calculation the potential risk is greatly increased. There are two main reasons for this.

1) Grit is likely to be relatively plentiful in the soil (perhaps 5000-50000 particles per m²) and the chances of mistakenly picking up a granule in mistake for grit are relatively low. Seeds are relatively scarce on arable soils (perhaps 250 per m²).

2) The number of particles of grit eaten per day is likely to be much less than the number of seeds. From our experimental data we found that sparrows ate 83 grit particles a day in the size range 0.5 - 0.8 mm. Wild sparrows might be expected to need 7g of weed seeds in a day. This is equivalent to more than 35000 seeds weighing 0.2mg (about the size of a granule). A bird would need to eat 1 seed a second for 10 hours to satisfy its daily requirement and therefore it is unlikely that birds will feed solely on such small seeds. However, several weed species important to birds produce seeds weighing less than 1mg and significant numbers of small seeds may be consumed.

In reality a bird is unlikely to treat granules, grit and seeds as equivalent, but will show preferences between them which may vary according to its needs and local conditions. Clay granules are likely to be poor substitutes for seeds and for grit and in the course of foraging birds will learn to avoid them. The consequences of such preferences are explored in more detail in the milestone report.
SUMMARY FOR OBJECTIVES 1-3

1. Granules may be ingested by birds in mistake for grit. Other authors have assumed that the risk of this happening is highest for granules of similar size to the grit found in the gizzards of wild birds. However, we measured grit consumption in grey partridges and house sparrows directly, and found that the range of grit sizes actually taken by birds was broader than that found in the gizzards of wild birds.

2. For partridges, the range of grit sizes found in the gizzard is narrower than that actually eaten by the bird. Larger grit particles may get broken down in the gizzard and smaller particles may pass through it.

3. For partridges and sparrows grit deprivation increases the consumption of both grit and granules. This suggests that in environments where natural sources of grit are limited then birds may take granules as a substitute.

4. For partridges we showed that birds would consume granules in a simulated natural environment where grit and granules were presented at realistic worst-case densities. Consumption of granules was highest when suitable grit was absent but occurred even when grit was present in abundant amounts.

5. For partridges, but not for sparrows we also showed that increasing the hunger of the birds, increased the likelihood that they would take granules. Again, in naturalistic simulations, presenting seeds and granules together against a soil background, more granules were taken when birds were hungry but some granules were taken even when seeds were abundant.

6. For sparrows, the increase in consumption of granules under grit-deprived and food-deprived conditions was small. But given that for some pesticides two or three granules is often enough to kill a small bird, then any factor which encourages increased consumption of granules is important.

7. Our experiments suggest that birds may mistake granules for seeds as well as grit and that regulatory practice should take this into account. The design of a laboratory test to evaluate the risks of granular formulations to birds should ensure that
   - the test species is likely to consume grit and seeds of similar size to the granular formulation
   - birds should be made familiar in the laboratory with grit and seeds of similar size to the granular formulation.
   - birds should be deprived of grit and seeds before exposure to granules
   - seeds, grit and granules should be presented at realistic densities in a naturalistic context.

8. Future research on risks posed by granular formulations should investigate the effects of avoidance on consumption and mortality.
SCIENTIFIC OBJECTIVE 4

"Determine the extent to which mammals and birds take pellets as food and how this depends on hunger and the availability of alternative foods."

Background

Pesticide treated pellets are often based on a substance that is a food in its own right (e.g. bran, wheat or barley) in order to attract the target pest. However there is a danger that these may also prove attractive to non-target wildlife. These experiments were conducted to provide more information about the attractiveness of pellets to birds and mammals and the factors which may affect uptake (and hence risk) such as the effects of hunger and the availability of alternative food.

Approach

Blank pellets (formulation without the active substance) were offered to captive starlings (*Sturnus vulgaris*) and house sparrows (*Passer domesticus*) under test conditions based on previous experience with treated seeds in project PN0902 to determine the potential for exposure under worst-case and more commonly encountered, less severe conditions. Factors varied were the degree of hunger, group size and the availability of alternative food. Similar studies were also conducted with captive bred wood mice (*Apodemus sylvaticus*) in order to establish the potential for such species to be exposed to pellet formulations and to provide initial information about test conditions suitable for use in standard test of avoidance of solid formulations by small mammals.

The initial test involved offering pellets under each combination of conditions for a six hour test period and measuring consumption. Animals were then offered the same type of pellets along with normal diet for three follow-on tests to determine whether the patterns seen on the test day would continue or if they would become accustomed to the pellets over time.

Initial exposure

Pellets consumed during the first six hour exposure period are shown in Table 2.

<table>
<thead>
<tr>
<th>Species</th>
<th>Group size</th>
<th>Hunger</th>
<th>Choice</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Starling</td>
<td>7.43</td>
<td>5.72</td>
<td>5.69</td>
<td>7.46</td>
</tr>
<tr>
<td>House sparrow</td>
<td>0.99</td>
<td>1.26</td>
<td>0.81</td>
<td>1.44</td>
</tr>
<tr>
<td>Wood mouse</td>
<td>-</td>
<td>-</td>
<td>1.24</td>
<td>1.76</td>
</tr>
</tbody>
</table>

Both bird species significantly avoided pellets if alternative food was available, but where there was no choice they consumed large quantities of pellets. Even so, small amounts were consumed by birds when alternative food was available. For both starlings and house sparrows, the mean consumption of pellets by no-choice test birds was approximately 65% of the normal diet consumption of choice test birds.

Both deprivation and lack of alternative food significantly increased consumption by wood mice, although the difference between choice and no-choice animals was less marked than for the bird species. Wood mice were generally more likely to consume substantial amounts of pellets than birds even in a choice situation.

Subsequent exposure

Both bird species showed a strong effect of previous experience on the consumption of pellets after no-choice tests, with a continuing high level of consumption relative to choice test birds. Choice test starlings showed gradually increasing consumption of pellets over time, but choice test house sparrows continued to almost completely avoid them. While blue pellets were less preferred on the test day by house sparrows, consumption of blue increased to undyed levels during the follow on period. The preference for blue pellets by starlings also increased but they appeared to be preferred over undyed pellets. However this difference was not significant.

The consumption of pellets by wood mice during the follow-on period was less affected by previous experience. While the no-choice wood mice appeared to consume slightly more pellets than the choice test mice this difference was not significant.

Conclusions

The results indicate that all three species will take pellets as food under some conditions but both bird species consumed only very small amounts if there was alternative food available. However, if the toxicity and concentration of the active substance are high enough, even this low level of consumption may be sufficient to lead to poisoning unless there is a strong and rapid avoidance response caused by the active substance or added repellents. Given these results the following recommendations can be made.
1) **Consider pellets as food items in risk assessment.** Blank pellets appeared palatable to small mammals under all conditions and to birds under no-choice conditions where quantities consumed approached normal food levels.

2) **Consider the likely availability of alternative food when considering risk from pellets.** By far the most important factor reducing intake of pellets, especially for birds was the availability of alternative food. Low food availability in the wild may increase the possibility of pellets being sampled.

3) **Obtain good data on avoidance if this is to be used as a basis for approval.** As the pellet material was not inherently unpalatable, it would be necessary to ensure that the active ingredient itself (or any added repellents) would lead to avoidance. This may be particularly important for small mammals which appear to be at most risk.
SCIENTIFIC OBJECTIVE 5
“Develop and test a method for measuring the avoidance response of small mammals to solid formulations (seeds, pellets and baits).”

