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**Environmental hazard/risk assessment of pesticides used
in agriculture for birds and mammals. The Dutch concept.
Part 2. Avian food avoidance behaviour.**

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Summary

The purpose of this report (the second one of a series in which the methodology for hazard/risk assessment of the use of pesticides for birds and mammals will be presented) is to give a method for taking into account the avoidance behaviour in the evaluation of LC50 studies and to provide a concept for the use of food avoidance behaviour in the environmental hazard/risk assessment of pesticides used in agriculture for birds.

An analysis is made of the standard dietary LC50 test and of 3 types of food avoidance tests. It is concluded that the standard dietary LC50 test provides most of the information necessary to understand the repellent properties of a pesticide. It is recommended to measure the food consumption daily, to provide information about the learning abilities of the species tested (when does food avoidance appear).

A method is proposed to calculate the No Repellent Concentration (NoRC). The NoRC is the concentration in the treated food at which the birds in the test will eat the same amount of treated food as the amount of (untreated) food in the control group. Data of the range finding tests can be used to design the LC50 test (which doses will be tested) to provide a better estimation of the NoRC value.

These small alterations of the standard LC50 test make other repellency tests with birds superfluous, because the standard LC50 test is providing enough information about the repellent properties of the substance. Those repellency tests can be used to provide information about the edibility of the granulates and seeds (coated or pillorized, but not treated with a substance), which results in less suffering of test animals.

Samenvatting

Dit rapport is het tweede van een serie waarin een methode voor de beoordeling van het risico van het gebruik van bestrijdingsmiddelen voor vogels en zoogdieren wordt gepresenteerd. Het doel van dit rapport is aan te geven hoe de repellente eigenschappen van bestrijdingsmiddelen kunnen worden meegenomen in de risicoschatting.

De standaard 5 dagen dieetstudie (LC50) en 3 typen vermijdingstesten werden geanalyseerd (repellency/food avoidance tests). De standaard LC50 geeft bijna alle informatie die nodig is om de repellente eigenschappen van stoffen te kunnen begrijpen. Het wordt aanbevolen de voedselconsumptie dagelijks te meten, zodat informatie beschikbaar komt over het tijdstip wanneer de vogel het vermijdingsgedrag gaat vertonen.

Er wordt een methode gepresenteerd waarmee de "No Repellent Concentration" (NoRC) kan worden berekend. De NoRC is die concentratie in het behandelde voer waarbij de vogels in de test net zoveel eten van het voer als in de controle test. Data van de "range finding test" kunnen gebruikt worden, voor de keuze van de doseringsreeks in de LC50 test, zodat een betere bepaling van de NoRC kan plaatsvinden.

Deze kleine veranderingen van de standaard LC50 test maken andere vermijdingstesten met het bestrijdingsmiddel overbodig. Deze testen kunnen wel gebruikt worden om te bepalen of een granulaat of een gecoat of gepilleerd zaad (dus zonder toevoeging van een bestrijdingsmiddel) door vogels worden opgegeten, met het resultaat dat de geteste vogels gedurende de test minder te lijden zullen hebben.

1. Introduction

The purpose of this report (the second one of a series in which the methodology for hazard/risk assessment of the use of pesticides for birds and mammals will be presented) is to give a method for taking into account the avoidance behaviour in the evaluation of LC50 studies and to provide a concept for the use of food avoidance behaviour in the environmental hazard/risk assessment of pesticides used in agriculture for birds.

In part 1 (Luttik 1992) a general introduction to the subject is presented and a synopsis of the decision scheme is given.

The ability of birds to detect and avoid toxic foods has been demonstrated with several pesticides (Bennett 1989A and 1989B, Conover 1984, Grue 1982 and Kononen et al. 1986 and 1987). In many 5-d avian dietary LC50 tests a dose related decrease in food consumption can be noticed (Table 1).

Table 1 Range of food consumption in % of the food consumption of the control group according to the dose range in LC50 tests.

Compound	Species	Dose range (mg/kg food)	Food consumption	Reference
Methiocarb	Mallard	265-5000	59 - 3	Kononen et al. 1986
Methiocarb	Bobwhite	265-2778	82 - 43	Kononen et al. 1986
Carbofuran	Mallard	33- 616	40 - 5	Kononen et al. 1987
Carbofuran	Bobwhite	86-1620	86 - 29	Kononen et al. 1987
Parathion	Bobwhite	79- 251	58 - 21	Bennett 1989A
Carbofuran	Bobwhite	520-2000	82 - 40	Bennett 1989A
Methiocarb	Bobwhite	200-2000	95 - 19	Bennett 1989A
Chlorpyrifos	Bobwhite	178-1778	92 - 12	Bennett 1989B
Methyl- parathion	Bobwhite	50- 159	46 - 13	Bennett 1989B
Dicrotophos	Common Grackles	10- 200	27 - 3	Grue 1982
Dicrotophos	Common Grackles	10- 200	49 - 4	Grue 1982
Methyl- parathion	Common Grackles	25- 400	87 - 6	Grue 1982
Fenthion	Common Grackles	25- 400	70 - 6	Grue 1982
Fenthion	Common Grackles	30- 120	28 - 7	Grue 1982
Fenitrothion	Common Grackles	100-1600	25 - 5	Grue 1982
Fenitrothion	Common Grackles	25- 100	87 - 37	Grue 1982

In controlled-feeding tests with groups of juvenile Mallards (*Anas platyrhynchos*) and Northern Bobwhite (*Colinus virginianus*) with a free choice between two feeders - one containing untreated food and the other containing one of several dietary concentrations of test chemical - birds responded to the presence of many chemicals at sublethal concentrations by decreasing the proportion of treated food consumed mostly without reducing the total food consumption, thereby reducing the occurrence of toxic effects (Table 2).

From the data presented in table 1 and 2 it is obvious that these bird species are able to detect the test chemicals in their food, which results in a food avoidance behaviour of these species.

Table 2 Food consumption in avian food avoidance behaviour tests (g/b-d).

Carbofuran Bobwhite			Methiocarb Bobwhite		
Dose (mg/kg food)	Food consumption Untreated (g/b-d)	Treated (g/b-d)	Dose (mg/kg food)	Food consumption Untreated (g/b-d)	Treated (g/b-d)
0	6.8	--	0	7.0	--
520	4.1	2.3	200	2.9	3.5
729	4.6	2.1	316	3.9	2.7
1020	5.2	1.2	501	4.2	2.4
1428	5.4	1.0	794	5.4	1.1
2000	4.6	1.4	1259	6.7	0.7
			2000	5.3	0.9

Carbofuran Mallard			Methiocarb Mallard		
Dose (mg/kg food)	Food consumption Untreated (g/b-d)	Treated (g/b-d)	Dose (mg/kg food)	Food consumption Untreated (g/b-d)	Treated (g/b-d)
0	56	--	0	50	--
33	32	14	265	42	4
59	37	6	476	51	3
106	43	5	857	43	2
190	43	3	1543	46	1
243	45	1	2778	42	1
616	35	1	5000	38	1

g/b-d = gram food per bird per day. Data on Bobwhite from Bennett (1989A) and on Mallards from Kononen et al. (1986).

2. Avian dietary LC50 tests

2.1. Standard LC50 test

The principle of the OECD guideline for testing of chemicals number 205 (Avian Dietary Toxicity Test) is that birds are fed a diet containing the test substance at a range of concentrations for a period of five days. Beginning on day 6, the birds are fed basal diet, free of the test substance, for a minimum of three additional days (OECD 1984).

Conditions for the validity of the test are:

- Two control groups, and one treatment group for each of the, at least, five dietary levels of the test substance should be used. Each group consists of 10 birds.
- Diets containing the test substance should be available ad libitum.
- *The mortality in the controls should not exceed 10 per cent at the end of the test.*
- There must be evidence that the concentration of the substance should be at least 80 per cent of the nominal concentration throughout the five days of the test period.
- The lowest treatment level should not result in compound-related mortality or other observable toxic effects.

The results should include the following information:

- Number of deaths at each treatment level and in the control groups.
- Average body weights for live birds in each pen at the beginning of the test, the end of the exposure period, and at the end of the test; individual weight of all birds that die during the test.
- Description of all signs of intoxication and other abnormal behaviour, including day of onset, duration, severity, and numbers affected in the different dietary concentrations and controls each day of the test period.
- Estimated food consumption, by weigh-back method, per pen for the exposure period and the postexposure period.
- Calculated LC50 value, 95 per cent confidence limits, slope of the concentration-response curve, the results of a goodness-of-fit test, highest concentration causing no mortality and lowest causing 100 per cent mortality. The statistical methods used should be described or the reference given.
- Anything unusual about the test, any deviation from the procedures, and any other relevant information.

