March 2001

Case Study 1 : Foliar Insecticide I

Monte Mayes

John D. Eisemann
USDA/APHIS/WS National Wildlife Research Center, John.D.Eisemann@aphis.usda.gov

Alain Baril

Tony Hawkes

Liesbeth Heijink

See next page for additional authors

Published in *Avian effects assessment: a framework for contaminant studies*. A. Hart, D. Balluff, R. Barfknecht, P. F. Chapman, T. Hawks, A. Leopold, and R. Luttik, editors. SETAC Press, Pensacola, Florida, USA, 2001.

http://www.setac.org/htdocs/pubs/avianeffects.html

http://www.setac.org/

CHAPTER 4

Case Study 1: Foliar Insecticide I

Monte Mayes, John Eisemann, Alain Baril,
Tony Hawkes, Liesbeth Heijink, Peter Lawlor

Case Study Reports

A major part of the Woudschoten workshop was conducted in the form of breakout groups dealing with a case study each. A case study consists of a set of test results, data, and information on a fictitious pesticide which could be part of an application for authorization. The groups were asked to take the role of regulators assessing the risk to birds for a specified use. Basic data packages for each case containing a description of the use, standard toxicity data, and background information were prepared by the case study authors and distributed in advance. The groups were then asked to apply the framework to their case step by step. When the uncertainty turned out to be too high, the group selected further information and higher-tier data that would be required for the assessment. The authors of the case studies were prepared to provide such additional data which then were subject for the discussion in the further rounds. Thus, Steps 3 to 6 forming a loop in the flowchart (Figure 1-1) might have been run through several times.

A basic idea of the framework is the separate consideration of 3 timescales: short-term, medium-term, and long-term. (This nomenclature was adopted during the workshop, although in the data provided and in these reports sometimes other terms are used, e.g., "acute exposure" instead of "short-term exposure" or "dietary toxicity" instead of "medium-term toxicity"). In each case study, different combinations of timescale and exposure route could have been identified as relevant. Due to time constraints, however, the groups often focused only on certain scenarios.

The framework structure was not fully established when the groups started working and was modified as a result of discussions during the workshop. Therefore, the case studies did not precisely follow the final version of the framework. However, the case study reports are designed to show how the sequence of assessment related to Steps 1 to 6 of the final framework.

The case studies were used to tune the framework concept and develop the effects assessment procedure. There was insufficient time to complete every aspect of the

Avian Effects Assessment: A Framework for Contaminants Studies. Andy Hart et al., editors.
 ISBN 1-880611-48-1

assessments, or resolve all issues to every participant's satisfaction. Therefore, the case studies should not be regarded as providing definitive guidance on specific issues.

Finally, it must be stressed that the subject of the workshop was effects assessment, so exposure issues were not dealt with in depth.

Case Study 1 presented an evaluation of the effects of an acutely toxic spray to avian wildlife.

Basic Data

General information and use patterns

Function:	Insecticide spray: wheat, corn, multiple pests
Mode of action:	Acetylcholinesterase (AChE) inhibitor
Type and composition of formulation:	Sprayable liquid
Application rate:	Typical rate 1.2 kg a.i./ha (maximum of 2 applications, minimum 14 days apart)
Application method:	Aerial or ground

Physical and chemical properties

Water solubility:	8 mg/L
Aqueous hydrolysis at 25°C:	Average half-life 65 days
Log K_{ow}:	4 to 5
K_d:	175
K_{oc}:	7500
Vapor pressure:	1.1 x 10^{-5} mm Hg (25°C)
Aerobic soil metabolism:	Average half-life 45 days (25°C)
Soil photolysis:	half-life 25 days (25°C)

Avian toxicity

Acute oral toxicity

Method:	USEPA 71-1
Species:	Bobwhite quail
Age:	14 weeks old
Birds per treatment:	5 males + 5 females
Test material:	Technical grade of a.i. (99%)

Mortality:	See Table 4-1
Observations:	Signs of toxicity, such as ataxia and wing droop, were observed within 3 hours at 75 mg/kg and at 8 hours at 45 mg/kg. Surviving birds at all treatments recovered by 5 days post-dosing. Food consumption was reduced at 27 mg/kg and higher and was associated with a significant reduction in weight gain. Mortality occurred within 3 days of dosing with 100% mortality at 75 mg/kg within 24 hours. Mortality at other doses ceased 72 hours post-dosing.
LD50:	31.6 mg/kg (25 to 40)
Probit slope:	6.77

Acute dietary toxicity - Mallard

Method:	USEPA 71-2
Species:	Mallard duck
Age:	8 days old
Birds per treatment:	10
Exposure period:	5 days on treated food, 3 days on control diet.
Test material:	Technical grade of a.i. (99%)
Mortality:	See Table 4-2
Observations:	Food consumption was dramatically reduced at 300 ppm and higher. Average food consumption for the controls was 27% of body