Background

Where formulations are identified as being high risk, PSD often find it necessary to request data on the potential for avoidance of the formulation by small mammals, either due to repellency or post-ingestional effects. There is no standard test of avoidance for small mammals, apart from some efficacy tests which are not suitable for this purpose. Without an established standard test it is necessary to negotiate the method to be used on a case by case basis and therefore difficult to ensure that the results obtained from different studies are either realistic or comparable. Before the test design could be developed and tested with an actual high risk formulation, it was necessary to address the following questions 1) what is the pattern of feeding in captive wood mice, how does this relate to the behaviour of wild mice and how could this affect the conduct or outcome of avoidance studies? 2) does deprivation increase initial feeding rate under laboratory conditions as for birds? and 3) what are consequences of complete avoidance on bodyweight and can we set reasonably safe deprivation limits?

Patterns of feeding

The consumption of normal diet by mice during the light and dark periods and patterns of feeding during the dark period were recorded. Some mice were also video recorded to determine patterns of behaviour without disturbance.

It was clear that most feeding occurred during the dark period but the behaviour of our captive wood mice was very variable and some individuals consumed a large proportion of their daily food when it was light. Given this and the potential effects on mouse condition and feeding behaviour at the start of the study, it was necessary to establish the effects of removing the food during the daylight period on subsequent consumption and bodyweight maintenance, in order to develop a suitable test.

Effects of food restriction on consumption and feeding rate

Food consumption and feeding rate were measured following different periods of deprivation up to 18h. Food was either not removed, removed during the previous light period (reversed daylight), during the first 6h of the dark period, or both (Table 3).

Table 3. Mean consumption in the first two hours of the test period expressed as actual consumption and as a percentage of normal 24h consumption (n = 6 except for 12h deprivation where n = 5).

<table>
<thead>
<tr>
<th>Deprived (h)</th>
<th>Light period Deprived 2100-0900</th>
<th>First 6h dark Deprived 0900-1500</th>
<th>First 2h (g)</th>
<th>6h test (g)</th>
<th>2h as % normal daily (24h)</th>
<th>6h as % normal daily (24h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No</td>
<td>No</td>
<td>0.24</td>
<td>0.93</td>
<td>7.5</td>
<td>29.0</td>
</tr>
<tr>
<td>6</td>
<td>No</td>
<td>Yes</td>
<td>0.82</td>
<td>2.27</td>
<td>27.7</td>
<td>72.9</td>
</tr>
<tr>
<td>12</td>
<td>Yes</td>
<td>No</td>
<td>0.49</td>
<td>1.20</td>
<td>12.9</td>
<td>33.9</td>
</tr>
<tr>
<td>18</td>
<td>Yes</td>
<td>Yes</td>
<td>0.97</td>
<td>2.29</td>
<td>32.0</td>
<td>73.7</td>
</tr>
</tbody>
</table>

Removing the food for a period at the beginning of the dark period significantly increased both the rate of consumption and the amount consumed as has previously been found for birds. Therefore, restrictions on feeding time may be an important factor when considering the potential for avoidance to protect animals in the wild. However, removing food during the previous light period did not affect feeding rate or bodyweight maintenance.

Effects of complete avoidance on weight loss

Bodyweight was monitored after periods of food deprivation designed to simulate the effects of complete avoidance on weight loss. This allowed us to define safe levels of deprivation for testing that would not cause significant weight loss. This indicated that removing food for a few hours at the beginning of the dark period would not lead to dangerous weight loss even if the test diet was completely avoided in a subsequent 6h test.
**Validation study with methiocarb treated pellets**

The preceding experiments indicated that restricting food at the beginning of the dark period and manipulating the availability of alternative food could safely be used to increase the motivation to feed in captive wood mice. This allows the strength of any avoidance response to be tested over a range of possible scenarios..

Two sets of test conditions (pellets only or pellets and untreated wheat seed) were used to test the palatability and risk posed by methiocarb treated slug pellets to wood mice. Other factors affecting the realism of the presentation of treated pellets (density and moisture content) were also tested (Table 4).

**Table 4. Factors tested.**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Level</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternative food</td>
<td>YES</td>
<td>= 5g wheat seeds in a dish at far end of cage</td>
</tr>
<tr>
<td></td>
<td>NO</td>
<td>= pellets only offered (empty wheat dish)</td>
</tr>
<tr>
<td>Density</td>
<td>HIGH</td>
<td>= 5g pellets in dish as for wheat (c 430 treated pellets)</td>
</tr>
<tr>
<td></td>
<td>MEDIUM</td>
<td>= 5g pellets spread on tray (c 430 treated pellets)</td>
</tr>
<tr>
<td></td>
<td>LOW</td>
<td>= 0.23g (5xLD) pellets spread on tray (20 pellets)</td>
</tr>
<tr>
<td>Moistened</td>
<td>YES</td>
<td>= Capillary matting on pellet tray/dish moistened</td>
</tr>
<tr>
<td></td>
<td>NO</td>
<td>= Capillary matting dry</td>
</tr>
</tbody>
</table>

The consumption of treated pellets was very low compared to untreated pellets and there was no significant effect of the availability of wheat or pellet moisture on exposure, although density of pellets did affect consumption (Figure 8). The reason for the effect of density is not clear, but it is possible that the medium density (where pellets were spread in large numbers over the cage floor) increased the encounter rate and hence sampling rate compared to the other levels. At high density, the pellets were contained in a small space and may have been more easily ignored. It is also possible that at such a high density the smell of the formulation may have deterred feeding.

**Figure 9. Mean consumption of treated pellets at each level of each factor.** Bars represent standard errors.

Despite the very low consumption of methiocarb treated pellets, eight out of 24 mice tested died following exposure (Table 5).
Table 5. Mortalities out of two mice tested at each set of conditions.

<table>
<thead>
<tr>
<th>Choice test</th>
<th>No-choice test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High density</td>
</tr>
<tr>
<td>Low density</td>
<td>1/2</td>
</tr>
<tr>
<td>Medium</td>
<td>1/2</td>
</tr>
<tr>
<td>High</td>
<td>0/2</td>
</tr>
</tbody>
</table>

The mortalities did not appear to be strongly related to any of the factors tested but tended to be among smaller individuals. The difference in weight between those that died and those that died was significant (t-test, T = 3.53, p = 0.003). This suggests that the pellets are initially attractive to the animals and some consumption is required before avoidance occurs. However, the toxicity of the material is such that sampling of only two to three 0.01g pellets can lead to mortality, especially in smaller individuals. Given these results, the effects of deprivation were not tested with methiocarb as it was clear that high levels of mortality would occur under more severe conditions.

**Recommendations**

1) **Conduct cage tests under conditions that are as realistic as possible.** When testing the avoidance response of small mammals it is necessary to use methods that as far as possible (within the limitations of a captive test) represent the behaviour of wild animals.

2) **Conduct tests under a range of conditions.** The results from this study, objective 4 in this project, and earlier projects on birds (PN0902, PN0909 and PN0914) suggest that the effects of various sets of conditions of food availability and hunger should be tested to determine the strength of any avoidance response and the conditions under which it may break down. This can then be used by the risk assessor to consider the frequency with which effects are expected in the field.