2.2. Evaluation of LC50 tests

The calculation of the LC50 and its confidence limits is based on the assumption that birds exposed to certain dietary concentrations of a test chemical will consume the same amount of food in each treatment level. Very often this is not true (see example in Table 3). The assumed daily food intake (DFI) can be quite different from the measured DFI. According to the differences between the assumed and measured DFIs the assumed and measured daily chemical intake (DCI) will be different. Some more examples can be found in Figure 1. This reduced food consumption can be induced by repellent properties of chemicals:

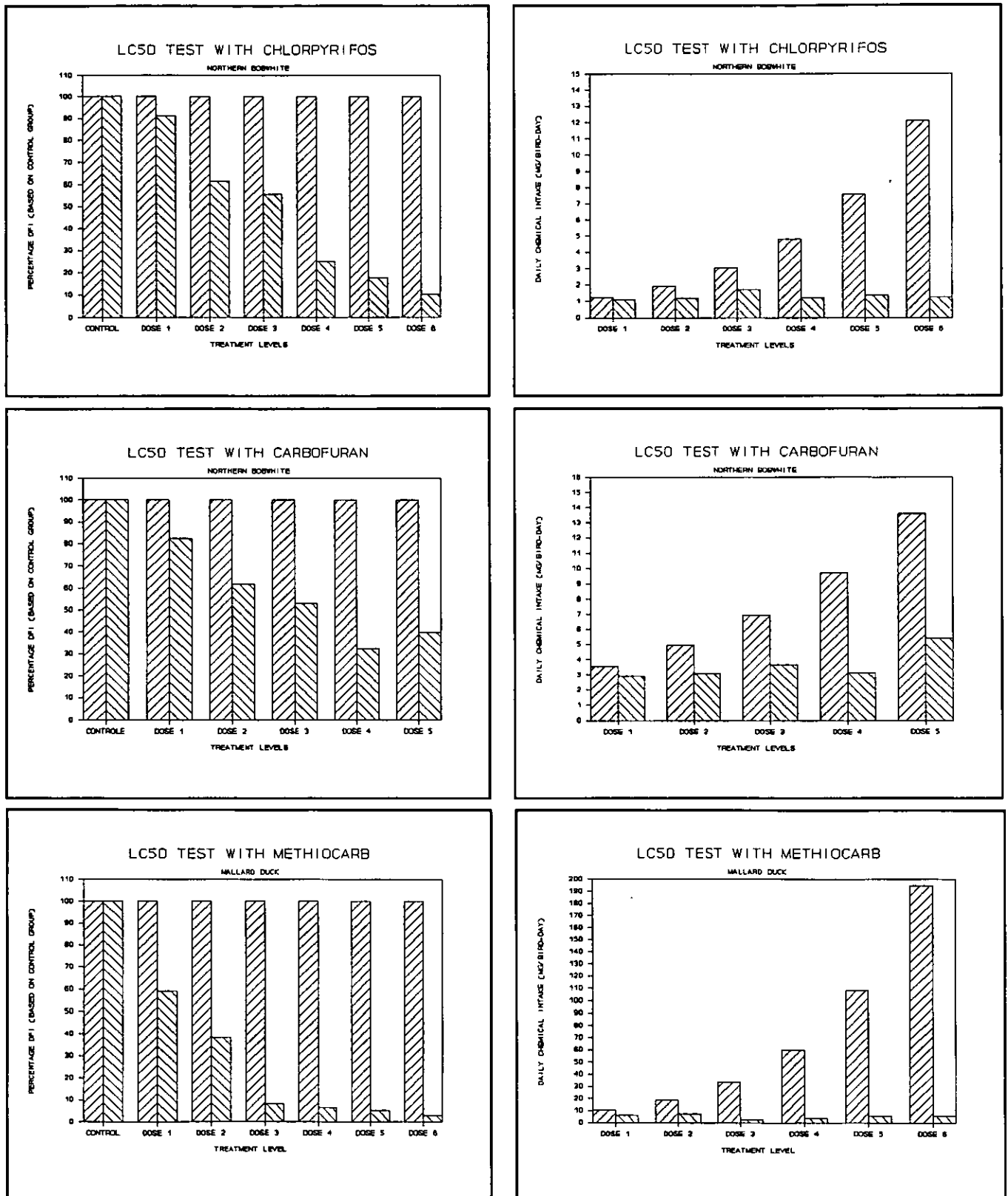
- taste repellents (chemicals that make food distasteful to the animals eating it, e.g. copper oxalate (Conover 1984)),
- aversive conditioners (animals experience illness after consuming treated food, associate the illness with the food and avoid it thereafter, e.g. lithium chloride (Conover 1984)),
- anorexia inducers (chemicals that reduce the food consumption by one or another way effecting the feeding behaviour of animals, e.g. methyl-parathion, fenthion (Grue 1982)),
- or chemicals with a combination of properties (e.g. methiocarb (Conover 1984)).

Table 3 Difference between assumed and measured DFI and DCI and percentage mortality in a LC50 test with Bobwhite and parathion (Bennett 1989A)

Dietary Concentration (mg/kg food)	Assumed DFI (g/b-d)	measured DFI (g/b-d)	Assumed DCI (mg/b-d)	Measured DCI (mg/b-d)	Mortality (%)
0	7.2	7.2	0.00	0.00	0
79	7.2	4.2	0.57	0.33	0
100	7.2	3.8	0.72	0.38	0
126	7.2	4.5	0.91	0.68	20
159	7.2	2.9	1.15	0.46	50
200	7.2	2.0	1.44	0.40	60
251	7.2	1.5	1.81	0.38	80

b-d = bird per day

Figure 1 Difference between assumed and measured DFI and DCI in LC50 tests with Bobwhite (Bennett 1989A and B) and Mallard (Kononen 1987). Left bar is assumed and right bar is measured DFI or DCI.



From Table 3, it is remarkable that a dose-related response is found in spite of the fact that the daily chemical intake does not differ much between the treatment levels. The same can be noticed in the examples of Figure 1 (see table 4).

Table 4 Measured daily chemical intake (DCI) and mortality in 3 LC50 studies with Bobwhite for chlorpyrifos, carbofuran and methiocarb (Bennett 1989A and B).

	Chlorpyrifos Bobwhite		Carbofuran Bobwhite		Methiocarb Mallard	
	DCI (mg/b-d)	Mort. (%)	DCI (mg/b-d)	Mort. (%)	DCI (mg/b-d)	Mort. (%)
Dose 1	1.10	0	2.91	20	6.10	0
Dose 2	1.18	0	3.06	20	7.09	17
Dose 3	1.70	6	3.67	30	2.74	25
Dose 4	1.20	56	3.14	60	3.86	17
Dose 5	1.35	94	5.40	90	5.56	33
Dose 6	1.24	100			5.50	42

Bennett (1989A) observed in the test with carbofuran (Bobwhite) two types of mortality:

- A) Acute poisoning within 1 day, with little or no weight loss (average loss 5%) and
- B) death occurring 3 to 6 days from test initiation, with significant weight loss (average loss 38%).

In the tests with parathion and methiocarb all bird mortality occurred between day 3 and 6, with an average weight loss of 37% (21-43%) for parathion and an average loss of 40% (35-47%) for methiocarb (Bennett 1989A).

The other study of Bennett (1989B) and the study of Grue (1982) do not mention the time of death of the test birds, but considering the average weight loss of the dead birds, a type B mortality must be the case. Kononen et al. (1986 and 1987) did not give body weights of dead birds.

Dead Bobwhite exposed to chlorpyrifos and methyl-parathion (Bennett 1989B)

had an average loss of 38% of their body weight (range 20-47%). Common Grackles that died during the LC50 tests carried out by Grue (1982) with dicotophos, methyl-parathion, fenthion and fenitrothion lost an average of 28-36% of their initial body weight. These values exceed those reported by Hill (1972) for House Sparrows (*Passer domesticus*) after starvation: average weight loss 21.4% (17-25%). Visible fat was absent in birds that died and muscle tissue was reduced on the sternum of the Common Crackles. Mortality following dietary exposure of dicotophos increased between May and August, although chemical intake remained relatively constant. The observed increase in mortality was associated by Grue (1984) with a natural decrease in fat and flesh condition in response to increased ambient temperatures (21-31 °C) and post-nuptial moult. The data presented in this chapter indicate that LC50s, when marked decrease in food consumption is noticed, should be considered as a measure of the relative vulnerability of a species to a dietary exposure to a toxicant and not as sensitivity.