Table 4-1 Mortality in acute oral test on Bobwhite quail

Dose level (mg/kg)	Mortality on days after dosing										
	1	2	3	4	5	6	8	10	12	14	Total
0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0
27	0	3	1	0	0	0	0	0	0	0	4
45	5	3	0	0	0	0	0	0	0	0	8
75	10	0	0	0	0	0	0	0	0	0	10

Table 4-2 Mortality in dietary toxicity test on Mallard duck

Treatment (ppm)	Cumulative mortality Day							
	1	2	3	4	5	6	7	8
75	0/10	0/10	0/10	0/I0	0/10	0/10	0/10	0/10
150	0/10	0/10	1/10	1/10	2/10	3/10	3/10	3/10
300	0/10	0/10	2/10	3/10	4/10	4/10	4/10	4/10
600	0/10	0/10	3/10	4/10	5/10	5/10	6/10	6/10
1200	0/10	2/10	6/10	8/10	8/10	8/10	10/10	10/10

weight per day. Birds at the 1200 ppm treatment level consumed approximately 8% of their body weight per day. Birds at the 75 ppm treatment level had food consumption similar to the controls. There was a decrease in body weight at the 150 ppm treatment level and greater. Birds that died during the test had partially empty to empty gastrointestinal tract. Signs of intoxication included wing droop, loss of coordination, and ruffled appearance.

LC50: 342 ppm (229 to 525)
Probit slope: 2.67

Dietary toxicity - Bobwhite

Method: USEPA 71-2
Species: Bobwhite quail
Age: 9 days old
Birds per treatment: 10
Exposure period: 5 days on treated food, 3 days on control diet.
Test material: Technical grade of a.i. (99%)
Mortality: See Table 4-3
Observations: At the 75 ppm dose level all birds were normal in appearance and behavior throughout the test period. At the 150 ppm dose level a few birds exhibited "hyperexcitibility" on days 3 and 4, followed by lethargy on days 5 and 6. There was a reduction of food consumption at 300 ppm and higher accompanied by a reduction in weight gain. Surviving birds at all dose levels appeared normal by day 7 (see Table 4-3).
LC50: 364 ppm (263 to 507)
Probit slope: 4.06

Reproductive toxicity - Mallard

Method: USEPA Method 72-4
Species: Mallard duck
Age: Approaching first laying season
Test substance: Technical grade of a.i. (99%)
Dose groups: 25, 75, and 125 ppm
Birds per Treatment: 16 male + 16 female

Table 4-3 Mortality in dietary toxicity test on Bobwhite quail

Treatment (ppm)	Cumulative mortality Day							
	1	2	3	4	5	6	7	8
75	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
150	0/10	0/10	0/10	0/10	1/10	1/10	1/10	1/10
300	0/10	0/10	0/10	1/10	3/10	3/10	3/10	3/10
600	0/10	0/10	2/10	4/10	7/10	8/10	8/10	8/10
1200	0/10	0/10	2/10	6/10	8/10	10/10	10/10	10/10

Exposure period: 20 weeks
Results: Birds at the 25 ppm treatment level showed no signs of toxicity and had no reproductive impairment based on the evaluation criteria. There were no signs of toxicity at the 75 ppm treatment level. At this concentration there was a reduction in food consumption and weight gain compared to the controls and a reduction in the number of eggs laid and the number of 14-day-old survivors per hen. The top dose of 125 ppm resulted in frank toxicity to the adults resulting in 36% mortality, reduced food consumption, and weight gain; additionally, there was a clear reduction in eggs laid, viable embryos, and 14-day-old survivors per hen. The no-observed-effect concentration (NOEC) was determined to be 25 ppm.

Reproductive toxicity - Bobwhite

Method: USEPA Method 72-4
Species: Bobwhite quail
Age: Approaching first laying season
Test substance: Technical grade of a.i. (99%)
Dose groups: 25, 75, and 125 ppm
Birds per treatment: 16 male + 16 female
Exposure period: 21 weeks
Results: Birds at all treatment levels showed no signs of toxicity and had no reproductive impairment based on the evaluation criteria. The NOEC was determined to be 125 ppm.

Mammalian toxicity

Oral LD50 mouse: 160 mg/kg
Oral LD50 rat: 185 mg/kg
Inhalation LC50 rat: 1000 mg/m^3 (= 1 mg/L)
Dermal LD50 rabbit: >5000 mg/kg

Exposure estimates

Initial estimates are based on application rate and vegetation residue analysis of Fletcher et al. (1994). Focal species were selected to represent species of the type of birds that may occur in the agroecosystem of concern and do not necessarily represent the most likely species to be encountered in such ecosystems.

Focal species

Pheasant, 1.1 kg (omnivore), and Sparrow, 25g (omnivore)

Food consumption

Values for these species are based on Nagy (1987), and the diet composition is based on Martin et al. (1951).

Exposure characterization - Pheasant

Dietary intake (grams dry weight) for a 1.1 kg bird is estimated at 58 grams per day.