3) **Where necessary conduct use larger scale tests to confirm conclusions.** Where realistic conditions (e.g. density of test material) cannot be achieved and a high risk is predicted from a cage test, it may be necessary to conduct a semi-field or large arena style study to confirm this.
SUMMARY OF FINDINGS FOR OBJECTIVES 4-5

1. Pellets used as the base material for slug pellets were shown to be attractive to both birds and mammals and should be considered as food items in risk assessment; strong evidence of avoidance may be necessary if they are to be considered safe.

2. Where tests of avoidance are conducted with small mammals, with either pellets or treated seeds, it would be advisable to test them under different conditions of availability of alternative food and hunger level to characterise the strength of the response. The presentation of the test material should be as realistic as possible.

3. Methiocarb treated slug pellets present a high risk to small mammals despite a strong avoidance response. Sampling of only one or two pellets before avoidance can lead to mortality and occurs even in the presence of alternative food.
SCIENTIFIC OBJECTIVE 6
"Improve estimates of pesticide residues in earthworms for use in risk assessments for worm-eating animals after pesticide applications."

Background

Earthworms form an important food source for a range of mammals and birds. If earthworms are exposed to pesticides, there is a risk that animals will be poisoned from consuming contaminated earthworms. Present risk assessment procedures for spray applications have often assumed that earthworms contain 30% soil by weight. The predicted pesticide concentration in the earthworm is therefore calculated to be 30% of the predicted environmental concentration (PEC) in the soil, assuming that the pesticide is evenly distributed in the top 5 cm of soil. There is a need to validate this method for sprays and test whether a similar approach is suitable for solid formulations, which are affected by additional complicating factors.

Approach

A microcosm study was conducted involving the use of six pesticides applied as spray formulations (Table 6) and two species of earthworm (Lambricus terrestris and Eisenia fetida) to determine the effects of exposure period, soil organic matter (OM) content and rainfall on the pesticide residues in earthworms. The pesticides were chosen to provide a range of chemical classes, solubilities, degradation times and octanol-water partition coefficient (log Kow).

Table 6. List of pesticides in spray formulations.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>log Kow</th>
<th>Solubility</th>
<th>DT50 (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethoate</td>
<td>0.704</td>
<td>23.8g/l</td>
<td>2-16</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>1.85</td>
<td>120mg/l</td>
<td>7-28</td>
</tr>
<tr>
<td>Malathion</td>
<td>2.75</td>
<td>145mg/l</td>
<td>1</td>
</tr>
<tr>
<td>Difenoconazole</td>
<td>4.20</td>
<td>15mg/l</td>
<td>145 (photolysis)</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>4.7</td>
<td>1.4mg/l</td>
<td>60-120</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>&gt;6</td>
<td>&lt;1µg/l</td>
<td>65-125</td>
</tr>
</tbody>
</table>

A second study investigated residues in earthworms following the surface application of three granular formulations (Table 7) under similar test conditions.

Table 7. List of pesticides in granular formulations.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>log Kow</th>
<th>Solubility</th>
<th>DT50 (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldicarb</td>
<td>0.053</td>
<td>4930mg/l</td>
<td>15-61</td>
</tr>
<tr>
<td>Ethoprophos</td>
<td>3.6</td>
<td>700mg/l</td>
<td>14-87</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>4.7</td>
<td>1.4mg/l</td>
<td>60-120</td>
</tr>
</tbody>
</table>

Spray formulations

Test containers consisted of plastic pots which provided a soil depth of 22cm and surface diameter of 23cm. Tests were conducted at a temperature of 15°C and a relative humidity of 70%. There was a 12:12h light:dark cycle to encourage normal daily cycles of earthworm activity. Test soil was based on the constituents of OECD soil (silver sand, kaolin clay, sphagnum moss peat) with either 2% peat (low OM) or 10% peat (high OM) and 1% horse manure. Calcium carbonate was used to adjust pH to 6.5 and de-ionised water was used to moisten soil to 60% of water holding capacity. Small discs of lettuce leaf were placed on the surface of the soil as food.

Five to six days before treatment sixteen E. fetida and four L. terrestris were placed in each container. The six pesticides were applied at rates similar to the maximum permitted application rate in the UK. The formulations were diluted so that they could be applied to the surface of the soil at these rates in a volume of 5ml. Containers not subjected to simulated rainfall were then left until sampling except for checks for dead worms and weekly replacement of lettuce discs during the exposure period. Rain bins were irrigated with the equivalent of 20mm of rain 24h after treatment. On the following day any excess water in the dish under the bin was removed to represent drainage.

Test bins were sampled 2, 7, 14 or 28 days following treatment. First the surface of the bin was checked for lettuce discs and earthworms on the surface. These were recorded and any worms removed, weighed and frozen for possible analysis. A core of soil approximately 22mm in diameter and 5cm in depth was removed from the centre of the bin for residue analysis. The soil was then
hand sorted to find the remaining earthworms. As each earthworm was recovered any loose soil was removed by gently shaking the worm and dropping it onto clean filter paper. The worms were not washed as it was necessary to determine the residue in and on the worm that would be ingested by birds. The species, condition, weight and approximate depth were then recorded before the earthworms were stored frozen until residue analysis.

Each combination of soil type (high or low organic matter content), rainfall (no rainfall or heavy rainfall on the day after application) and sampling time (2, 7, 14 or 28 days after application) was tested, requiring 16 microcosms for each pesticide (96 in total).

**Granular formulations**

This study was conducted in the same way as the sprays experiment above, except that due to the high level of mortality and consequent small sample sizes seen in the spray study at the longer periods, sampling was conducted on days 2, 7 and 14 only. Also, an attempt was made to determine the relative amounts of residue in the adhered soil, the worm tissue and in the gut contents. Worms of each species were subdivided into i) the same condition as collected in the previous study (uncleaned), ii) washed to remove adhering soil (cleaned) and iii) washed and allowed to empty gut contents for 30 minutes (washed and emptied). To achieve this it was necessary to add a larger number of worms to each bin in turn necessitating the use of larger containers so that earthworm density was similar to that in the previous study. Thus six *L. terrestris* and 24 *E. fetida* were added to pots with a soil surface diameter of 27cm and soil depth of 23cm.

**Residues after spray applications**

Very few worms (2.5%) were recovered from the soil surface after spray treatment, so analysis was restricted to the worms recovered from the soil at each sampling point. Given the poor condition of most dead earthworms, which decayed rapidly in the soil, only live earthworms were analysed. It was assumed that these are most likely to be taken by birds. For each container one sample of soil and one sample of each species of earthworm was analysed (288 samples in total).

Overall residues found in soil and each species of earthworm for each pesticide are shown in Figure 10. Residues were adjusted to take account of recovery from spiked samples and are expressed as a percentage of predicted soil residues. These were calculated from the application rates by assuming all pesticide is contained in the top 5cm of soil, and using the appropriate bulk density for soil in each case (1.63 g/cm³ for low OM soil and 1.13 g/cm³ for high OM soil as calculated from soil weight and bin volume).