3. Food avoidance tests

3.1. Introduction

The search for bird repellents to protect seeds and agricultural crops and the evaluation of chemicals as repellents for protecting paper, plastics, and textile packaging materials from rodent damage has been in progress for many decades. Tigner and Besser (1962) developed a method, a concentration-repellent effect determination, to express quantitatively rodent repellency of chemicals on packaging materials. The method yields an expression of effectiveness in terms of the concentration required to repel 50% of test rodents (R_{50}), the confidence limits (95%) and a regression line that allows the estimation of the concentration that repels any given percentage of test animals. Starr et al. (1964) adapted this method to birds.

In recent years several acceptance/palatability tests have been developed for birds: BBA 25-1 (BBA 1981), INRA test (INRA 1990) and Median food avoidance test (Kononen 1986 and Bennett 1989A).

3.2. BBA 25-1 and INRA test

Both tests are designed to give information about the behaviour of birds when they are exposed to a formulation of a chemical at the normal field dosage. A short description of the BBA 25-1 and INRA test is given in table 5.

The BBA 25-1 test has two exposure conditions: the aggravated test in which the

birds have a choice between 75% treated and 25% untreated food and the non-aggravated choice test of 10% treated and 90% untreated food. The food is spread out on the floors of the aviaries. The INRA test has also an aggravated test in which the birds have no choice (100% treated food) and a non-aggravated test in which the birds have a choice between 50% treated and 50% untreated food in separate feeders. These two tests have one serious shortcoming: only one concentration is tested. When more concentrations are used in the field more tests have to be performed. An other shortcoming is the fact that birds in the field have no choice at the treated area but have only the choice of eating and not eating (and looking for an other feeding place). This situation is partly covered in the aggravated experiment of the INRA test.

Table 5 Design of BBA 25-1 and INRA test.

Test design/parameter	BBA 25-1	INRA
Species	Japanese quail Ring-necked Pheasant	Common Partridge Red-legged Partridge
Minimum number of replicates	3 (quail) 2 (pheasant)	1
Control group	no	yes
Number of birds per test	8 (quail) 5 (pheasant)	10-12
Age of birds	adults	not specified
Preexposure treatment	16 h starvation	no starvation
Duration of exposure period	8 hours for quails 7 days for pheasant	24 hours
Observation period	14 days	14 days
Parameters to be examined	Mortality, toxic signs, behaviour, body weight, (gross pathology).	Mortality, toxic signs body weight, food consumption, (gross pathology).

Table 5 Continued

Test design/parameter	BBA 25-1	INRA
Exposure conditions	<p>Exposure in groups. Food spread out on sandy floor of aviaries.</p> <p>1. Aggravated test: choice test, 75% treated + 25% untreated food.</p> <p>2. Non-aggravated test: choice test, 10% treated + 90% untreated food</p>	<p>Exposure individually in cages. Food given in feeders.</p> <p>1. Aggravated test: no choice test, 100% treated food.</p> <p>2. Non-aggravated test: choice test, 50% treated + 50% untreated food in separate feeders.</p>

3.3. Median food avoidance test

In analogy to the median lethal concentration (LC50) of the avian dietary test, a medium food avoidance concentration is the maximum concentration of a test compound that is expected to result in equal consumption of treated and untreated feed by a group of animals given free access to both clean and contaminated feed (Kononen et al. 1986). This concentration is the highest expected dietary concentration of toxicant that will not result in detectable avoidance behaviour by an exposed population. This concentration is named by Kononen et al. (1986) the FAC50 value (median food avoidance concentration) and by Bennett (1989A) the DT value (discrimination threshold).

A short description of the test performed by Kononen et al. (1986 and 1987) and Bennett (1989A and B) is given in Table 6.

3.4. Evaluation of food avoidance test of Kononen et al. and Bennett

The FAC50 or DT values of the studies carried out by Kononen et al. and Bennett are presented in Table 7. The FAC50 value of Kononen et al. were calculated in the same manner as the LC50s (probit analysis) except the response variable was the proportion of total food that was treated food (see also Kononen 1988). The DT values of Bennett were calculated as the intersec-

Table 6 Description of food avoidance tests according to Kononen et al. (1986 and 1987) and Bennett (1989A and B).

Test design	Kononen et al.	Bennett
Species	Mallard Bobwhite	Bobwhite
Number of different doses	5-6	5-6
Number of birds per dose	10-12	10
Number of birds in control	24	10 or 20
Age of birds	juveniles	juveniles
Preexposure treatment	no starvation	no starvation
Duration of exposure period	5 days	5 days
Observation period	14 days	14 days
Exposure conditions	Exposure in groups. Food given in feeders. Choice test: 50% treated + 50% untreated food.	Exposure in groups. Food given in feeders. Choice tests: 50% treated + 50% untreated food and 90% treated + 10% untreated food.
Number of feeders in test (treated:untreated)	1:1	1:1, 5:5 and 9:1
Parameters to be examined	Mortality, toxic signs, behaviour, body weight, food consumption.	Mortality, toxic signs behaviour, body weight, food con- sumption.

tion of a two-phase regression analysis using the log of the ratio of untreated to treated food consumption as the dependent variable and the log of the concentration of treated food as the independent variable (Bennett and Schafer 1988). For convenience the concentration of the compound by which the bird species will not make a difference between treated and untreated food will be called the discrimination threshold (DT) in this report.

In all cases where birds were offered the choice between treated and untreated food in two feeders per cage (1:1) a dose related food avoidance was noticed and DT values could be calculated (see also Figure 2A and 2B). When the same species was tested by Bennett and Kononen et al. the difference between the DT values were a factor 1.1 and 2.4 for carbofuran and methiocarb, respectively. A marked difference was found in the case of the study with methiocarb and Mallards carried out with 2 birds per pen and with 6 birds per pen. When 6 birds are put in one pen the DT value was approximately 100 times lower than when 2 birds were put in one pen. Although only information from one study is available these results suggest that the avoidance response of young Mallards to methiocarb-treated food is strongly influenced by factors such as the number of birds per pen or the amount of floor space allotted to each bird.

In all studies of Bennett (1989A and 1989B) little or no reduction in total food consumption was observed from control to treated groups because increased consumption of untreated food compensated for reduction in consumption of treated food (see also Table 8). No mortalities or signs of intoxication were observed in the tests with chlorpyrifos, methyl-parathion and parathion. In the test with methiocarb only 1 bird died at the highest concentration tested (this was the smallest bird; day 0 weight = 20 g, compared to a mean weight = 32 g for all birds). In the test with carbofuran 3 mortalities on day 1 from acute poisoning were observed.

In the studies of Kononen et al. (1986 and 1987) little reduction in food consumption was noticed (not significant, $P \geq 0.05$) in the studies with the Bobwhite. In the studies with Mallards little or no reduction in food consumption was noticed except at the highest concentration tested (significant, $P < 0.01$), but a not doses related decrease in food intake was measured in the study with 2 birds per pen with methiocarb (3 cases significant less than the control, $P < 0.01$). Mortalities were noticed in 4 of the five tests carried out (2 to 9 dead birds) except the test with methiocarb with 6 Mallards per pen.

In the studies of Bennett (1989A and 1989B) where Bobwhite were offered treated and untreated food in 5 feeders each per cage (5:5) a dose related food avoidance was noticed and DT values could be calculated. The DT values for

