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INCIDENCE OF LETHAL BIRD POISONING REDUCED BY REGURGITATION OF PESTICIDE-TREATED FOOD

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Abstract—Regurgitation by birds feeding on pesticide-treated seed was quantified in two experiments, and its role in reducing the risk of acute poisoning from an organophosphorus pesticide was assessed. Captive feral pigeons (*Columba livia*) were offered fonofos-treated seed ad libitum on a test day after a 6-d period in which one group was given free access to untreated seed and another group was given no food in one experiment or 15% of normal intake in the other. Avoidance of treated seed reduced intake substantially, preventing the ingestion of lethal doses for all birds fed ad libitum and some but not all of the food-restricted birds. Some of the latter regurgitated most of the seed taken in (>60%), reducing by ~50% the dose of pesticide assimilated and lowering the dose from above to below lethal levels. Regurgitation was the most likely explanation for the survival of at least 12 and 50% of the food-restricted birds in these experiments. Regurgitation reduced but did not prevent mortality, because some birds died without regurgitating and others despite doing so. This study provides the first clear evidence that regurgitation can directly reduce mortality of birds exposed to pesticides in their food. Implications for the risk of poisoning wild birds and avian toxicity testing are discussed.

Keywords—Birds Pesticides Regurgitation Food avoidance Toxicity testing

INTRODUCTION

The risk of birds being poisoned by pesticides is determined by the toxicity of the active ingredient and the likelihood and degree of exposure [1]. Risk is increased when birds feed on items treated with pesticides, such as seeds treated with insecticidal seed treatments that have caused bird mortality [2,3]. Exposure may be reduced if birds find treated food unpalatable and reduce consumption or if they vomit treated food, reducing the final dose absorbed. Although avoidance of pesticide-treated food and its potential to reduce exposure have been widely demonstrated in many laboratory studies [4–6], little is known about how regurgitation affects the risk of pesticide poisoning. Birds can vomit after consuming food containing distasteful or harmful substances, both naturally occurring [7–9] and synthetic, including pesticides [10–12]. Regurgitation can substantially reduce the dose of pesticide absorbed when chemicals are placed directly into the gut by gavage, as in standard acute oral toxicity tests [13]. However, it is not known whether regurgitation contributes significantly to a reduction in exposure in birds feeding voluntarily on pesticide-treated food.

In previous experiments in our laboratory in which wheat seed treated with the organophosphorus (OP) insecticide fonofos was offered to feral pigeons (*Columba livia*) in outdoor aviaries, it was observed that some birds vomited treated seeds. Because those experiments were not primarily designed to detect and measure regurgitated seed, the actual frequency and intensity of regurgitation could not be accurately determined. However, these observations suggested that regurgitation might be a factor affecting exposure; therefore, follow-up experiments were planned to quantify the extent of regurgitation and its effects on the dose absorbed. This article presents results on the importance of regurgitation in reducing bird mortality from two experiments with captive birds and assesses the implications for toxicity testing and risk assessment.

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MATERIAL AND METHODS

Subjects, housing, and schedule of experiments

Wild-caught adult feral pigeons of unknown sex were held in outdoor aviaries for several weeks before being moved to indoor rooms, where the experiments were performed. Sixteen birds in experiment 1 and another 16 in experiment 2 were housed individually in cages (45 cm long × 90 cm wide × 35 cm high) in a room under a 8:16-h light:dark cycle. Mean daily room temperature ranged from 13 to 15°C in experiment 1 and from 14 to 17°C in experiment 2. Water and grit were available ad libitum throughout the experiments. Birds were fed ad libitum with a mixture of wheat, maize, chicken layer pellets, and maple peas (maintenance diet) during the period of acclimatization to room conditions and feeding regimen. Maintenance diet was available for 7 to 8 h/d in experiment 1. In experiment 2, food was initially available for the same period but later reduced to 2 h/d for the rest of the experiment. Main procedures began once both food consumption and body weight had stabilized. The schedule of the experiments simulated conditions of daylight and daily feeding time experienced by wild pigeons in England during the winter months, when acute bird poisoning has been caused by OP-treated seeds [3]. Daylight (8 h) was slightly longer than outdoors in winter to allow for at least 6 h of undisturbed food availability and enough time for the routine care of animals, body weight measurements, and preparation of equipment in the room. The restricted feeding time of 2 h used in experiment 2 is realistic for the field situation, as suggested by estimations based on observations of wild woodpigeons feeding on cereal seed on newly sown fields in England in winter [14].