**Figure 10. Boxplot (median and interquartile range) of residue expressed as percent of predicted soil residue in soil and both species of earthworm for each pesticide** (Dim = dimethoate, Car = carbaryl, Mal = malathion, Dif = difenoconazole, Chl = chlorpyrifos, Bif = bifenthrin, S = soil, Ef = *E. fetida*, Lt = *L. terrestris*). Horizontal lines represent 30% and 100% of predicted soil residues.

In most cases, median soil residues were below the predicted level and *E. fetida* residues were lower than those for *L. terrestris*. Also, for those pesticides with low Kow or persistence (e.g. dimethoate, carbaryl and malathion) residues appear not only lower than predicted using the 30% estimate, but also lower than the actual measured soil residues. For bifenthrin, the range of residues in *L. terrestris* appears to exceed both predicted and measured levels indicating bioconcentration. This is consistent with the high Kow of this compound.
Residues expressed as a percentage of predicted values for each species at each factor level are shown in Figures 11 to 13.

**Figure 11.** Boxplot of residues as a percentage of predicted soil concentration for each species at each level of organic matter content. Boxes represent median and inter quartile range (E.f. = *E. fetida*, L.t. = *L. terrestris*).

![Boxplot of residues as a percentage of predicted soil concentration for each species at each level of organic matter content.](image)

**Figure 12.** Boxplot of residues as a percentage of predicted soil concentration for each species at each level of rainfall. Boxes represent median and inter quartile range (E.f. = *E. fetida*, L.t. = *L. terrestris*).

![Boxplot of residues as a percentage of predicted soil concentration for each species at each level of rainfall.](image)
Overall residues in *L. terrestris* were higher than those in *E. fetida* and there was some evidence that residues were higher in low OM soil and after rainfall. Residues in earthworms appeared relatively stable across the sampling periods except in the case of malathion where rapid degradation led to lower residues at the longer exposure periods.

Statistical analysis was conducted on the percentage values. Log values were used for all parametric analyses due to the distribution (strong positive skew) of residue data. There was a strong correlation between residues in both species of worm (Pearson correlation coefficient = 0.749, p < 0.001) but a paired t-test indicated an overall significant difference between the species (t = 5.45, p <0.001). Closer examination comparing the data for each species and each chemical indicated that these differences were only significant for three of the chemicals, bifenthrin, difenoconazole and carbaryl. In all cases the E.f. residues were significantly lower than those for L.t.. There were no significant differences for any of the OP compounds.

Given these differences subsequent analysed were conducted separately for each species. The effects of all main factors and two-way interactions on the residues in each species and soil samples were tested using separate univariate ANOVAs. The most important factor was chemical which significantly affected all three measurements of residue (*E.f. - F = 73.3, p < 0.001; L.t. - F = 89.8, p < 0.001; soil - F = 7.9, p < 0.001*). The next most important factor was soil organic matter content which also had a significant effect on all three measures (*E.f. - F =, p 19.< 0.001; L.t. - F = 7.1, p =0.010; soil - F = 10.9, p = 0.002*). Days after treatment appeared to significantly affect both *L. terrestris* and soil sample residues (*L.t. - F = 3.0, p =0.041; soil - F = 3.2, p = 0.030*). Rainfall alone appeared not to have an effect but had an almost significant effect on *E. fetida* residues and there was a significant two-way interaction with days for this species. There was also a significant two-way interaction between chemical and days for soil residues.

Given that chemical appeared to have the largest effect on residues and that residues were in most cases higher in *L. terrestris*, the residues found in *L. terrestris* in relation to the physical properties of the pesticides are illustrated below (Figures 14 and 15).

Overall residues in *L. terrestris* were higher than those in *E. fetida* and there was some evidence that residues were higher in low OM soil and after rainfall. Residues in earthworms appeared relatively stable across the sampling periods except in the case of malathion where rapid degradation led to lower residues at the longer exposure periods.

Statistical analysis was conducted on the percentage values. Log values were used for all parametric analyses due to the distribution (strong positive skew) of residue data. There was a strong correlation between residues in both species of worm (Pearson correlation coefficient = 0.749, p < 0.001) but a paired t-test indicated an overall significant difference between the species (t = 5.45, p <0.001). Closer examination comparing the data for each species and each chemical indicated that these differences were only significant for three of the chemicals, bifenthrin, difenoconazole and carbaryl. In all cases the E.f. residues were significantly lower than those for L.t.. There were no significant differences for any of the OP compounds.

Given these differences subsequent analysed were conducted separately for each species. The effects of all main factors and two-way interactions on the residues in each species and soil samples were tested using separate univariate ANOVAs. The most important factor was chemical which significantly affected all three measurements of residue (*E.f. - F = 73.3, p < 0.001; L.t. - F = 89.8, p < 0.001; soil - F = 7.9, p < 0.001*). The next most important factor was soil organic matter content which also had a significant effect on all three measures (*E.f. - F =, p 19.< 0.001; L.t. - F = 7.1, p =0.010; soil - F = 10.9, p = 0.002*). Days after treatment appeared to significantly affect both *L. terrestris* and soil sample residues (*L.t. - F = 3.0, p =0.041; soil - F = 3.2, p = 0.030*). Rainfall alone appeared not to have an effect but had an almost significant effect on *E. fetida* residues and there was a significant two-way interaction with days for this species. There was also a significant two-way interaction between chemical and days for soil residues.

Given that chemical appeared to have the largest effect on residues and that residues were in most cases higher in *L. terrestris*, the residues found in *L. terrestris* in relation to the physical properties of the pesticides are illustrated below (Figures 14 and 15).
There appear to be large differences between chemicals based on their physical properties when comparing residues in earthworms with expected residues. For bifenthrin, which has high log Kow and persistence, median residues in *L. terrestris* exceeded the predicted soil residue. For the less persistent compounds residues were much lower than that predicted. The low residues observed for malathion did not seem to relate to the log Kow for this compound but seems to be due to its low persistence in the soil. Similar patterns were seen in the *E. fetida* residues.
Residues after granular applications

The number of earthworms found dead and dying on the soil surface was higher than in the spray formulation trials. Dead and dying worms were collected from the surface of all aldicarb bins, 11 out of the 12 ethoprophos bins while only one E.f. was collected from the surface of two of the chlorpyrifos bins. Most of these were E.f. with a maximum of one L.t. collected from any bin. Total numbers collected of each species are shown in Table 8.

Table 8. Number of dead and dying earthworms collected from the surface of bins for each treatment.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>E. fetida</th>
<th>L. terrestris</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldicarb</td>
<td>104</td>
<td>7</td>
<td>111</td>
</tr>
<tr>
<td>Ethoprophos</td>
<td>88</td>
<td>2</td>
<td>90</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

The only effect on numbers appeared to be the active substance with no clear influence of other factors such as rainfall or soil organic matter content.

Residues measured in worms as collected from within the soil at each sampling point are shown in Table 9. As for the sprays data the residues were expressed as a percentage of expected soil residue and log values used in all parametric analyses.

Table 9. Residues in earthworms after granular applications expressed as a percentage of predicted soil residues.