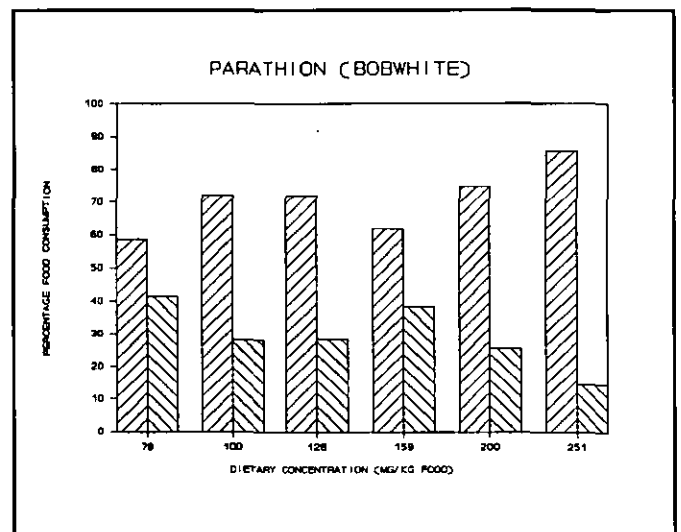
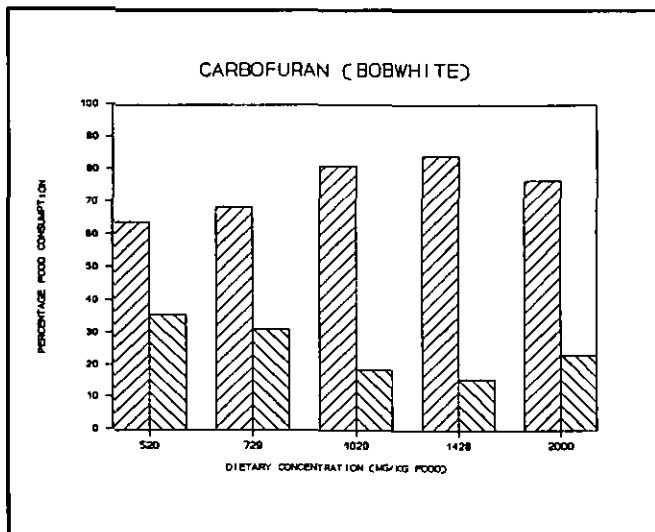
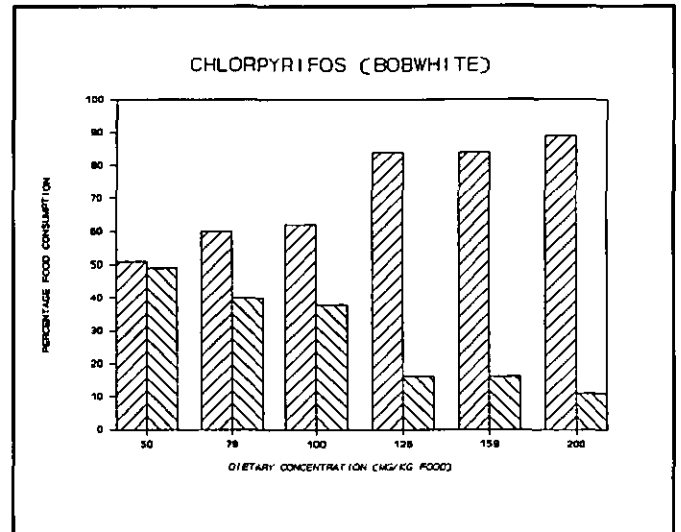
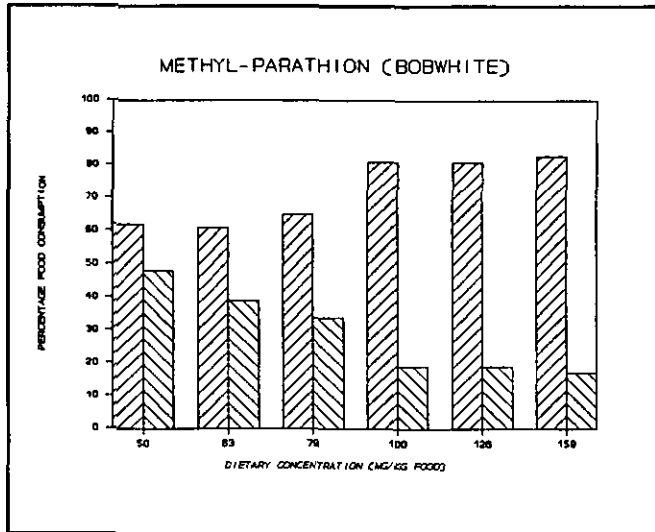
Table 7 Discrimination threshold values and LC50 values (mg/kg food) from studies carried out by Kononen et al. and Bennett.

Compound	Species	DT value (mg/kg fd)	LC50 (mg/kg fd)	Reference
Two feeders/cage, one containing treated food and one untreated food:				
Carbofuran	Bobwhite	170	1144	Bennett 1989A
	Bobwhite	159	917	Kononen et al. 1987
	Mallard	10	242	Kononen et al. 1987
Chlorpyrifos	Bobwhite	63	647	Bennett 1989B
Methiocarb	Bobwhite	224	1457	Bennett 1989A
	Bobwhite	95	827	Kononen et al. 1986
	Mallard ¹	145	7469	Kononen et al. 1986
	Mallard ²	1.5		Kononen et al. 1986
Methyl-parathion	Bobwhite	10	91	Bennett 1989B
Parathion	Bobwhite	33	177	Bennett 1989A
Ten feeders/cage, five containing treated food and five untreated food:				
Carbofuran	Bobwhite	400	1144	Bennett 1989A
Chlorpyrifos	Bobwhite	26	647	Bennett 1989B
Methiocarb	Bobwhite	222	1457	Bennett 1989A
Methyl-parathion	Bobwhite	45	91	Bennett 1989B
Parathion	Bobwhite	38	177	Bennett 1989A
Ten feeders/cage, nine containing treated food and one untreated food:				
Carbofuran	Bobwhite	--	1144	Bennett 1989A
Chlorpyrifos	Bobwhite	71	647	Bennett 1989B
Methiocarb	Bobwhite	>1259	1457	Bennett 1989A
Methyl-parathion	Bobwhite	> 126	91	Bennett 1989B
Parathion	Bobwhite	--	177	Bennett 1989A

¹ = 2 birds per pen. ² = 6 birds per pen.

Figure 2A

Percentages of food consumption by Bobwhite in food avoidance tests
(Bennett 1989A and B).



Legend:

Left bar: untreated food

Right bar: treated food

10 birds per pen.

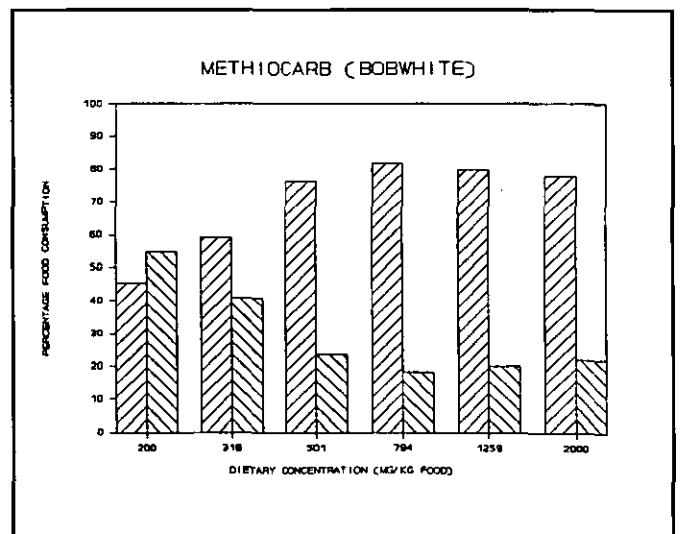


Figure 2B

Percentages of food consumption by Bobwhite and Mallards in food avoidance tests (Kononen 1986 & 1987).