The experiments were completed in five consecutive phases

Table 1. Schedule of experiments 1 and 2 with food type and regimen for each phase and experimental group

	Experiment 1		Experiment 2	
	Nondeprivation group	Deprivation group	Nonrestriction group	Restriction group
Phase 1 (5 d)	Dyed blank 0900–1500		Dyed blank 0900–1100	
Phase 2	Maintenance diet 4 d, 0900–1500		Maintenance diet 2 d, 0900–1100	
Phase 3 (2 d)	Dyed blank 0900–1500		Dyed blank 0900–1100	
Phase 4 (6 d)	Dyed blank 0900–1100	No food	Dyed blank 0900–1100	15% normal intake 0900–1100
Phase 5 (test day)	Treated seed 0900–1500		Treated seed 0900–1100, 1130–1530	

as shown in Table 1. In the first phase, each bird was given 35 to 50 g/d of untreated dyed wheat seeds for 6 h in experiment 1 or 2 h in experiment 2. This was repeated for 5 d, and seed consumption was measured daily. At the end of phase 1, birds were assigned to two experimental groups in each experiment. To minimize the influence of individual differences, the experimental groups were balanced for seed consumption (first parameter) and body weight change (second parameter) in phase 1. This was done by ranking the birds using these parameters, assigning at random one of the top two birds to one experimental group, and the second bird to the other group, and repeating this for each pair of birds in rank order until all birds were assigned. The experimental groups are termed nondeprivation and deprivation in experiment 1 and nonrestriction and restriction in experiment 2. In phase 2, all birds were given free access to maintenance diet. In phase 3, each bird was given 35 g/d (experiment 1) or 50 g/d (experiment 2) of untreated dyed seeds for 2 d. In phase 4, the same daily regimen as in phase 3 was kept for one group (nondeprivation and nonrestriction groups for experiments 1 and 2, respectively), and in the other group, birds had no food (experiment 1, deprivation group) or each bird was given 15% (2.4–3.9 g) of its mean consumption during phase 1 (experiment 2, restriction group). Phase 5 consisted of one single test day in which all birds were offered fonofos-treated seeds. In experiment 1, a 35-g sample per bird was presented for 6 h. In experiment 2, a 35-g sample per bird was presented for 2 h, remaining seeds were retrieved, and a fresh 35-g sample per bird was presented for an additional 4 h. Birds were checked for mortality and symptoms of poisoning every 2 h from ~1100 until ~1730, and then survivors were killed humanely and stored at or below −18°C until dissection. Birds were weighed to the nearest 1 g at 0800 to 0900 every 1 to 5 d and at 1500 to 1600 on several days during the five phases. After thawing overnight at room temperature, birds were dissected, their crop and gizzard contents were examined and determined, and found seed was stored at or below −18°C until analysis for pesticide residues.

In experiment 2, 12 birds (six from each group) were video recorded continuously on the test day during the periods in which seeds were available to determine feeding and regurgitation behaviors. Koster [15] separates the emetic response into vomiting (active expulsion of either solids or fluids) and retching (display of vomiting movements without the expulsion of matter). Retching movements were easy to detect in our recordings, but in some cases it was not possible to determine whether matter had been expelled. Therefore, we did not differentiate between vomiting and retching, and all emesis episodes were included to characterize the emetic response.

Measurement of seeds consumed and regurgitated

For feeding, seeds were scattered on filter paper on a tray (45 × 45 × 1 cm high). Food consumption, expressed as grams fresh weight, was calculated as the difference in fresh weight between the amount of food given and the amount left at the end of the feeding session, corrected for changes in moisture content based on two control samples placed in empty cages. A different procedure was used when seeds were moistened by water, saliva, or feces. On these occasions, all samples from that session were oven-dried for 24 h at 70°C. All samples from the test day in both experiments were oven-dried as above, with the exception of seeds vomited within the first 2 h of the test day in experiment 2, which were counted and frozen immediately at or below −18°C to await analysis of pesticide residues. The fresh weight of seeds from oven-dried samples was estimated using the average ratio of wet weight to dry weight for the two control samples in each session. Seeds given in this session were standardized in size by sieving, and the mean weight of a seed (0.0559 ± 0.00041 g, $n = 270$) was used to estimate the fresh weight of the known number of seeds vomited. The estimated average weights of the seeds used on the test day in experiment 1 and in the second session of experiment 2 were 0.0483 and 0.0506 g, respectively. Most vomited seeds were regurgitated in clumps that were easily detected. Vomited seeds scattered among noningested seeds were differentiated because they were swollen and adhered by saliva to the filter paper.