<table>
<thead>
<tr>
<th>Type</th>
<th>Chemical</th>
<th>Mean (%)</th>
<th>Median (%)</th>
<th>Minimum (%)</th>
<th>Maximum (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. fetida</td>
<td>Aldicarb</td>
<td>35.6</td>
<td>17.7</td>
<td>0.3</td>
<td>128.2</td>
</tr>
<tr>
<td></td>
<td>Ethoprophos</td>
<td>39.2</td>
<td>18.3</td>
<td>0.5</td>
<td>166.6</td>
</tr>
<tr>
<td></td>
<td>Chlorpyrifos</td>
<td>27.2</td>
<td>11.6</td>
<td>4.1</td>
<td>147.6</td>
</tr>
<tr>
<td>L. terrestris</td>
<td>Aldicarb</td>
<td>13.2</td>
<td>3.3</td>
<td>0.2</td>
<td>77.4</td>
</tr>
<tr>
<td></td>
<td>Ethoprophos</td>
<td>2.4</td>
<td>0.8</td>
<td>0.1</td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td>Chlorpyrifos</td>
<td>52.0</td>
<td>26.5</td>
<td>6.9</td>
<td>179.0</td>
</tr>
</tbody>
</table>

The residues in each species from each bin were compared using a paired t-test. There was a significant difference \( T = 2.38, p = 0.023 \) with mean E.f. residues higher than mean L.t. residues. Tests for each compound showed that for aldicarb and chlorpyrifos the differences were not significant but for ethoprophos there was a large significant difference \( T = 3.77, p = 0.004 \).

Given the differences between species the analysis was conducted on each separately. While there significant effects of time after application \( F = 6.1, p = 0.006 \) and soil OM \( F = 21.2, p < 0.001 \)content on E.f. residues, this did not appear the same for L.t. with chemical as the most important factor. \( F = 18.2, p < 0.001 \).

The overall effects of each factor on the residues in each species are shown in Figures 16 to 19.
Figure 16. Boxplot of residues as a percentage of predicted soil concentration for each species at each level of exposure period. Data for all chemicals are pooled (E.f. = *E. fetida*, L.t. = *L. terrestris*).

![Boxplot of residues as a percentage of predicted soil concentration for each species at each level of exposure period.](image)

Figure 17. Boxplot of residues as a percentage of predicted soil concentration for each species at each level of organic matter content. Data for all chemicals are pooled. Boxes represent median and inter quartile range (E.f. = *E. fetida*, L.t. = *L. terrestris*).

![Boxplot of residues as a percentage of predicted soil concentration for each species at each level of organic matter content.](image)

Figure 18. Boxplot of residues as a percentage of predicted soil concentration for each species at each level rainfall on the day after treatment. Data for all chemicals are pooled. Boxes represent median and inter quartile range (E.f. = *E. fetida*, L.t. = *L. terrestris*).

![Boxplot of residues as a percentage of predicted soil concentration for each species at each level rainfall on the day after treatment.](image)
During sampling when worms were allowed time to empty void their gut contents before freezing, it was found that none of the *L. terrestris* appeared to void any material. Also, aldicarb and ethoprophos exposed *E. fetida* voided relatively little compared to chlorpyrifos exposed ones. This combined with the relatively high numbers of earthworms removed from the surface meant that only chlorpyrifos exposed earthworms were analysed to determine the effects of washing and emptying.

The reasons for the differences in amount of voided material are unclear but it is possible given the higher mortality in aldicarb and ethoprophos bins that these worms were either too severely affected either to empty their gut contents or possibly to feed. For *L. terrestris* it is also possible that as they are dark period surface feeders on vegetable matter, that they had already empty guts when they were sampled. The residues found in each type of worm are shown in Figure 20.

**Figure 20.** Effects of the various treatments (washing of soil, washing off of soil and allowing emptying of gut contents) on the residues found in earthworms exposed to chlorpyrifos. Washed and emptied *L. terrestris* were not analysed due to the lack of voided gut contents.

Data for each type of sample were compared using paired t-tests. While the residues in unwashed E.f. samples appeared slightly higher than the other types, these differences were not significant. However, there was a large difference between unwashed and washed L.t. samples ($T = 2.79, p = 0.018$) indicating that washing had significantly reduced the measured residue. Indeed residues on washed worms were on average around 50% of that in unwashed worms.

**Conclusions**

Residues after spray formulations indicate that chemical and soil organic matter content had the greatest effects on residues in earthworms. The measured values also indicate that a simple prediction of earthworm residues as 30% of soil residues is not appropriate for all chemicals. For some of the chemicals, particularly those with high logKow values and rapid decay rates in soil, the 30% prediction would exceed the measured residues and provide a safe estimate. For other chemicals where logKow has a higher...
value and persistence is higher then this method may well underestimate residues in earthworms and hence risk. An improved estimate, particularly for compounds with higher Kow values (e.g. 3 or above), would be to assume that the residue in earthworms is the same as that in the soil unless there is evidence that it is less than this. However it appears that even this approach may underestimate residues and risk. These results appear to agree with other approaches under development which identify logKow and concentration in the various soil compartments of soil as the most important factors in models that predict bioconcentration in earthworms (e.g. Jager 1998).

The results obtained for granular formulations were less clear but the data for *L. terrestris* appeared to indicate the same effect of logKow on residues. It is possible that the different type of application affected the pattern of residues but the large number of earthworms found on the surface may also have affected the observed patterns. However, the results obtained for unwashed and washed earthworms did indicate that washing earthworms before analysis could substantially reduce the measured residue at least in *L. terrestris*. This highlights the importance of adhered soil in the total pesticide loading of earthworms collected by predators and perhaps should be included in any estimate of earthworm residue which currently consider only the earthworm tissues and gut contents.
SCIENTIFIC OBJECTIVE 7
“Determine whether dead and dying earthworms and molluscs are more or less attractive to birds and mammals than unaffected prey.”

Background

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 paralysed state until death. They are therefore more available and easier to catch by predators such as worm-eating birds, increasing the risk of secondary poisoning. However it is not known to what extent predators may in fact avoid these dying and dead (and decaying) invertebrates in favour of normal unaffected prey. This project aimed to provide more information about the responses of predators to these prey to further refine the estimate of risk.

Invertebrate prey

All earthworms used in the tests with wood mice and blackbirds were adult *Lumbricus terrestris*. They were presented either alive (as collected from the soil), fresh dead (immersed in water at 40°C for 10 minutes), or decaying (killed as before and placed on a moist soil surface for 24h + 2h before presentation). All slugs used were grey field slugs (*Deroceras reticulatum*) and were presented either alive, fresh dead (placed in a freezer for 30 minutes or water at 40°C for five minutes), or decaying (killed as before and placed on a moist soil surface for 24h + 2h).

Palatability of live and dead earthworms to wood mice

Wood mice (*Apodemus sylvaticus*) were offered were offered live or fresh dead earthworms in both choice and no-choice tests and consumption measured. The consumption of each type of worm is shown in Figure 21.

Figure 21. Mean consumption of live and dead earthworms in choice and no-choice tests. For choice tests the data are total consumption over two days for each type of worm. For no-choice test the data represent consumption in one day (n = 12 in both cases).

Under choice test conditions, live worms appeared to be slightly preferred over dead ones and this difference was significant (Wilcoxon signed rank test, p = 0.045). However, under no-choice conditions the difference was less marked and was not significant (Wilcoxon signed rank test, p = 0.126).

The mice were then offered decaying earthworms in choice tests with live or fresh dead worms (Figure 22).
Both live and fresh dead worms were significantly preferred over decaying ones (Wilcoxon signed rank tests, $p = 0.004$ and $0.003$ respectively).

