Bull. Environ. Contam. Toxicol. (1998) 61:448-454

Bulletin of Environmental Contamination and Toxicology

Presentation Method and the Avoidance of Fonofos-Treated Seed by Captive Birds

S. L. Fryday, S. A. Chandler-Morris, A. D. M. Hart

Central Science Laboratory, Ministry of Agriculture Fisheries and Food, Sand Hutton, York YO41 1 LZ, UK

Received: 1 February 1998/Accepted: 31 August 1998

Tests of avoidance are sometimes required in risk assessment to establish whether reduced consumption of pesticide-treated food will protect birds that may otherwise be at risk of poisoning. At present there is no internationally accepted guideline for avoidance testing and designs are often agreed on a case by case basis between registrants and the regulators. These tests are therefore conducted in several different ways ranging from simple cage tests with food in bowls (INRA 1990) to tests in aviaries with food spread on the floor (BBA 1993). Species used range from standard laboratory quail species to wild birds from the species considered to be at risk. There is therefore a need for new international guidelines on how avoidance tests should be conducted (OECD 1996) so that the results of tests in different countries are more comparable thus reducing the need for repeated testing.

One important aspect when considering the development and use of tests designed to predict dietary exposure in the wild is the presentation of the treated food. While it may be desirable to present the test diet in the same way that it would be encountered in the wild, this is not always possible and the food is presented in some sort of container. Whatever method is used, the presentation of the food must be realistic enough that no mechanisms other than those which would occur in the wild can lead to avoidance. For example previous work at this laboratory has shown that seed treated with the organophosphorus insecticide fonofos at normal application rate (1080 mg/kg) is almost completely avoided by birds in cage tests when it is presented in bowls, but significant quantities are eaten when the same seed is presented on trays in aviaries (*Fryday et al., in prep*). A possible explanation for this difference may be mechanisms such as smell, or nasal or ocular exposure or irritation if vapour from the test substance builds up in the air inside the food container. The olfactory abilities of birds have been described for a wide range of species (Tucker 1965) including pigeons (Henton, Smith and Tucker 1966; Michelson 1959; Snyder and Peterson 1979). The potential for odour alone to bring about an aversive response in birds has also

Correspondence to: S.L. Fryday

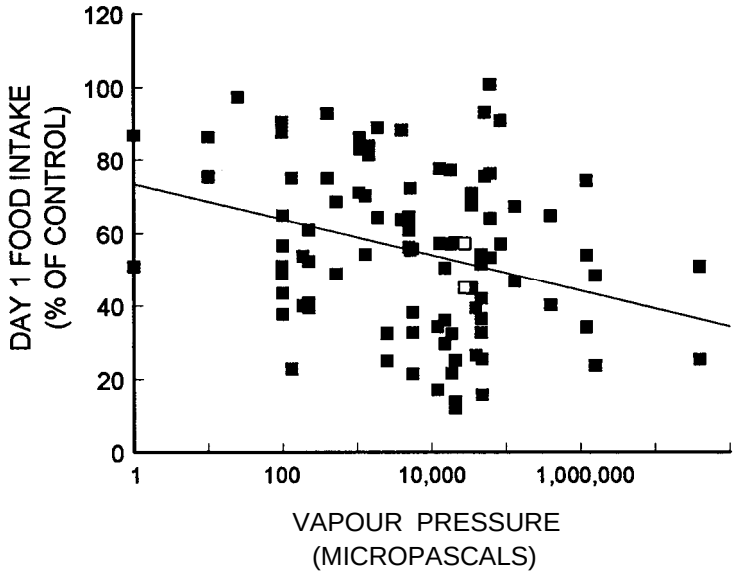

Figure 1. Relationship between the vapour pressure of pesticides and the consumption of treated food as a percentage of control birds in the first day of 5-day dietary toxicity tests with Japanese quail. Consumption data from Hill and Camardese (1986) vapour pressures from The Pesticide Manual (BCPC 1983). Data for treatment levels causing mortality during the first day are excluded. The correlation was significant ($r = -0.31$, $n = 100$, $p < 0.01$). Open markers indicate values for fonofos (vapour pressure = 28 millipascals at 25 °C).

been demonstrated (Marples and Roper 1997). This suggests that at least under certain conditions smell could be a factor in the avoidance response to compounds such as fonofos. Indeed, there is a negative correlation between the vapour pressure of a substance and the degree of avoidance seen in dietary toxicity tests (Figure 1). Thus presentation of treated seed in bowls as recommended by some test methods (e.g. INRA) may overestimate the likely level of avoidance and greatly underestimate risk. This study was conducted to directly compare the effects of the two presentation methods (bowl and tray) on the avoidance of fonofos-treated seed. The effects of cage and aviary testing were also compared.