Preparation and residue analysis of seed samples

Seeds given in the first and second phases were treated with blank formulation (containing rhodamine dye) to accustom birds to feeding on colored seeds. Pesticide-treated seeds were coated 2 d before the test day by mixing batches of 1 kg of seed with 2.5 ml of fonofos seed treatment (433 g active ingredient [a.i.]/L) (Zeneca, Fernhurst, Surrey, UK) for 2 min in a dressing machine (Hege II, Hans-Ulrich Hege Maschinenbau, Waldenburg, Württemberg, Germany). Seeds were dried at room temperature for 24 h and bagged in 35-g samples. Four samples of the same batch given to birds were taken at the start of each feeding session on the test day and frozen at or below −18°C for residue analyses. To determine residue decay over the testing session, seeds from another two samples

Table 2. Daily consumption (g fresh weight) of untreated seed during pretest days (mean of 5 consecutive days before deprivation begun) and of fonofos-treated seed on the test day by feral pigeons in experiment 1[a]

	Non-deprivation group	Deprivation group	p
Untreated seed ingested	18.90 ± 0.94	17.15 ± 0.95	NS[b]
Treated seed taken in	2.21 ± 0.42	5.27 ± 0.76	<0.01[b]
Treated seed vomited	0.17 ± 0.13	3.15 ± 0.74	<0.01[c]
Treated seed retained	2.04 ± 0.35	2.11 ± 0.60	NS[b]

[a] Data are mean ± SE (n = 8).
[b] Student's t test.
[c] Mann-Whitney U test.

were spread on filter paper in empty cages in the testing room at the start of each session, left until the end of the feeding session, and then retrieved and frozen for analysis. Fonofos concentration in seeds was analyzed by gas chomatography after homogenization of samples in acetone [16]. Residues of fonofos in seeds given to birds at the start of the test day were 823 ± 28.8 mg/kg seed (n = 4) in experiment 1 and 693 ± 38.1 mg/kg seed (n = 4) in experiment 2.

The level of significance in all statistical analyses was $p < 0.05$. Values shown in text and tables are mean ± SE.

RESULTS

Experiment 1

Birds took in significantly less treated seeds on the test day than the average daily intake of untreated seeds during pretrial days (Table 2). Seed intake on the test day was reduced to 12 and 31% of normal daily intake for nondeprived and deprived birds, respectively. These results show that birds stopped feeding on treated seed long before fulfilling daily requirements. This avoidance response was sufficient to prevent lethal poisoning in the nondeprived birds because the dose of fonofos in the seed taken in (3.78 ± 0.74 mg a.i./kg body weight, range = 1.40–7.07, n = 8) was below lethal levels (median acute oral lethal dose [LD50] of fonofos to pigeons, 13.3 mg a.i./kg body weight; 95% confidence limits, 7.5–23.7 [17]), and, in fact, no bird died in this group. The avoidance response in the deprived group did not fully prevent exposure to lethal doses. The mean dose of fonofos in seed taken in by deprived birds (9.73 ± 1.40 mg a.i./kg body weight, range = 5.44–16.01, n = 8) approached the median lethal dose and was above the lower 95% confidence limit of the lethal dose for five birds, two of which died.

Two nondeprived birds regurgitated little treated seed (<1 g) (Fig. 1a), but the effect of this on the dose they absorbed was small compared to the effect of the avoidance response. Mean amounts of seed regurgitated by the deprived group were significantly larger (Table 2). The percentage of seed regurgitated by individual deprived birds increased with the amount ingested but was highly variable (Fig. 2a). All surviving birds in the deprivation group vomited a substantial proportion of the seed taken in (72 ± 5%, n = 6, Fig. 2a).

The two birds that died retained larger quantities of seed in the gut than any of the other 14 birds in this experiment (Fig. 1a). These birds died within the first 2 h of the experiment and still had substantial amounts of seed remaining undigested in the crop after death (3.14 g in bird 15 and 4.20 g in bird 293), whereas the crops of surviving birds contained only negligible amounts. Three birds took in more seed than the amount retained by the two birds that died but survived, apparently by vomiting most of the seed.