**Palatability of live and dead slugs to wood mice**

Wood mice were offered live or fresh dead slugs (*Deroceras reticulatum*) under choice test conditions and the number of prey of each type taken was recorded. 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 mean number of each type of prey taken is shown in Figure 23.

**Figure 23. Mean consumption of live and dead slugs in one-day choice tests.** Bars represent standard errors.

Both ‘freeze killed’ and ‘water killed’ slugs were significantly preferred over live ones (Wilcoxon signed rank tests, $p = 0.008$ and $0.003$ respectively)

The test was repeated using decaying slugs (Figure 24).
Figure 24. Mean consumption of live and decaying slugs in one-day choice tests. Bars represent standard errors.

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 more palatable than the water killed slugs which would appear to contradict the possibility that the difference seen for fresh dead slugs may be caused by the effective washing of slugs killed using water. However, the decaying freezer killed slugs were washed before presentation to remove adhered soil which may also remove surface secretions.

To confirm the apparent low palatability of live slugs the mice were given a no-choice test with live slugs only. The mean number of slugs taken was 2.08, slightly higher than observed in the choice tests but still far lower than the consumption of dead slugs in the above trials.

**Palatability of live and dead earthworms to blackbirds**

Blackbirds (*Turdus merula*) were offered earthworms in three conditions (live, fresh dead or decaying) in a three way choice test. This was conducted over three days with the type of worm in each tray changed each so that each type was offered in each tray (Table 10). The pattern used for each bird was selected at random.

<table>
<thead>
<tr>
<th>Tray 1</th>
<th>Tray 2</th>
<th>Tray 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>Decay</td>
<td>Live</td>
</tr>
<tr>
<td>Day 2</td>
<td>Dead</td>
<td>Decay</td>
</tr>
<tr>
<td>Day 3</td>
<td>Live</td>
<td>Dead</td>
</tr>
</tbody>
</table>

There was an apparent strong preference for live worms over dead worms with few dead worms and no decaying worms taken. To determine whether blackbirds would consume dead worms in the absence of live ones they were then offered fresh dead and decaying under no choice test conditions and the consumption compared to the consumption of live worms when presented under the same conditions. The mean consumption of each type of worm is shown in Figure 25.
Figure 25. Mean number of live, fresh dead and decaying earthworms consumed per day during a three way choice test (conducted over three days) and one day no-choice tests. Bars represent standard errors.

In the choice test the consumption of live worms was similar to that observed in earlier trials. The consumption of dead worms was low and there was a significant effect of worm condition on total consumption over three days consumption (Friedman test, Chi-square = 15.2, p = 0.001) and a significant difference between the consumption of live and dead worms (Wilcoxon signed rank test, p = 0.012). In the no-choice test there was no significant effect of worm condition on consumption when fresh dead and decaying consumption were compared to live consumption measured under the same conditions (Friedman test, Chi-square = 3.9, p = 0.140). These results suggest that all types of worms are palatable to blackbirds but that live ones may be preferred if there is a choice even though they are harder to detect as they are covered in soil.

Effects of the appearance of live and dead worms

In the above trials, the appearance of the three types of worm was different. Live worms would move to the edges of the trays and cover themselves with soil, which made them harder to detect. To test the effects of appearance on the results of the choice test, a further test was conducted where birds were offered live worms as before along with dead worms covered in soil to mimic live ones. Before trays were presented to the birds it was ensured that all worms in each tray appeared similar by ensuring that both live and dead worms were covered in soil and were positioned around the edge of the tray. The mean number of each type of worm taken is shown in Figure 26.

Figure 26. Mean consumption of live and dead earthworms under choice conditions manipulated so that the appearance of both types was similar. Bars represent standard errors.

Under these conditions there was a higher level of consumption of dead worms than would be expected from the results of the initial choice test although live worms still appear to be preferred. However, this difference was not significant (Wilcoxon signed rank test, p = 0.205) suggesting that altering the appearance did encourage feeding on dead worms under these conditions over that seen in the earlier test. While appearance does seem to be a factor in the choice it would appear that other factors such as movement may still make live worms slightly more attractive.

Palatability of live and dead slugs to blackbirds

Wild caught adult blackbirds were offered under choice test conditions for two days with the position of each type of slug switched on the second day. Unlike the worm studies where the quantity of material was in excess of normal daily consumption, this was not possible with the slugs due to their small size and the large numbers that would be required to achieve this. Therefore each bird was
presented with 10 slugs of each type and the number of prey of each type taken over two days was recorded. Dead slugs were killed by freezing.

It appeared that more live slugs were taken than dead ones but this was complicated by the fact that some of the live slugs (15%) had escaped from control trays by the end of each trial. The number of slugs taken during each day and over the two days are shown in Table 11.

Table 11. Number of live and dead slugs taken during choice tests.

<table>
<thead>
<tr>
<th>Pen</th>
<th>Dead</th>
<th>Live</th>
<th>Dead</th>
<th>Live</th>
<th>Dead</th>
<th>Live</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>8</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>9</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>9</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Mean</td>
<td>3.4</td>
<td>5.8</td>
<td>3.4</td>
<td>6.1</td>
<td>6.8</td>
<td>11.9</td>
</tr>
</tbody>
</table>

These results indicated a higher consumption of live slugs than dead ones and the results was significant (Wilcoxon signed rank tests, p= 0.042) although it is possible that this difference may be slightly smaller as some live slugs may have escaped from the test trays (as seen in controls). However, the results were variable and there was a strong correlation between the numbers of live and dead slugs consumed (Pearson correlation coefficient = 0.804, p = 0.016 for tray counts) with those birds that fed heavily on live slugs also consuming several dead ones suggesting that the dead prey were not unpalatable. As for worms, it is possible that movement of the prey initiated attacks by the blackbirds. Video recordings of the trials were observed and it was noted that several of the slugs that appeared to be escaping from the test trays made themselves more obvious by moving into the open and were quickly picked off by the birds (five slugs on Day 1 and seven slugs on Day 2). It is therefore possible that all escaping slugs would have been detected and eaten and that this may go some way to explaining the differences in consumption.

Blackbirds were then offered fresh dead and decaying slugs in a one-day choice test using a grid of four trays as used in the final earthworm experiment. All slugs were killed by the freezing method and were not washed before presentation to the birds. Two trays contained five fresh dead slugs with the remaining two containing five decaying slugs. Mean numbers of dead and decaying slugs taken were 4.8 and 5.0 respectively. As in the previous experiment there was a large individual variation between birds in the number of slugs taken with half of the birds refusing all those presented while the remainder consumed almost all of the slugs of both types. Comparison with the previous experiment shows that the three birds that ate most dead slugs in that experiment were also among the highest consumers here. Clearly there was no significant preference for one type of slug over the other.

Conclusions

The data obtained for wood mice seem to 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 this would do little to reduce risk in the wild. Despite the apparent attractiveness of dead invertebrates to wood mice, they seemed to find decaying worms and live slugs less palatable. Given the rapid breakdown of the decaying worms and the strong odour they produce, this is perhaps not surprising despite the apparent palatability of decaying slugs. The reason for the apparent unpalatability 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.