MATERIALS AND METHODS

Twenty four wild-caught feral pigeons were used in this study. All birds had been maintained in captivity for at least three months before the start of the study. Birds were moved to indoor test cages (0.9m x 0.45m x 0.35m high) or outdoor test aviaries (2.7m x 1.8m x 1.9m high) at least 10 days before testing. The indoor test room was set to a 16/8hr light/dark cycle and a temperature of 16°C to approximately mimic the conditions prevailing in outdoor aviaries (early June daylength, mean maximum/minimum temperatures 22/9°C). Food, water and grit were available *ad libitum* during this period. Birds were accustomed to feeding on untreated wheat alone before the pre-trial period.

Dyed blank seed was prepared by adding 2.5mL of blank formulation (rhodamine dye solution supplied by Zeneca) to 1kg samples of re-cleaned wheat ('Axona') in a small experimental seed dresser. Treated seed was prepared at the nominal application rate (1080mg/kg) by adding 2.5mL of fonofos seed treatment (4339 a.i./L) to 1 kg samples of seed in the same way. All seed was air dried for at least 24hr before use. Concentrations in the test diet were confirmed by gas chromatography after homogenisation of samples in acetone.

In the first phase of the study 16 feral pigeons were used, eight in indoor cages and eight in outdoor aviaries (Table 1). Half of each group were offered food in circular glass bowls (18cm wide x 7cm high) and half in metal trays (45cm x 45cm x 1cm high) of a similar surface area to those used in previous studies (*Fryday et al., in prep.*). Due to the level of variability observed in the results from the first phase of the experiment, especially the results for consumption from bowls outdoors, the outdoor part of the study was repeated with a further eight birds, four with bowls and four with trays. The bowls and trays used indoors and outdoors were identical.

Table 1. Number of birds tested under each combination of housing type/food container type in each phase of the experiment.

		BOWL	TRAY
PHASE 1	INDOOR CAGES	4	4
	OUTDOOR AVIARIES	4	4
PHASE 2	OUTDOOR AVIARIES	4	4

After acclimatisation to test cages/aviaries and wheat, birds were offered dyed blank wheat only for two days followed by treated wheat only for two days and consumption monitored daily. Each bird was offered 70g of the appropriate type of wheat on each test day which formed a layer approximately 1 cm deep in bowls and a patchy monolayer on trays.

RESULTS AND DISCUSSION

Mean consumption by each group of birds in each phase of the experiment is shown in Table 2. Mean fonofos residue at the start of the first phase of the experiment was 700mg/kg, and at the start of the second phase (outdoor aviaries only) was 739mg/kg. There was also no significant difference in consumption between the two outdoor stages. These data were therefore pooled. The mean consumption by each test group over the two test days is shown in Figure 2. There was a marked effect of presentation method indoors where very little seed was taken from bowls compared to trays. Outdoors this difference was also evident, but was less marked. The level of consumption from trays was similar both indoors and outdoors.

Table 2. Mean consumption (g) and range (in brackets) for each group of birds during each stage of the experiment. Mean daily consumption on each test day is also shown.

	INDOOR CAGES		OUTDOOR AVIARIES	
STAGE	BOWL	TRAY	BOWL	TRAY
Pre-treatment (mean)	27.7 (23.7 - 33.8)	23.5 (16.5 - 31.0)	23.5 (15.9 - 30.6)	27.1 (12.5 - 37.6)
Test Day 1	0.12 (0.1 - 0.2)	2.3 (0.85 - 4.0)	0.9 (0.0 - 3.0)	1.3 (0.5 - 2.5)
Test Day 2	0.4 (0.0 - 1.8)	1.4 (0.0 - 2.9)	1.1 (0.0 - 4.5)	2.5 (0.0 - 5.6)
Test Mean	0.3 (0.0 - 1.0)	1.8 (0.7 - 3.4)	1.0 (0.0 - 2.8)	1.9 (0.0 - 3.3)

Test day consumption was analysed using a repeated measure analysis of variance with test day as the within subjects factor and presentation method and indoors or outdoors testing as between subjects factors. Mean pre-treatment consumption was included as a covariate. There