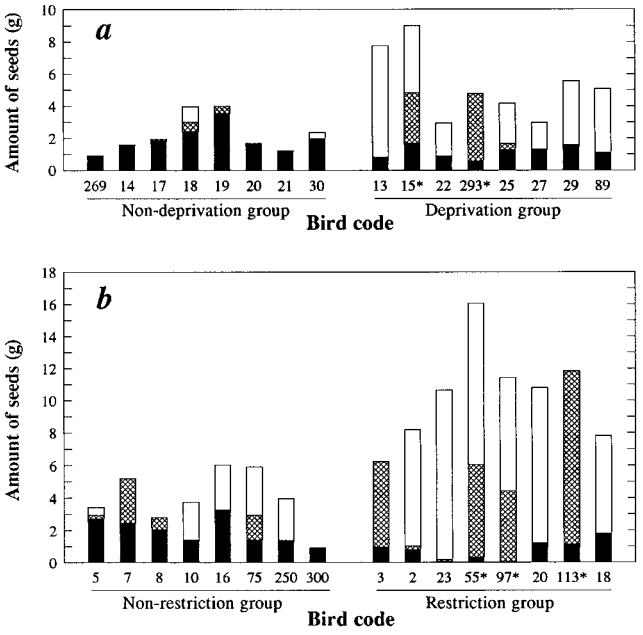

Fig. 1. Amount of fonofos-treated seed digested (■), retained in the crop or gizzard (☒), and regurgitated (□) by individual birds on the test day of experiments 1 (**a**) and 2 (**b**). *Bird died of acute poisoning within ~2 h of the start of the test period.

These results suggested that the actual dose of pesticide

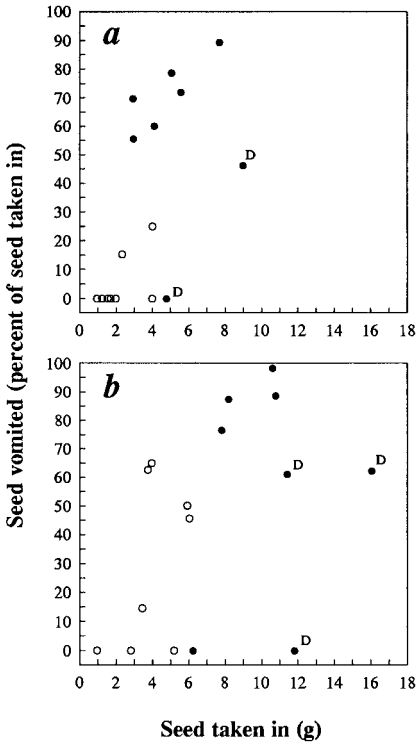

Fig. 2. Percentage of seed vomited in relation to the total amount of seed taken in by birds in experiments 1 (**a**) and 2 (**b**). ○ = Birds from the nondeprivation or nonrestriction groups; ● = birds from the deprivation or restriction groups. D indicates that the bird died of acute poisoning within ~2 h of the start of test period.

Table 3. Daily consumption (g fresh weight) of untreated seed during pretest days (mean of 5 consecutive days before restriction begun) and of fonofos-treated seed on the test day by feral pigeons in experiment 2[a]

	Non-restriction group	Restriction group	*p*
Untreated seed ingested	20.68 ± 0.93	20.99 ± 1.05	NS[b]
Treated seed taken in	4.00 ± 0.60	10.37 ± 1.07	<0.001[b]
Treated seed vomited	1.39 ± 0.49	6.27 ± 1.48	<0.05[c]
Treated seed retained	2.61 ± 0 48	4.11 ± 1.38	NS[c]

[a] Data are mean ± SE (n = 8).
[b] Student's t test.
[c] Mann-Whitney U test.

assimilated by some birds of the deprivation group was critically reduced by the discharge of considerable amounts of pesticide with the vomited seed. This suggested that regurgitation was critical in reducing mortality among deprived birds but was not conclusive because the vomited seed was not analyzed for fonofos residues and hence the actual doses of fonofos assimilated were not determined.