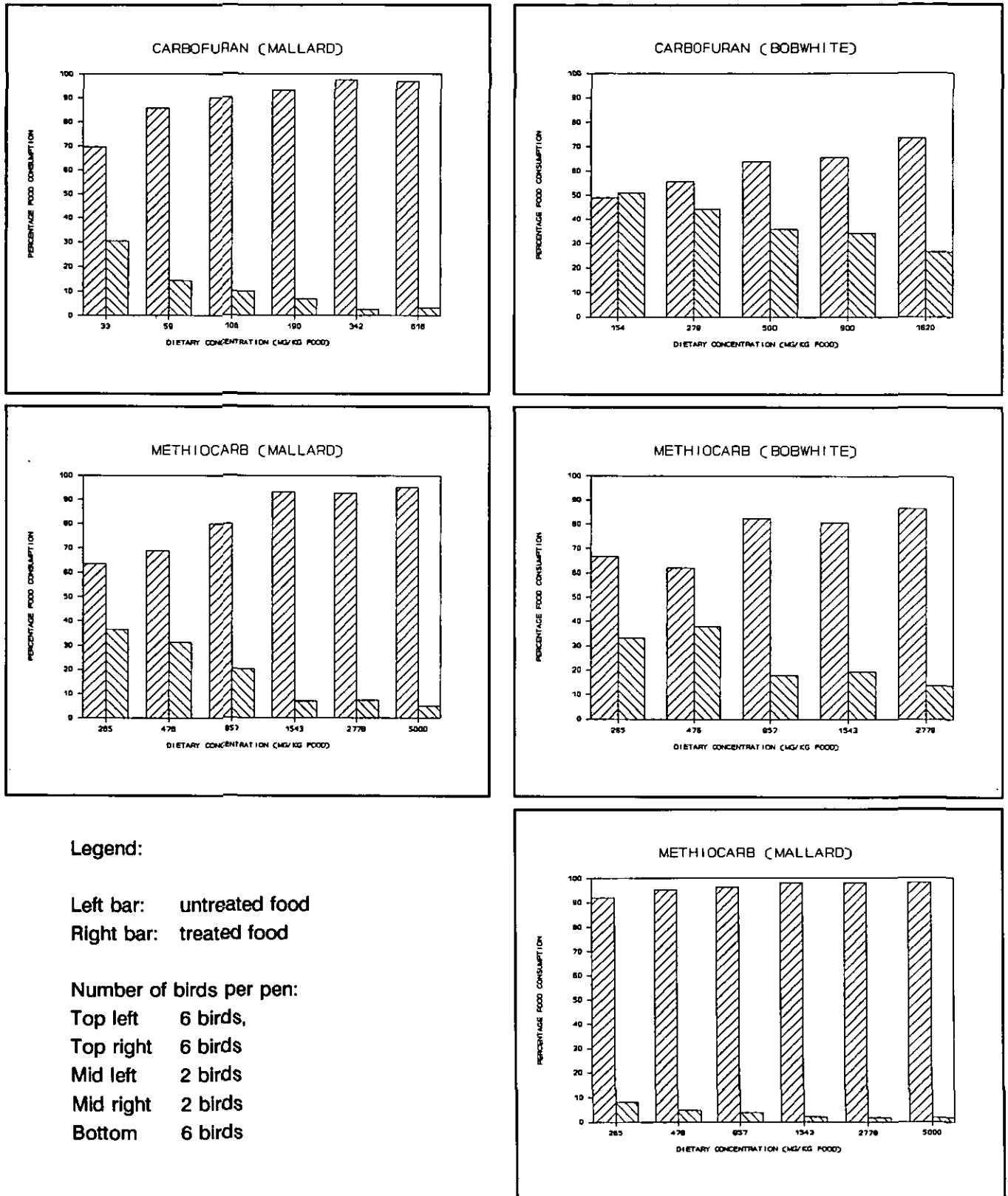


Table 8 Daily food intake in percentages of the Daily food intake of the control group according to the dose range.

Compound	Species	Ratio feeders T:UNT	Dose range (mg/kg food)	Birds/ pen	DFI range in % of control	Reference
Carbofuran	Bobwhite	1:1	154-1620	2	86 - 62	Kononen et al. 1987
	Bobwhite	1:1	520-2000	10	99 - 88	Bennett 1989A
	Bobwhite	5:5	520-2000	10	103 - 69	Bennett 1989A
	Bobwhite	9:1	520-2000	10	83 - 43	Bennett 1989A
	Mallard	1:1	33 - 616	2	87 - 74	Kononen et al. 1987
Chlorpyrifos	Bobwhite	1:1	40 -1280	10	108 - 102	Bennett 1989B
	Bobwhite	5:5	45 -4467	10	97 - 81	Bennett 1989B
	Bobwhite	9:1	45 -4467	10	104 - 50	Bennett 1989B
Methiocarb	Bobwhite	1:1	265-2778	3	88 - 72	Kononen et al. 1986
	Bobwhite	1:1	200-2000	10	106 - 89	Bennett 1989B
	Bobwhite	5:5	200-2000	10	99 - 74	Bennett 1989B
	Bobwhite	9:1	200-2000	10	97 - 50	Bennett 1989B
	Mallard	1:1	265-5000	2	84 - 61	Kononen et al. 1986
	Mallard	1:1	265-5000	6	108 - 78	Kononen et al. 1986
Methyl-parathion	Bobwhite	1:1	13 - 126	10	101 - 96	Bennett 1989B
	Bobwhite	5:5	20 - 200	10	95 - 19	Bennett 1989B
	Bobwhite	9:1	20 - 200	10	72 - 23	Bennett 1989B
Parathion	Bobwhite	1:1	50 - 200	10	99 - 76	Bennett 1989A
	Bobwhite	5:5	50 - 200	10	102 - 79	Bennett 1989A
	Bobwhite	9:1	50 - 200	10	82 - 28	Bennett 1989A

methiocarb and parathion found in the 1:1 choice did not differ from those in the 5:5 choice (224 and 222 mg/kg food for methiocarb and 33 and 38 mg/kg food for parathion). For carbofuran and methyl-parathion lower DT values were found in the 1:1 choice than in the 5:5 choice (170 versus 400 mg/kg food for carbofuran and 10 versus 45 mg/kg food for methyl-parathion). For chlorpyrifos a lower DT value was found in the 5:5 choice than in the 1:1 choice (26 versus 63 mg/kg food).

Little or no food reduction was noticed in the tests with parathion, chlorpyrifos and carbofuran. In the tests with methiocarb and methyl parathion a dose related decrease in food consumption was observed (Table 8). No dead birds were found in the tests with parathion and methiocarb, 1 dead bird in the test with chlorpyrifos and 8 dead birds with methyl-parathion at the highest concen-

tration. In the test with carbofuran 5 day 1 mortalities occurred and 3 dead birds were found on day 3 and 4 of the test.

In the studies of Bennett (1989A and 1989B) were Bobwhite were offered treated and untreated food in 10 feeders per cage (9:1) a dose related food avoidance was only found in the study with chlorpyrifos (DT = 71 mg/kg food). In the tests with the other compounds the birds did not discriminate between the feeders, except in the highest concentration tested with methiocarb and methyl-parathion. In all tests a dose related decrease in food consumption was noticed (Table 8). The responses in these tests (except chlorpyrifos) were comparable to the LC50 tests, i.e. mortality, food consumption and body weight changes. Although the birds were able to detect the presence of chlorpyrifos, some mortality did occur in the highest dietary concentrations in this test. In case of carbofuran 4 day 1 mortalities and 7 day 3-5 mortalities were observed and for methiocarb 1 day 1 and 4 day 3-5 mortalities.

The ability to detect and avoid lethal dietary exposures can be influenced by the number and relative proportion of treated and untreated food choices. The DT remained at sublethal concentrations in all chlorpyrifos tests, while increasing significantly in methyl-parathion tests as the number of feeders or relative proportion of treated feeders increased. The DTs of parathion, carbofuran and methiocarb remained at sublethal concentrations in the 1:1 and 5:5 tests, but increased significantly in the 9:1 test with methiocarb and no DTs could be estimated for the compounds parathion and carbofuran in the 9:1 tests. In contrast to the 1:1 and 5:5 tests, there was little or no discrimination in the 9:1 tests (except chlorpyrifos), and mortality approached levels observed in the LC50 tests, even though untreated food was available in ad libitum quantities.

Another important aspect to understanding food avoidance behaviour is the timing of formation of aversions. Bennett (1989A) noticed in the carbofuran choice tests that 50% of the mortality occurred on day 1, usually within the first few hours. Birds seemed to consume lethal quantities of the chemical before any food aversion was formed.

Kononen et al. (1986 and 1987) measured the food consumption of the test animals at each day of the food avoidance tests with methiocarb and carbofuran. The results of these measurements are graphically presented in Figure 3a and 3B. The Mallards exhibited definite avoidance behaviour on day 1 and throughout the remainder of the exposure periods. The Mallard test with methiocarb that utilized six birds per pen resulted in a stronger daily avoidance response

Figure 3A

Food consumption by Bobwhite and Mallards at a certain dietary concentration at each day of methiocarb food avoidance tests (Kononen 1986) in % of the control group.