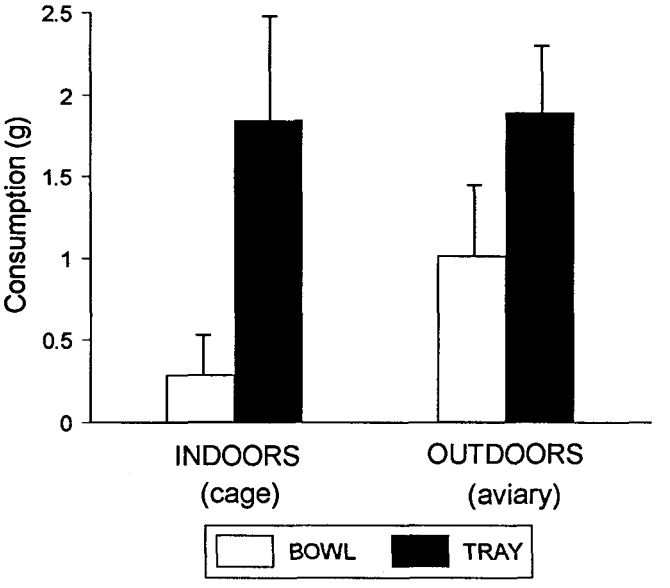

Figure 2. The effects of presentation method on the avoidance of fonofos-treated seed by pigeons. Mean consumption (and standard errors) for each test group over the two test days.

was a significant effect of presentation method ($F = 7.04$, $p=0.016$), but no significant effect of indoor or outdoor testing ($F = 0.74$, $p = 0.402$). There was also no significant effect of test day on consumption ($F = 0.22$, $p = 0.643$).

There was clearly an effect of presentation method on the responses of birds to the treated seed. In view of the apparent relationship between avoidance and vapour pressure (Figure 1), it is possible that the effect of presentation method is due to higher levels of vapour in bowls. This could also explain why the effect tended to be less marked in outdoor aviaries, as greater air movement outdoors would make the vapour concentration in the bowls more similar to that over the trays. Further experiments would be required to confirm the role of vapour in these results.

The results show that cage tests with food in bowls may significantly overestimate the extent to which fonofos-treated seed is avoided by birds in the wild. If this is caused by the effects of vapour, as we postulate, then still larger differences might be expected for pesticides with higher vapour pressures than fonofos. Whatever the mechanism involved, it is

clearly advisable that small food containers should not be used in avoidance tests and that treated seed should be spread out on trays or on the floor of the test enclosure. When this was done, there was no difference between results obtained indoors and outdoors. The choice of location will therefore depend on the balance between the needs for realism and standardisation in testing.

These results also have implications for other tests, such as dietary and reproduction tests, where treated diet is offered to birds. These tests are usually done indoors and involve larger numbers of birds in standard cages, so generally it may not be practical to present food in trays. However, the use of trays should be considered for highly volatile compounds especially if they are known to have a strong odour or irritant properties. In any case, the possibility of vapour influencing consumption should be borne in mind when interpreting results.

Acknowledgments. We are very grateful to the UK Pesticides Safety Directorate, Ministry of Agriculture Fisheries and Food, for funding this study, to Sarah Quinn for practical assistance and to Zeneca for supplying blank formulation.

REFERENCES

BBA (1993) Guidelines for testing plant protection products in the authorization procedure. Part IV, 25-1, Testing of baits, granules and treated seeds for hazards to birds - acceptance tests (2nd edition). Published in German by the Department of Plant Protection Products and Application Techniques of the Federal Biological Research Centre for Agriculture and Forestry, Germany.

BCPC (1983) The pesticide manual - 7th edition, British Crop Protection Council, London

Henton WW, Smith JC, Tucker D (1966) Odor discrimination in pigeons. Science 153: 1138-1139

Hill EF, Camardese MB (1986) Lethal dietary toxicities of environmental contaminants and pesticides to *Coturnix.* United States Department of the Interior, Fish and Wildlife Service, Technical Report 2, Washington DC.

INRA (1990) Method for acceptance of feed or seeds treated with a repellent, by captive birds. Available in French from G Grolleau, National Institute for Agronomic Research, Versailles, France.

Marples NM, Roper TJ (1997) Response of domestic chicks to methyl anthranilate odour. Anim. Behav. 53(6):1263-1270

Michelson WJ (1959) Procedure for studying olfactory discrimination in the pigeon. Science 130:630-631

OECD (1996) Report of the SETAC/OECD Workshop on Avian Toxicity Testing, Report No. OECD/GD(96)166, OECD Environmental Health and Safety Publications, Series on Testing and Assessment No.5, OECD, Paris

Snyder GK, Peterson TT (1979) Olfactory sensitivity in the black-billed magpie and the pigeon. Comp Biochem Physiol 62:921-925

Tucker D (1965) Electrophysiological evidence for olfactory function in birds. Nature 207:34-36