Experiment 2

As in experiment 1, the intake of treated seed on the test day of experiment 2 was below the average intake of untreated seed (Table 3). The amount of treated seed taken in was higher in experiment 2 than in experiment 1 (Fig. 1), and, as a result, the dose of fonofos in seed taken in was higher. The mean dose for nonrestricted birds was 6.70 ± 1.03 mg a.i./kg body weight (n = 8) and for restricted birds was 19.96 ± 1.91 mg a.i./kg body weight (n = 8). This dose was below lethal levels for most nonrestricted birds but near or above the median acute oral toxicity dose for all restricted birds (Fig. 3). Five nonrestricted and six restricted birds vomited substantial amounts of seeds (nonrestricted, 2.23 ± 0.44 g, range = 0.50–2.96, n = 5; restricted, 8.36 ± 0.76 g, range = 5.99–10.46, n = 6) (Fig. 1b).

Regurgitation reduced the final dose of fonofos assimilated by birds, but partial assimilation of pesticide from vomited seeds occurred while they remained in the gut. The concentration of fonofos in seeds vomited was 56.2 ± 2.9% (range = 43.9–74.8, n = 9) of the initial pesticide content. The amount of fonofos absorbed by birds from vomited seed was estimated by subtracting the measured residues in the vomited seed from the initial residues in that quantity of seed, which were based on the concentration measured at the start of the testing period. The amount actually absorbed may have been lower because some pesticide may have been lost before the vomited seed was collected and some may have been lost with regurgitated mucus. However, these losses should be relatively minor because residue decay in control samples was only 9% over the first 2 h of the test day and care was taken to retrieve as much mucus as possible with the vomited seed.

The amount of fonofos absorbed from digested and retained seed was estimated by calculating the amount in the total quantity of seed taken in, then subtracting (1) the amount in vomited seed, (2) the amount absorbed from vomited seed, and (3) the amount of fonofos remaining unabsorbed in the gut contents. The results of these measurements and calculations are shown in Figure 3. As a consequence of the vomiting and partial assimilation, the maximum possible dose of fonofos absorbed

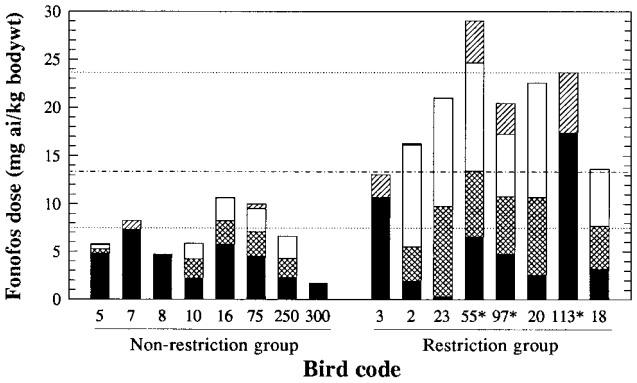

Fig. 3. Doses of pesticide assimilated and not assimilated from seed retained in the gut and seed vomited by individual birds in experiment 2. Full bar = dose of pesticide in the seed taken in; □ = dose not assimilated, expelled in vomited seed (measured); ▨ = dose not assimilated remaining in seed retained in the gut (measured); ■ = dose assimilated from seed digested and seed retained in the gut (estimated by subtraction); ☒ = dose assimilated from vomited seed (estimated by subtraction). Vomit from all birds except two (5 and 16, nonrestricted) were analyzed for fonofos residues. Values for birds 5 and 16 were estimated using the mean residue (340 mg/kg) in seeds vomited by three other nonrestricted birds (10, 75, and 250). ai = active ingredient; - - - - = median acute oral lethal dose; ·····= upper and lower 95% confidence limits of fonofos for feral pigeons [17]. *Bird died of acute poisoning within ~2 h of the start of the test period.

by restricted birds was significantly reduced by 50% from a potential 20.50 ± 2.18 mg a.i./kg body weight (n = 6), assuming no regurgitation, to the actual value of 10.96 ± 1.79 (n = 6), which takes regurgitation into account (Student's paired t test, t = 8.89, df = 5, p < 0.001).