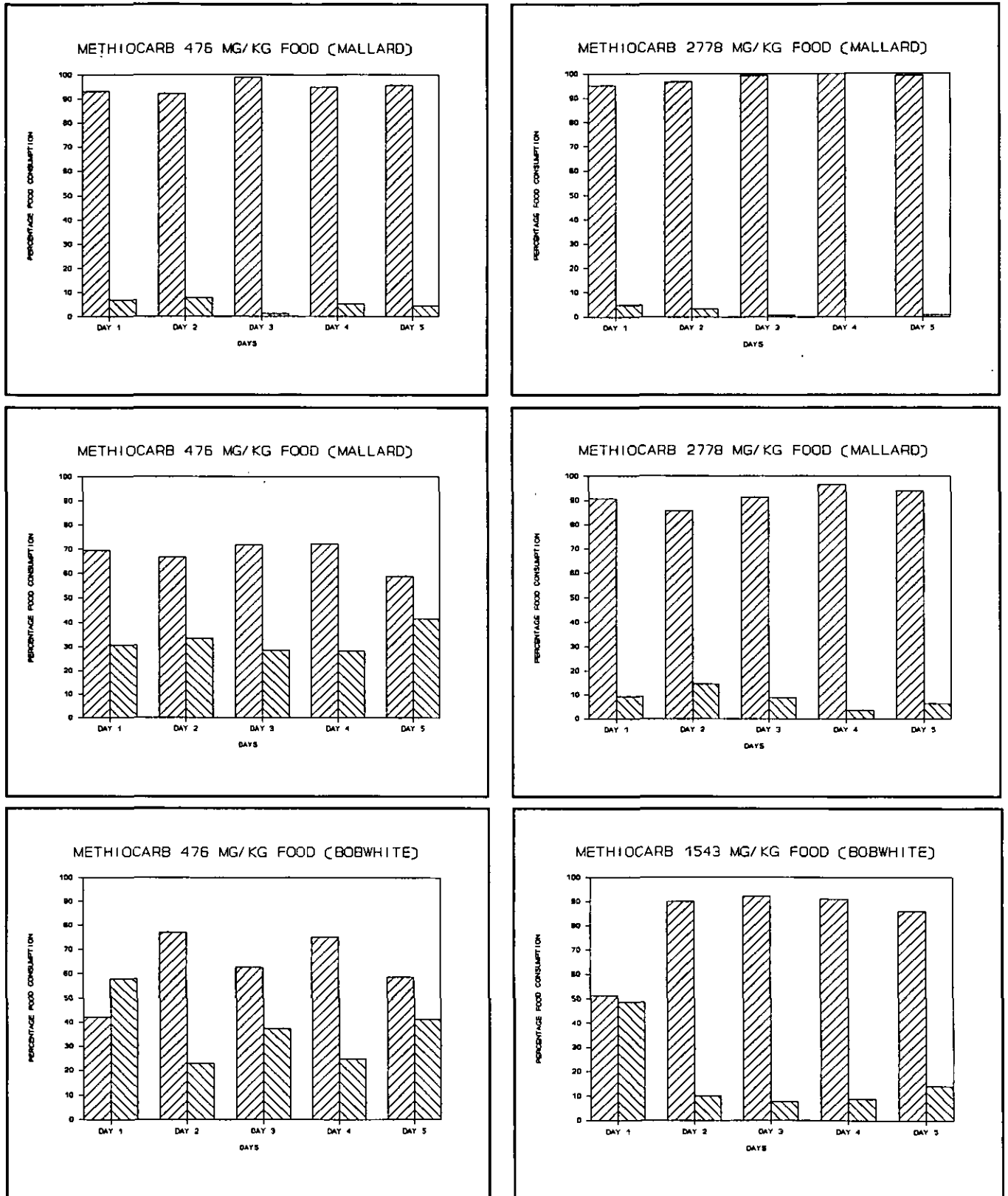
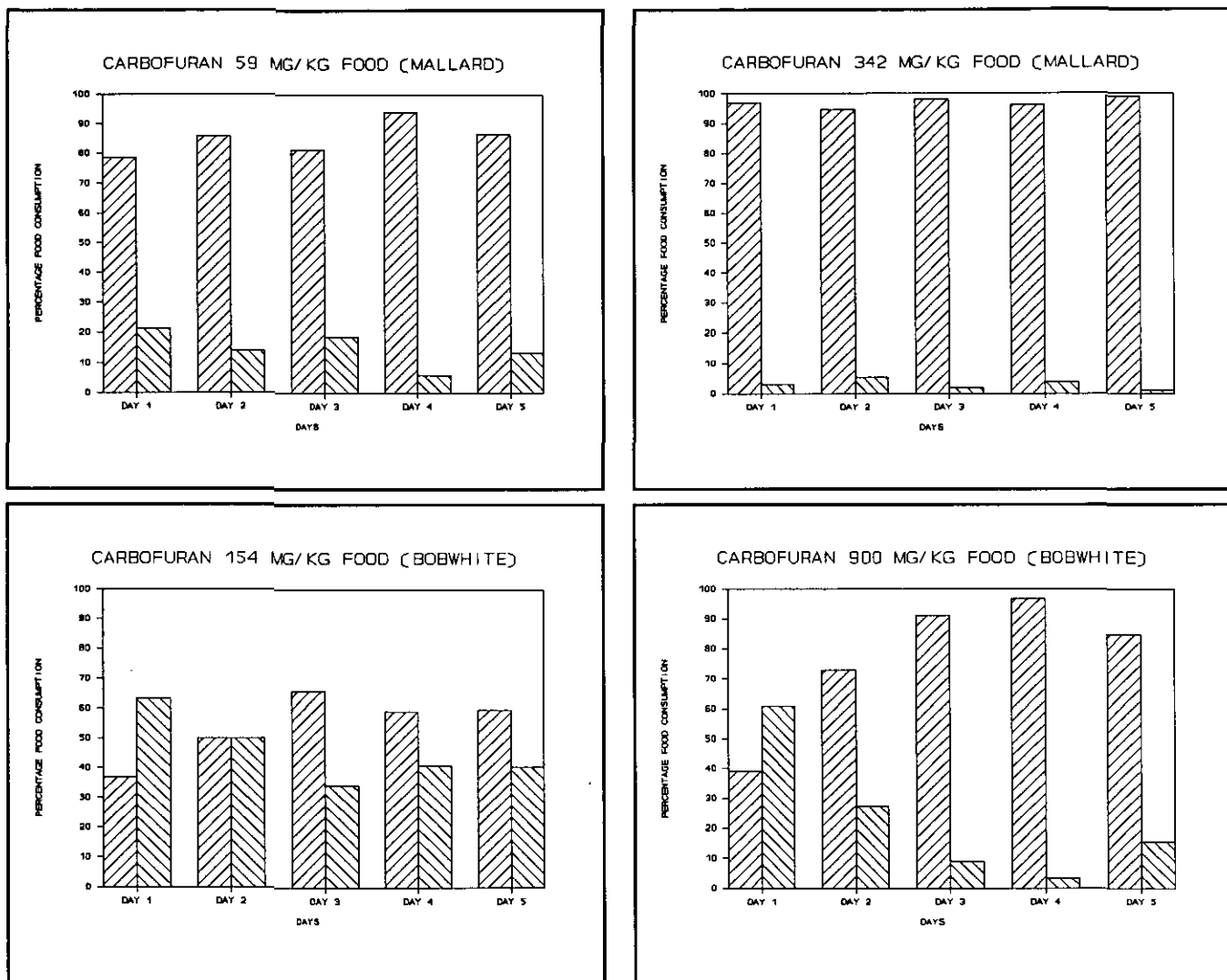


Figure 3B

Food consumption by Bobwhite and Mallards at a certain dietary concentration at each day of carbofuran food avoidance tests (Kononen 1987) in % of the control group.



Legend Figure 3A and 3B:

Left bar: untreated food
Right bar: treated food

Number of birds per pen:
Figure 3a top graphs: 6 birds
mid + bottom graphs: 2 birds
Figure 3b all graphs: 6 birds

than in the study with two birds per pen. Bobwhite demonstrated no avoidance behaviour on the first day of exposure, avoidance occurred on the second day of the exposure period and continued throughout the remainder of the exposure period. In three of the four examples they actually consumed more treated food than clean food (lowest carbofuran concentration is below the DT).

Discrimination thresholds can be used in the risk assessment by comparing these DTs with the concentrations that birds can encounter in the environment. It is assumed that the results of the Bobwhite can be used for the Common Quail (*Coturnix coturnix*). The diet of the Common Quail consists of small seeds and small insects (Glutz von Blotzheim et al., 1973). By using the relation between the concentration on crops/insects and the pesticide dosage immediately after application given by Hoerger and Kenaga (1972) and Kenaga (1973), an estimation of the environmental concentration can be made for the Common Quail (see Table 9).

Table 9 The predicted environmental concentrations (PEC) for Common Quails and the DTs for Bobwhite.

Compound	PEC ¹ (mg/kg food)	Discrimination thresholds (1:1)	
		Bennett	Kononen et al.
Carbofuran	73 - 218	159	170
Chlorpyrifos	22 - 116	63	
Methiocarb	7.3 - 29	224	96
Methyl-parathion	4.4 - 109	33	
Parathion	4.4 - 109	10	

1 = According to normal agricultural use in the Netherlands.

The lowest predicted environmental concentrations of the tested chemicals are below the DTs of these compounds. This would mean that Common Quails will not discriminate between treated and untreated food. Discrimination between treated and untreated food can occur in the highest predicted environmental concentrations, except for methiocarb (highest PEC below DT).