The data obtained for blackbirds suggest that earthworms in any condition may be taken but that the birds appear to have a preference for live worms when they are present. This appeared to be the case even though the live worms were harder to see and possibly find due to their behaviour of covering themselves in soil and moving to the edge of the tray. While appearance alone appeared to be important given the results of the test where dead worms were presented in a similar fashion, it would appear to be more than this. One possibility is that the birds were responding to the movement of the worms and this made them more attractive than dead ones under choice conditions. However, these birds were in good condition and were not forced by risk of starvation to consume the dead worms so it would appear that all types may present a risk. The response to slugs by blackbirds appeared to be very variable between individuals with some birds taking very few or no prey. Again live prey appeared preferable to dead in choice tests and there was some indication that again movement of the prey may be important. As for worms, those birds that did consume slugs appeared to take all types again suggesting that there was no strong palatability issue and that all prey types may be taken.
In summary it is concluded that dead and dying invertebrate prey should be considered a risk to birds and small mammals unless there is evidence (for example from a specialised avoidance study) that this is affected by the palatability of the pesticide formulation itself.)
**Scientific Objective 8**

“Determine whether birds and mammals remove adhering granules or pellets before eating earthworms or molluscs.”

**Background**

After application of solid formulations, there is a possibility that some of the particles may become adhered to the surface of invertebrates feeding on the surface. Should these then be eaten by predatory birds or mammals, then the risk to the non-target animals would be significantly increased if they were to ingest the formulation along with any residues in the tissues of the prey. This presents problems in risk assessment, as simple rules of thumb to estimate residues in invertebrates could significantly underestimate the actual pesticide loading. However, it is not known to what extent predators may remove adhering particles during manipulation of food before ingestion. This project aimed to provide more information about the way birds and mammals handle invertebrate food and the potential for removal of the formulation before ingestion.

**Adherence of granular material to earthworms**

During the course of work on residues in earthworms after granular applications (Objective 6) it was noted that 31% of the worms that were exposed to Temik 10G (aldicarb) were found dead or dying on the surface of the soil. Of these, 77% had a number of Temik granules adhered to them. Mean number of adhered granules was 4.1 and the maximum on any individual worm was 20. The appearance of the worms suggested that the Temik granules were firmly adhered as they appeared to be surrounded by, and sometimes covered with mucous secretions. This may have made removal by predators before ingestion less likely than for other material such as soil. This may also have been the case for the other formulations tested (Spannit granules and Mocap) but the size and colour of these granules made detection difficult.

The results of this study combined with the size and appearance of Temik granules suggested that blank Temik granules would be the most appropriate test material for this study as they were the most likely to be detected if they were removed from the worms during feeding. However, given the altered appearance of the worms that had been exposed to the real formulation, it was necessary to test whether blank granules would adhere in the same way as treated ones.

A study was conducted to compare the relative strength of adherence of treated and blank granules and to establish the best blank material for use in test with animals. Earthworms (*L. terrestris*) were allowed to come into contact with either Temik 10G, blank Temik (both clay granule based), or silica particles of a similar size (500-800 microns) for 30 minutes. The number of particles adhered to each worm was counted and worms exposed to each type of granular material were divided into two groups. One group was returned to a clean petri-dish for 30 minutes to determine how many of the granules would fall off without intervention (e.g. during the course of a trial before they were handled by predators). The remaining worms were shaken briefly as might occur when collected by a foraging bird before ingestion. Following these manipulations the number of granules remaining on each worm was counted.

These tests indicated that treated Temik granules adhered more strongly than blank ones and that silica particles of a similar size also adhered more strongly than blank Temik (Table 12).

**Table 12. Mean number of granules adhered per worm and proportion remaining after each type of removal method.** Each combination of removal method and granule type was replicated eight times.

<table>
<thead>
<tr>
<th>Removal method</th>
<th>Temik 10G</th>
<th>Blank Temik</th>
<th>Silica</th>
<th>Temik 10G</th>
<th>Blank Temik</th>
<th>Silica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left for 30 minutes.</td>
<td>7.5</td>
<td>14.9</td>
<td>17.9</td>
<td>44.2</td>
<td>30.6</td>
<td>42.7</td>
</tr>
<tr>
<td>Shaken</td>
<td>11.3</td>
<td>14.0</td>
<td>19.4</td>
<td>66.1</td>
<td>38.2</td>
<td>58.0</td>
</tr>
<tr>
<td>Total</td>
<td>9.4</td>
<td>14.4</td>
<td>18.6</td>
<td>55.14</td>
<td>34.4</td>
<td>50.3</td>
</tr>
</tbody>
</table>

The proportion of blank Temik granules that remained after manipulation was significantly lower than the proportion of treated granules. Also it appeared that fewer granules remained if worms were left for 30 minutes than were removed by shaking the worms, but this difference was not significant. Silica granules adhered more strongly than clay ones under the same conditions. It is not clear why fewer treated granules adhere to earthworms but it is possible that as they become affected by the chemical they are less active and therefore come into contact with fewer particles. Further work would be necessary to establish whether this is the case.

The Temik trials highlighted two problems with the approach of presenting worms covered with blank pellets during trials with predators, which was the original intent in this project.

1. **Loss of material with time.** Most particles will fall off the worms when they are left on the presentation tray meaning that the effectiveness of removal would be difficult to establish by counting remaining particles as it will not be known how many if any...
were adhered to the worms. Control samples may partially get around this but these would not necessarily reflect the situation at the time of feeding.

2. **Differential adherence during handling.** During simulated predation and agitation of the worms it was found that actual formulation adhered more strongly to the worms than blank material although silica particles did adhere more strongly in a way similar to the treated granules. Silica could be used as a surrogate material but it is not known how animals would react to them compared to granules and this material would be less easy to detect at the end of any trial.

The trial was repeated using Mocap 10G (Ethoprophos) and Spannit granules (Chlorpyrifos) to test whether these formulations would behave in a similar fashion (Tables 13 and 14).

### Table 13. Mean number of granules adhered and proportion remaining after each type of removal method during trials with Mocap. Each combination of removal method and granule type was replicated four times.

<table>
<thead>
<tr>
<th>Removal method</th>
<th>Number adhered (before removal)</th>
<th>Remaining (% of adhered)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mocap 10G</td>
<td>Blank Mocap</td>
</tr>
<tr>
<td>Left for 30 minutes</td>
<td>10.5</td>
<td>13.8</td>
</tr>
<tr>
<td>Shaken</td>
<td>9.5</td>
<td>14.5</td>
</tr>
<tr>
<td>Total</td>
<td>10.00</td>
<td>14.1</td>
</tr>
</tbody>
</table>

There were no large differences between the % adhesion of the three types of material during the Mocap trial, although again initial adhesion of treated material appeared lower than that of untreated particles.

### Table 14. Mean number of granules adhered and proportion remaining after each type of removal method after trials with Spannit granules. Each combination of removal method and granule type was replicated four times.

<table>
<thead>
<tr>
<th>Removal method</th>
<th>Number adhered (before removal)</th>
<th>Remaining (% of adhered)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spannit granules</td>
<td>Fullers earth</td>
</tr>
<tr>
<td>Left for 30 minutes</td>
<td>20.0</td>
<td>19.8</td>
</tr>
<tr>
<td>Shaken</td>
<td>16.3</td>
<td>17.8</td>
</tr>
<tr>
<td>Total</td>
<td>18.1</td>
<td>18.8</td>
</tr>
</tbody>
</table>

For Spannit no blank material was available so Fullers earth particles of a similar size were used as a surrogate. Again differences between treated granules and silica were not large but fullers earth appeared to adhere more firmly than either.