The implications of regurgitation for individual birds are shown in Figure 3. The two restricted birds for which the dose of fonofos assimilated remained above the LD50 value died (birds 55 and 113). Regurgitation was crucial in reducing the final dose assimilated to below the LD50 value for five birds, of which one died (bird 97) and four survived. These four birds vomited more than 75% of seed taken in (Fig. 2b). The three casualties died within ~2 h of the start of the test period without digesting or regurgitating all the seed taken in (Fig. 1b). Undigested seed was found in the gut of these three birds at dissection (6.43 ± 1.94 g in the crop and 0.50 ± 0.23 g in the gizzard) but also in survivors. One bird (bird 3) that did not vomit and survived had 6.24 g of seed in the crop (84% of seed taken in) which remained undigested for ~9 h (from ~0900 when the bird stopped feeding until it was killed at ~1800 at the end of the experiment). Regurgitation also reduced the dose of fonofos absorbed by birds in the nonrestriction group, but its contribution to survival was not critical for three birds (5, 10, and 250) and uncertain for another two (16 and 75).

Data from video recordings showed differences in feeding and emetic patterns between nonrestricted and restricted birds on the test day. The feeding pattern of all six videotaped birds from the restriction group was similar. These birds fed during the first 9 min after the seed was presented and showed no (five birds) or negligible (one bird) feeding activity during the rest of the day. The feeding pattern in the nonrestriction group was more variable; four birds consumed seed almost exclusively in the initial feeding bout, whereas the remaining two had several feeding bouts spread over the 6 h of testing.

Data on the emetic response of videotaped birds that vomited are summarized in Table 4. The duration of individual

Table 4. Emetic response to fonofos-treated seed by videotaped feral pigeons that vomited seed (four nonrestricted and four restricted birds) in experiment 2[a]

	Non-restricted birds	Restricted birds	*p*
Latency to emesis[b] (min)	99.9 ± 26.5 (46.7–157.9)	23.2 ± 4.6 (13.1–34.1)	<0.05[c]
No. of emetic events	5.0 ± 3.3 (1–15)	8.0 ± 1.5 (4–11)	NS[d]
Mean duration of single emetic events[e] (s)	10.6 ± 0.8 (8.5–12.0)	10.1 ± 1.0 (7.8–12.1)	NS[f]
Total duration of emetic events (s)	52.8 ± 35.5 (12–159)	80.5 ± 18.9 (46–133)	NS[d]
Time from start to end of emetic events[g]	29.0 ± 20.6 (0.2–88.7)	175.3 ± 49.8 (39.0–277.2)	<0.05[c]

[a] Data are mean ± SE (n = 4); minimum and maximum values are in parentheses.
[b] Time from start of feeding to onset of retching in the first emetic event.
[c] Student's t test with square-root-transformed data.
[d] Mann-Whitney U test.
[e] From onset to the end of retching movements.
[f] Student's t test.
[g] Time from start of retching in the first emetic event to end of retching in the last emetic event.

emetic events was similar in all birds from both experimental groups. The number of emetic episodes and their total duration tended to be higher in restricted than in nonrestricted birds, but the differences between groups were not significant, possibly because of interindividual variation and low sample size. Regurgitation started significantly earlier in the restriction group and occurred over a longer period than in the nonrestriction group.

DISCUSSION

Vomiting is a physiological response to distasteful or harmful substances that affects the risk of poisoning for birds consuming food containing toxic substances. It has been demonstrated that, as a mechanism involved in the development of conditioned food aversions in birds, vomiting has a secondary role in preventing prolonged feeding on toxic food and thus poisoning [9,18]. Although results from previous studies have suggested that regurgitation also has a primary role in preventing or reducing mortality by allowing the expulsion of toxic food before a lethal dose is absorbed [8,9], no previous study has demonstrated quantitatively that vomiting can lower the dose absorbed from lethal to nonlethal levels. This is the main finding of the present study, in which we found that pigeons ingesting food bearing a lethal dose of a pesticide can survive by regurgitating enough food to reduce the absorbed dose below lethal levels.

Under many conditions, feral pigeons feeding on fonofos-treated seed are able to avoid poisoning by stopping feeding before a lethal dose is ingested. This was found in both of our experiments, mainly among birds fed ad libitum, in which results were broadly similar to those found previously in our laboratory in other experiments with feral pigeons. However, avoidance was insufficient to prevent ingestion of potentially lethal amounts of seed among food-restricted birds, mainly in experiment 2. Regurgitation of treated seed before all the pesticide was assimilated reduced the final dose absorbed substantially and was the most likely explanation for the survival of some birds, at least one (12%) and four (50%) of the food-restricted birds in experiments 1 and 2, respectively. These results show that regurgitation can be crucial in reducing acute poisoning when the avoidance response is insufficient.