4. No Repellent Concentration (NoRC)

4.1. Introduction

Kononen et al. (1986 and 1987) and Bennet (1989A and 1989B) have defined the DT as the concentration in the treated food at which the birds are not discriminating between treated and untreated food when both types of food are available ad libitum. From the LC50 tests it is possible to estimate the concentration in the treated food at which the birds in the test will eat the same amount of treated food as the amount of (untreated) food in the control group. This discrimination threshold will be called the No Repellent Concentration (NoRC).

4.2. Calculation of NoRC

The dose-response relationship in the LC50 test is calculated using the log of the food consumption (FC) of the control group and the food consumption of the treated groups in gram per bird per day over the 5 days test period as the response-dependent variable, and the log of chemical concentration of treated food as the independent variable ($y = \log \text{ concentration in food}$, see also Figure 4). At dietary concentrations below the NoRC, the expected value of response, $y = \log(\text{FC}_{\text{control}}/\text{FC}_{\text{treated}})$, should equal 0, reflecting no difference between the food consumption in the control group and the treated group. The dose response relationship was calculated with the nonlinear regression analysis of Genstat 5 release 1.3 (Genstat 1988).

Because Kononen et al. (1986 and 1987) used the probit analysis for calculating the DT and Bennett and Schafer (1988) a two-phase regression analysis, the DTs are recalculated in the same way as for the NoRC values, using the log of the ratio of untreated to treated food consumption as the response-dependent variable ($y = \log(\text{untreated FC}/\text{treated FC})$). A description of the programs used in Genstat can be found in the appendix.

4.3. Results

In Table 10 the results of the calculations of the NoRCs for the LC50 studies and the food choice studies and the 95% confidence limits are presented (see also Figure 5).

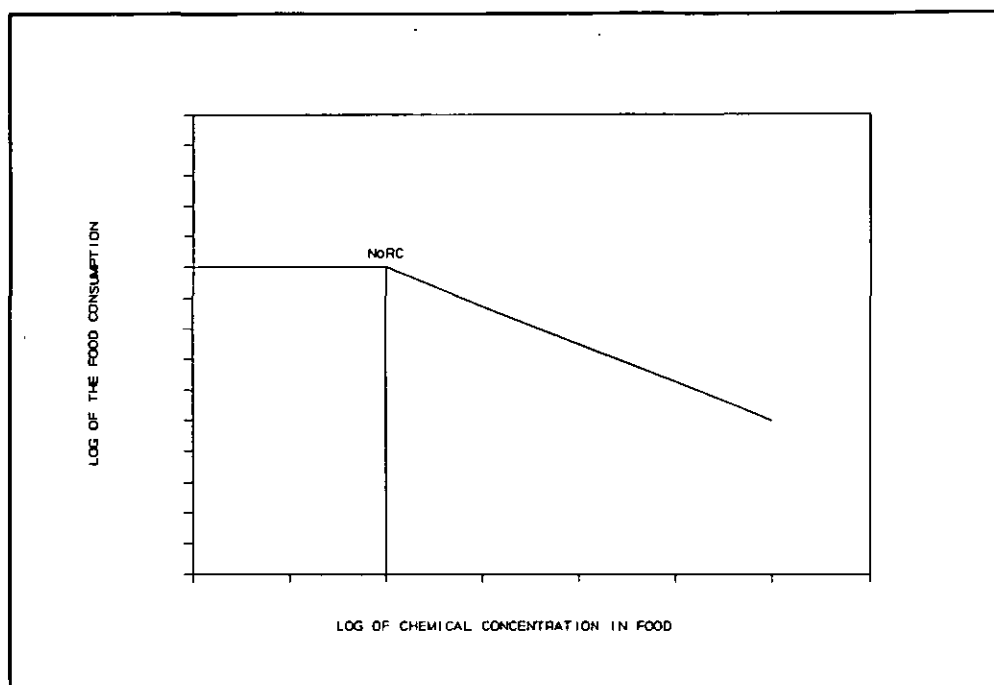


Figure 4 Relationship of the log of the dietary concentration and the log of the food consumption in the 2 phase regression model for the LC50 study.

Table 10 Calculated NoRC-values (mg/kg food)

Compound	Species	----- LC50 test -----		----- Choice test -----	
		NoRC	95% confidence limits	NoRC	95% confidence limits
Carbofuran	Bobwhite	67	4 - 1033	150	93 - 244
Carbofuran	Bobwhite	348	91 - 1329	167	7 - 4212
Carbofuran	Mallard	11	1 - 159	11	4 - 27
Chlorpyrifos	Bobwhite	213	138 - 303	48	18 - 130
Methiocarb	Bobwhite*	390	193 - 789	208	65 - 670
Methiocarb	Bobwhite	297	63 - 1395	98	11 - 898
Methiocarb	Mallard	138	33 - 571	159	66 - 382
Methyl-parathion	Bobwhite	23	18 - 27	10	5 - 20
Parathion	Bobwhite	53	21 - 133	33	7 - 151

* = data on highest concentration tested not used for the nonlinear regression analysis (outlier).

There is no scientific reason to believe that the NoRCs based on the LC50 studies are different from the NoRCs based on the food choice experiments. The 95% confidence intervals are overlapping to a high degree. There is a small tendency that the NoRCs based on the LC50 studies are a little higher than the NoRCs based on the food choice experiments (the mean ratio is 1.96).

The NoRC based on the LC50 study with Mallards for carbofuran is equal to the NoRC based on the food choice experiment. The NoRCs based on the LC50 study with Bobwhite for carbofuran and with Mallards for methiocarb are lower than the NoRCs based on the food choice experiments. The 6 other NoRCs based on the LC50 studies are higher than those based on the food choice experiments.

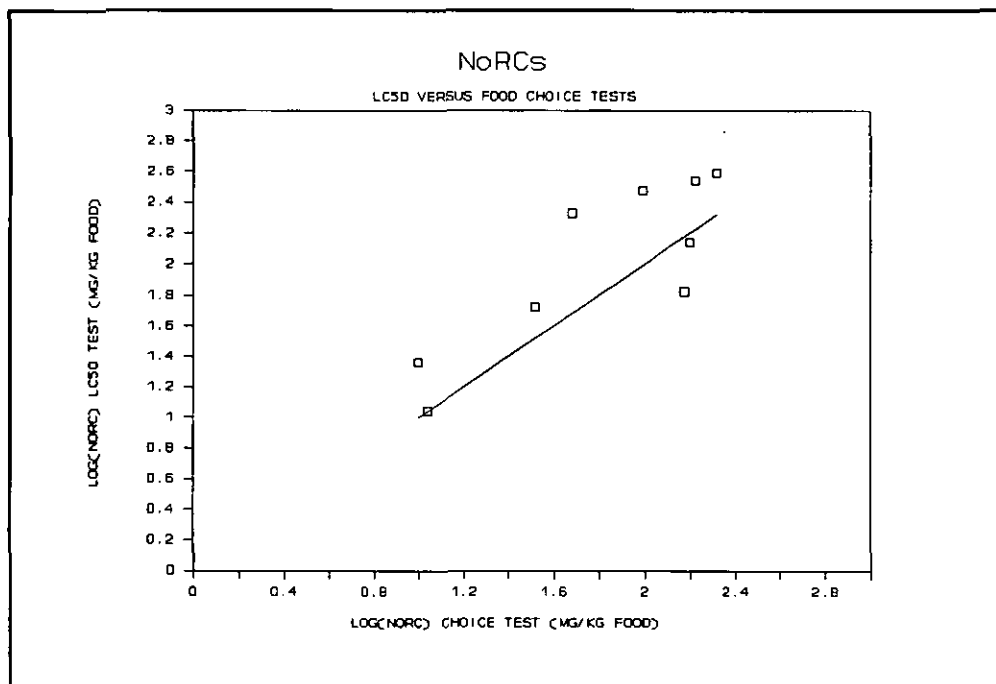


Figure 5 NoRC-values for the LC50 tests and the food choice tests.
(line in graph represents the case that the NoRC-values are equal).

5. Discussion and recommendations

From the studies analyzed in this report it is clear that the outcome of a LC50 test is not only the reflection of the intrinsic properties of the tested chemicals,

but also the reflection of the vulnerability of the species tested. The outcome is very often not the result of increasing dietary concentrations, but the result of decreasing food consumption. This food avoidance behaviour can be induced by repellent properties of the chemicals. Because repellency dictates sometimes to a high degree the outcome of the LC50 value, corrections for repellency in the hazard assessment must be applied with care.

When evaluating LC50 studies for for instance registration purposes it is necessary to pay attention to the following points:

- food consumption patterns,
- time dependent mortality and
- the No Repellent Concentration.