The trend of initial adherence levels compared to silica (approximately 50% for Temik, 75% for Mocap and 100% for Spannit) may provide more evidence that this is affected by the worms becoming immobile after exposure. The compounds with lower toxicity adhere in numbers closer to the silica values in each case. However, other explanations for these differences may be the shape and surface coating of the three types of granule. Temik granules tended to be rounded and have a dark, slightly dusty surface coating which may reduce the likelihood of adherence. Mocap granules are smaller with a more irregular shape which may promote adherence. Spannit granules are also irregular in shape with more flat surfaces which may also increase initial adherence.

If the strength of adherence of treated material is affected by the reaction of the epidermis and the secretion of mucous then it might also be expected that there would also be a trend with toxicity opposite to the one observed for initial adherence. This also appears to be the case with an overall value of 55% for Temik, 47% for Mocap and 41% for Spannit.

**Response of blackbirds to ‘clean’ and ‘adhered silica’ earthworms**

A study was conducted with blackbirds (*Turdus merula*) to test whether any difference in behaviour could be detected when feeding on clean earthworms or earthworms with adhering material. The behaviour of the birds when feeding would also provide information on the feasibility of measuring the removal of adhered material. Eight birds were each given a one day test with clean worms (fresh *L. terrestris* placed on a metal tray) or adhered worms (earthworms that were coated in silica and placed on a tray containing 5g of silica). Each bird was given both types on two consecutive days. The type offered on the first day was selected randomly. Trials were video recorded to try and detect any obvious behavioural differences between the treatment of each type. Numbers of worms taken and consumption were also recorded.

There were no obvious effects of either test day or worm type on the consumption of worms or their behaviour towards them. It was not possible to obtain complete records of feeding activity due to the behaviour of the birds which often carried the worms away for the feeding area or were oriented in such a way as pecks etc. could not be seen. However, from the data that was obtained and other observations there were no obvious differences with birds appearing to treat both types similarly without prolonged feeding or...
attempted cleaning of the coated worms. Mean values for consumption (numbers and weight of worms consumed) and measured behaviour (delay to the onset of feeding, pecks and time taken per worm) are shown in Table 15.

Table 15. Means of consumption and behavioural measurements for clean and silica coated worms.

<table>
<thead>
<tr>
<th>Type of worm</th>
<th>Number of worms taken</th>
<th>Consumption (g)</th>
<th>Delay to first worm (s)</th>
<th>Pecks per worm</th>
<th>Time per worm (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean</td>
<td>5.3</td>
<td>26.8</td>
<td>1381</td>
<td>41</td>
<td>108</td>
</tr>
<tr>
<td>Adhered silica</td>
<td>5.6</td>
<td>26.2</td>
<td>482</td>
<td>43</td>
<td>109</td>
</tr>
</tbody>
</table>

There were no significant differences between the values obtained for each test day or type of worm (Wilcoxon signed rank test) except for delay to feeding which was higher for clean worms. However, this appeared to be mainly due to the behaviour of one bird which took a long time to start feeding on that day.

Under the conditions of this study blackbirds did not appear to show any preference for clean or dead prey and no behaviour was observed that suggested that they were handling each type differently. These is consistent with the results of the earlier study (Objective 7) where birds were given a choice between live and dead earthworms and preferred the live ones even though they were covered in soil. Measurements of the amount of adhered soil showed that for live worms, soil constituted around 10% of the worms as collected while, for dead worms this was only around 2.5%. Given these data it does not appear that blackbirds are affected greatly by adhering material even in amounts that greatly exceed the amounts of granules that may be adhered.

Conclusions

These results suggest that some treated granules may adhere more strongly to earthworms than similar particles that do not contain pesticide. However we cannot separate out whether the treated pellets stick more securely because the worms are sick or whether it is because of the reaction with the epidermis. If the latter is the case, then it is possible that treated granules may adhere more firmly to the earthworm than other surrounding soil and be harder to remove before ingestion. These results also show that the use of blank clay granules may not be suitable for tests with mammals and birds to determine whether adhered granules are removed. While silica particles appear to better represent the treated granules, any interpretation of results with this material to predict removal of real granules would be speculative as animals may not respond to the presence of such a material which is a normal constituent of the soil on which these prey are found. Even when material is adhered to the worms the trials also showed that much of it is removed by worms as they move around. Again, this may not occur to such an extent with highly toxic formulations as the worms are likely to become immobile after exposure.

The study with blackbirds and the data from the earlier study (Objective 7) suggest that this species at least did not demonstrate any obvious preference for clean prey or any obvious attempts to remove adhering material. The behaviour of the birds also showed that it would not be practical to collect and quantify the amount of material removed. Given these results it was not possible to conduct studies that would provide meaningful adjustment values that could be used in risk assessment to predict the risk posed by adhering granules. However, given the strength of adherence observed for aldicarb and the lack of evidence that birds at least attempt to clean the prey, it would be reasonable to assume that all of the adhered material would be ingested by the bird unless the presence of the active material itself elicited removal of granules or avoidance of the prey. This would require assessment of the likely number of adhered granules and the behaviour of animals to worms exposed to the formulation. Both of these measurements fall outside the original scope of this study and would need to be addressed in specific studies for each formulation if this information was required.
SUMMARY OF FINDINGS FOR OBJECTIVES 6-8

1. Pesticide residues in earthworms can be affected by factors such as soil OM content, rainfall, time of exposure and properties of the active substance and may vary greatly from the level predicted by current methods. The best predictor seems to be the logKow of the substance. There is therefore a need to consider the properties of the active substance along with the conditions under which exposure may take place and to adapt predictions accordingly.

2. After a granular application, measurement of residues in unwashed worms with adhering soil and washed worms indicated that washing reduced residues significantly. It is recommended that the potential for adhered soil to increase the pesticide loading of earthworms collected by predators be considered when estimating risk.

3. Palatability tests have shown that wood mice will readily take dead invertebrate prey even when live prey and normal food are available. At this stage it must be recommended that dead and dying prey should be considered to be palatable to wood mice unless there is strong evidence (for example from a specialised avoidance study) that this is affected by the palatability of the pesticide itself.

4. Palatability tests have shown that when dead or decaying earthworms only are presented that they are readily taken by blackbirds although if there is a choice live ones are preferred, even though they are apparently more difficult to detect. In tests with slugs there was greater variation between birds but those birds that did take slugs appeared to readily take live, dead and decaying prey. It must therefore be recommended that dead and dying prey also be considered as a risk to blackbirds and possibly other worm eating birds unless other factors such as palatability of the formulation affect this.

5. Some treated pesticide granules appear to adhere more firmly to earthworms than untreated granules of the same material. This may have implications for the likelihood that birds and mammals will be able to remove them from exposed prey before ingestion. Where the potential removal of adhered material is considered important when assessing risk it would be best to use actual formulation and monitor the behaviour of the species at risk towards the prey.

6. Captive blackbirds did not appear to be affected by adhered material such as soil or silica when feeding on earthworms and made no overt attempt to remove it. This suggests, at least for this species, that any adhered material is likely to be ingested along with the prey.