Regurgitation reduced the incidence of lethal poisoning but did not prevent it. Mortality occurred among food-restricted birds under two different situations in our study: Some birds died despite vomiting, and others died without regurgitating.

Of the six food-restricted birds that took in lethal amounts of seed and vomited in experiment 2, four survived and two died. Survivors digested, on average, 11% of seed taken in and expelled the rest (88%), leaving the crop and gizzard practically empty. The two birds that died vomited 62% of the seed taken in and failed to expel all the contents of the crop. It is likely that these two birds absorbed a lethal dose and died before emesis had finished, leaving undigested seed in the crop.

The concentration of fonofos in vomited seed was reduced by approximately half of the initial level. This occurred because the seed vomited stayed in the gut long enough to allow absorption of substantial amounts of pesticide. The retention time of seed in the gut was therefore an important factor affecting the final dose absorbed. The duration of this period depends on the latency from ingestion to the onset of regurgitation and the duration of the period over which vomiting occurs. Regurgitation started relatively soon in restricted birds (mean, 23 min) but much later in nonrestricted birds (mean, 100 min). This difference was probably caused by the difference between groups in the amount of seed taken in and consequently in the maximum possible dose of pesticide, because it has been demonstrated in pigeons given a single oral dose that emesis starts earlier at the highest doses of emetic [19]. The rate of pesticide uptake through the gut could have been another factor, for example, if the feeding regimens caused differences in gut blood flow.

Taking into account the relatively high rate of absorption of pesticide from vomited seed, early vomiting was important to reduce the likelihood of absorbing a lethal dose, and it probably would have been sufficient to prevent mortality had birds vomited all the seed immediately after the start of the emetic response. If all undigested seed were discharged at once in the first regurgitation episode, the final dose absorbed by restricted birds that vomited would have been reduced by an additional 28%. However, the emetic response in these birds was not single and short-lived but occurred intermittently during a considerable period of time. The intermittent nature of the emetic response therefore restricted its effectiveness in preventing mortality. Similar patterns of emesis have been described for feral pigeons given oral doses of copper sulphate [19] and starlings given chlorfenvinphos [13] or feeding on food treated with methiocarb [12]. These observations indicate that the expulsion of liquids or discrete and relatively small food items such as seeds is not generally completed at once but requires several successful regurgitation episodes. Observations of gulls vomiting toxic bivalves [9] suggest that larger food items may require a shorter emetic response, which would enhance the protective value of regurgitation for large in comparison to small food items.

The other situation in which mortality occurred in this study was dying without regurgitating. These birds took in as much seed as other birds that had a strong regurgitation response. The time from the feeding period to the manifestation of the first severe symptoms of acute poisoning in one bird (~1 h) and to death in both birds (~2 h) was longer than the time to the onset of regurgitation in other restricted birds (<35 min).

It therefore appears that pigeons differ in their physiological capacity to vomit. This lack of a generalized regurgitation response in birds tested under similar conditions is a common feature reported in studies with pigeons [15,19–21]. It can be thus concluded that within a population, some pigeons would have limited or no capacity to vomit, making them more prone to acute poisoning if they encounter toxic food with emetic properties.

Results from several studies demonstrate that a wide range of birds regurgitate for different causes and purposes and that the capacity to vomit and the intensity of the emetic response can be highly variable within and between species (see Hart and Thompson [13] for detailed references). Pigeons may be a good model for species with a well-developed crop. The crop allows high feeding rates and the fulfilment of food requirements in short feeding periods. The high feeding rates favored by the presence of a crop may facilitate the regurgitation response. After feeding on treated seeds, pigeons may be able to regurgitate a high proportion of the meal because much of it is retained in the crop. Other species with limited capacity to store food temporarily in a crop need to feed more continuously and probably at lower feeding rates, which may imply that regurgitation is less likely.