Food consumption patterns can give an impression of the role of repellent properties of the chemical. The time (in combination with changes in body weight) at which mortalities occur (day 1 versus day 3-5) gives information about whether the mortalities are caused directly by the chemical or are the result of starvation, or both. The No Repellent Concentration can be used for the hazard assessment by comparing this value with the predicted or measured concentration in the food of animals which they can encounter in their environment.

The INRA test is providing information about the mortality, toxic signs, body weight, food consumption and sometimes on gross pathology of two species of partridge exposed for 24 hours for two exposure conditions (100% treated and 50% treated and 50% untreated food; food given in feeders).

The BBA test is providing information about the mortality, toxic signs, behaviour, body weight and sometimes on gross pathology of two species (adult Japanese quail and Ring-necked Pheasant) exposed for respectively 8 hours or 7 days for two exposure conditions (75% treated and 25% untreated and 10% treated and 90% untreated food; food spread out on sandy floor of aviaries).

Both tests are only providing information about a certain formulation tested at the field concentration for a particular treatment, mostly a granulate or a seed treatment. Because only special treatments are tested, this information is hardly usable for the hazard assessment of a concentration on the food which a bird can encounter in the field after spraying.

The experiments carried out by Bennett (1989A and 1989B) and Kononen et al. (1986 and 1987) provide information about the mortality, toxic signs, body weight and food consumption and about the discrimination threshold of the species

tested, exposed for 5 days for one exposure condition (50% treated and 50% untreated food; food given in feeders). In the tests carried out by Bennett (1989A and 1989B) also the choice between 90% treated and 10% untreated food was investigated. The studies carried out by Kononen et al. (1986 and 1987) provide also information about the time when the test species is starting to show food avoidance behaviour, because they measured daily the food consumption.

The imperfection of these tests and also of the BBA and INRA tests is the choice between treated and untreated food (exposure treatments) in the experiment. In the field birds usually will have no choice at the treated area but have only the choice of eating and not eating (and looking for an other feeding place).

The standard LC50 test can provide already answers to some of the aspects of repellent properties of the compound tested:

- 1) the time at which the mortalities occur,
- 2) the body weight changes of the birds during the test,
- 3) the estimated food consumption, by weigh-back method, per pen for the exposure period and the postexposure period, and
- 4) the No Repellent Concentration.

By measuring the food consumption daily the learning abilities of the bird species can be investigated. Up to now the food consumption (mg/bird per day) is mostly based on a weigh-back method over the whole exposure period.

The LC50 test is strongly focused on the determination of the LC50 value. When the choice of the dosage range is also focused on the NoRC value better information can be made available for the calculation of the NoRC. By measuring the daily food intake of the birds during the range finding test the NoRC can be approximately determined. This data can be used for the design of the LC50 test.

These small alterations of the standard LC50 test make other repellency tests with birds superfluous, because the standard LC50 test is providing enough information about the repellent properties of the substance. Those repellency tests can be used to provide information about the edibility of the granulates and seeds (coated or pillorized, but not treated with a substance), which results in less suffering of test animals.

6. References

- BBA (1981) Richtlinie zur Prüfung von Pflanzenbehandlungsmitteln auf Vogel gefährdung - Annahmeveruche -. Richtlinien für die amtliche Prüfung von Pflanzenbehandlungsmitteln 25-1. Biologische Bundesanstalt für Land- und Forstwirtschaft, Berlin, Germany.
- Bennett, R.S. and D.W. Schafer (1988) Procedure for evaluating the potential ability of birds to avoid chemically contaminated food. *Environ. Toxicol. Chem.* 7: 359-362.
- Bennett, R.S. (1989A) Factors influencing discrimination between insecticide-treated and untreated foods by northern bobwhite. *Arch. Environ. Contam. Toxicol.* 18: 697-705.
- Bennett, R.S. (1989B) Role of dietary choices in the ability of bobwhite to discriminate between insecticide-treated and untreated food. *Environ. Toxicol. Chem.* 8: 731-738.
- Conover, M.R. (1984) Response of birds to different types of food repellents. *J. Appl. Ecol.* 21: 437-443.
- Genstat (1988) Genstat 5 Release 1.3 (DOS/386) Implemented by Marketing Risk Management. Copyright 1988. Lawes Agricultural Trust (Rothamsted Experimental Station).
- Grue, C.E. (1982) Response of common grackles to dietary concentrations of four organophosphate Pesticides. *Arch. Environm. Contam. Toxicol.* 11: 617-626.
- Hoerger, F.D. and E.E. Kenaga (1972) Pesticides residues on plants, correlation of representative data as basis for estimation of their magnitude in the environment. *Environmental Quality*. Academic Press, New York, I: 9-28.
- INRA (1990) Methodologie d'acceptation de nourriture ou de semences traitees par une substance repulsive, par des oiseaux en conditions de captivite. G. Grolleau, 1/06/1990, Laboratoire de phytopharmacie unite d'eco-etho-toxicologie, Versailles Cedex.
- Kenaga, E.E. (1973) Factors to be considered in the evaluation of toxicity of pesticides to birds in their environment. *Environmental Quality and Safety*, Academic Press, New York, II: 166-181.
- Kononen, D.W., J.R. Hochstein and R.K. Ringer (1986) A quantitative method for evaluating avian food avoidance behavior. *Environ. Toxicol. Chem.* 5: 823-830.
- Kononen, D.W., J.R. Hochstein and R.K. Ringer (1987) Avoidance behavior of mallards and northern bobwhite exposed to carbofuran-contaminated food

- and water. *Environ. Toxicol. Chem.* 6: 41-50.
- Kononen, D.W. (1988) Two methods for evaluating avian food avoidance data. *Bull. Environ. Contam. Toxicol.* 40: 619-625.
- Luttik, R. (1992) Environmental hazard/risk assessment of pesticides used in agriculture for birds and mammals. The Dutch concept. Part 1. Introduction and synopsis of the decision scheme. RIVM-report 679101006, pp 30.
- OECD (1984) Avian Dietary Toxicity Test. OECD Guideline for testing of chemicals number 205 (Adopted 4 April 1984). Paris, France.
- Starr, R.I., J.F. Besser and R.B. Brunton (1964) A laboratory method for evaluating chemicals as bird repellents. *Agr. Food Chem.* 12: 342-344.
- Tigner, J.R. and J.F. Besser (1962) A quantitative method for evaluating chemicals as rodent repellents on packaging materials. *Agr. Food. Chem.* 10: 484-486.

Appendix

Programs used for calculating NoRC values with Genstat 5 Release 1.3 (DOS/-386) Implemented by Marketing Risk Management. Copyright 1988, Lawes Agriculture Trust (Rothamsted Experimental Station).

A) Program for calculating the NoRC with data from LC50 experiments.

```

job " program for estimating the NoRC from LC50 experiment"
"conc = dietary concentration"
"intake = food consumption"
open name = 'foodup.dat'; filetype = input; ch = 2
read [ch = 2; setnval = yes] conc, intake
print conc, intake
scal unitlength, beta, NoRC, alpha
calc unitlength = nval(conc)
calc alpha = elem(intake; 1)
calc beta = 0 & NoRC = 1
calc logconc = log10(conc + 0.0001 * (conc.eq.0))
calc logintake = log10(intake)
expression mu2; value = !e(fitted = alpha \
+ beta * (logconc - NoRC) * (logconc.gt.NoRC))
model logintake; fitted = fitted
rcycle parameter = alpha, beta, NoRC
fitnonlinear [calculation = mu2; print = model, s, est, fitted]
grap [ncol = 61; nrow = 16] logintake, fitted; logconc; method = p, l
stop

```

B) Program for calculating the NoRC with data from food avoidance experiments.

```

job "program for estimating the NoRC from food avoidance experiments"
"conc = dietary concentration"
"clean = clean food consumption"
"treated = treated food consumption"
open name = 'unttrt.dat'; filetype=input; ch=2
read [ch=2;setnval=yes] conc,clean,treated
calc unttrt=clean/treated
print conc,clean,treated,unttrt
scal unitlength,beta,NoRC
calc unitlength=nval(conc)
calc logunttrt=log10(unttrt)
calc beta=0 & NoRC=1
calc logconc=log10(conc+0.0001*(conc.eq.0))
calc logunttrt=logunttrt+0/logunttrt
expression mu1,value=!e(fitted=beta*(logconc-NoRC)*(logconc.gt.NoRC))
model logunttrt;fitted=fitted
rcycle parameter=beta,NoRC
fitnonlinear [calculation=mu1;print=model,s,est,fitted]
grap[ncol=61;nrow=16] logunttrt,fitted;logconc;method=p,l
stop

```