The inter- and intraspecific variation in the capacity of birds to regurgitate makes it difficult to establish the general importance of regurgitation in reducing the risk of poisoning from food containing toxic pesticides. The difficulty lies in determining the specific role that regurgitation may have in different situations. This may be affected by species, feeding behavior, body condition, food type and size, pesticide type, formulation, and concentration, among others. Our study has identified a situation in which regurgitation was critical in reducing bird mortality, indicating that more attention needs to be given to the characterization and quantification of regurgitation when birds are exposed to pesticides in their food. Vomiting has been observed in captive birds of several species [10–13] and in wild birds, such as gulls feeding on leatherjackets possibly poisoned by chlorpyrifos (see Hart and Thompson [13]) and free-living feral pigeons feeding on cereal seed treated with fonofos (M.R. Fletcher, personal communication). The implications of these vomitings on bird survival are unknown. Laboratory studies can provide a better understanding of what factors determine the role of regurgitation in affecting the risk of bird poisoning from pesticides before broad extrapolations to the field can be made.

Our study identified some interrelated factors (total amount of food eaten, occurrence of vomiting, proportion of food vomited, retention time of vomited food in the gut, and rate of absorption of pesticide) that affect the effectiveness of regurgitation in reducing the dose of pesticide assimilated. Organophosphorus pesticides have rapid effects on birds (detectable within hours of initial exposure [22–24]), which requires fast pesticide uptake. At such a high absorption rate, vomiting needs to occur rapidly and to be massive to be effective if highly toxic pesticides are involved. In our study, birds that ingested lethal amounts of food survived when they vomited nearly all the seed taken in, whereas death occurred in those that did not vomit or eliminate a sufficient amount of seeds before a lethal dose was assimilated. The narrow margin between lethal and sublethal effects for some birds suggests that the way these factors interact is critical in determining the success of regurgitation.

Implications for avian toxicity testing and risk assessment

The results of this study have implications for avian toxicity testing in general and dietary toxicity tests in particular. Little consideration is given to regurgitation in current avian toxicity tests. The current U.S. Environmental Protection Agency guideline for acute avian toxicity states that ''any regurgitation should be noted and reported'' [25]. Guidelines for dietary toxicity tests do not mention regurgitation [26,27]. Hart and Thompson [13] found that regurgitation can reduce considerably the dose of pesticide absorbed in birds dosed as they are in acute oral toxicity tests. These authors pointed out that the type of exposure in their experiment (forced dosing) is very different from that occurring in the wild. A major contribution of the present study has been to show that regurgitation can also have an important role in captive birds feeding freely on treated food, which better simulates the way exposure occurs in the wild [28]. Therefore, regurgitation may also affect the results of avian dietary toxicity tests in which birds are exposed via the oral route to chemicals by feeding voluntarily on treated food available ad libitum [27,28]. The way these tests are typically conducted (several animals grouped in pens with no detailed observation of behavior or measurements of food intake during the critical period at the start of the test period [27]) makes it difficult to detect and quantify regurgitation.

The potential for the extent of regurgitation to differ between captive and wild birds introduces additional uncertainty into risk assessment. If regurgitation does not occur in tests with captive birds (whether acute oral or dietary) for a particular pesticide but does occur in the wild, then the risk to birds in the wild would be overestimated. In this case, it may be prudent to ignore the potential discrepancy, because our results show it is unlikely that all individuals will regurgitate to the same extent. However, if regurgitation occurs in tests with captive birds but not in the wild, then the risk in the wild would be underestimated, perhaps to a substantial degree. One option for dealing with this uncertainty would be to repeat the tests with a different species that does not regurgitate the pesticide in question. Selection of appropriate species would be easier if it could be shown that some species were consistently unlikely to regurgitate. An alternative option might be to attempt to measure the extent of regurgitation and use these data to adjust the assessment of risk. Either way, it is clearly very important to detect the occurrence of regurgitation in captive studies. In future tests, it would be prudent to undertake additional observations for this purpose for a period of 1 to 2 h immediately after dosing or the start of exposure, at least for chemicals known to have emetic properties [10,12,13]. In the longer term, when new test guidelines are being developed [29], consideration should be given to including suitable observations to ensure that any regurgitation is detected.

Our findings establish regurgitation as an important factor in understanding risks to birds from the use of pesticides and thus suggest that regurgitation should be considered in risk assessment. Disregarding the potential of regurgitation to reduce risk might lead to unnecessary restrictions being placed upon useful agrochemicals. Alternatively, risks to some species might be underestimated if assessment is based on toxicity tests with species that regurgitate easily, because the capacity and intensity of vomiting vary between species. Further research is needed to determine which of the species at most risk from pesticides vomit, how much of the treated food is

vomited and how quickly, and how this information can be used in routine risk assessment.

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