Food Intake [FI] (g dry weight/day) = $0.302\ Wt.^{0.751}$ (Equation 4-1).

In spring and summer the diet consists of approximately 60% seeds, 20% foliage, and 20% insects.

Predicted concentrations on these food items based on a maximum application rate of 2.4 kg/ha are 28 ppm (seeds), 576 ppm (foliage), and 28 ppm (insects). Note: These are wet-weight values; insects are assumed to have a concentration similar to seeds.

Based on the above data, and assuming all food items came from the treated area, exposure can be estimated (see Table 4-4).

For the pheasant total exposure is estimated to be ~28 mg/kg/day.

Exposure characterization - Sparrow

Dietary intake (grams dry-weight) for the sparrow bird is estimated to be 7 grams.

Food Intake [FI] (g dry-weight/day) = $0.398\ Wt.^{0.850}$ (Equation 4-2).

In spring and summer, the diet consists of 70% insects and 30% seeds. Predicted concentrations on these food items based on a 1.2 kg/ha application rate are 28 ppm (seeds and insects). Note: these are wet-weight values.

Based on the above data, and assuming all food items came from the treated area, exposure can be estimated (see Table 4-5).

For the 25 g sparrow exposure is estimated to be ~58 mg/kg/day.

Table 4-4 Estimation of exposure in pheasant based on collected data and assuming all food items came from the treated area

Diet component	Proportion in diet (%)	% of dry matter	Consumption* (g/day)	Residues mg/kg food	Toxicant intake mg/day
Foliage	20	25	46	0.576	26.5
Insects	20	10	116	0.028	3.2
Seeds	60	88	40	0.028	1.1
Total					30.8

* Based on total consumption of 58 grams/day

Table 4-5 Estimation of exposure in sparrow based on collected data and assuming all food items came from the treated area

Diet component	Proportion in diet (%)	Percent (%) of dry matter	Consumption* (g/day)	Residues mg/kg food	Toxicant intake mg/day
Insects	70	10	49	0.028	1.37
Seeds	30	88	2.4	0.028	0.067
Total					1.437

* Based on total consumption of 7 grams/day

Framework Analysis

Step 1: Problem formulation

Issue: Evaluation of the product concept allowed the group to judge if an effects assessment will be required.

Reason: To determine the minimum dataset needed

Results: Table 4-6 exemplifies the thought process of our team during this evaluation.

Conclusion: Based on the use pattern, environmental fate, and potential toxicity of this compound, the group determined that the insecticide might present an unacceptable acute and chronic hazard to birds inhabiting corn and wheat agroecosystems. Therefore an effects assessment was required.

Three specific exposure scenarios were identified:

1) acute exposure (exposure within the first 24 hours of application),
2) subchronic exposure (exposure between 2 and 28 days), and
3) exposure during reproduction (within the acute-to-subchronic time frame).

Table 4-6 Step 1 considerations of product concept by the case study group

Property	Parameter	Implication	Determination
Application rate	Environmental loading	Magnitude of exposure	Potential for moderate–high level of exposure
Application frequency and interval	Environmental loading	Magnitude and temporal characteristics of exposure	Must consider acute (1 day) and subchronic (2–28 days) exposure
Application method	Characteristic of chemical deposition	Identification of potential route(s) of exposure	Oral exposure through contaminated food and water; dermal and inhalation exposure also possible
Designated crops	Define season of application and agroecosystems	Identification of focal species and life stages potentially at risk	Birds that live in and beside corn and wheat habitats potentially at risk; timing suggests need to assess reproductive effects
Chemical class	Known or unknown mechanism of action	May provide intuitive perspective on potential hazard	Pesticides that exhibit AChE inhibition activity have relatively high toxicity to birds
Environmental fate properties	Definition of magnitude and duration of residues	With application rate and application interval, will provide a refined estimate of the temporal exposure characteristics	Must consider acute (1 day), subchronic (2–28 days); exposure to metabolites possible

Acute Exposure

Based on the use pattern, environmental fate, and potential toxicity of this compound, the group determined that it may present an unacceptable acute risk to birds inhabiting corn and wheat agroecosystems. Birds may consume acutely toxic amounts associated with contaminated food items. Therefore, an acute effects assessment is required.

Step 2: Obtain minimum dataset for initial assessment

Issue: Assess acute oral toxicity
Reason: Understand intrinsic toxicity to birds
Outcome: Data on at least 1 species required. (Note: If limit test indicates LD50 > 2,000 mg/kg, proceed with assessment using LD50 = 2,000 mg/kg as the toxicity value.) Data from base dataset indicated a LD50 of 31.6 mg/kg for the northern bobwhite.
Issue: Is acute dermal or inhalation toxicity a concern?
Reason: Potential for dermal and inhalation exposure.
Outcome: Use mammalian data if available. Rabbit and rat data indicate low concern for dermal and inhalation toxicity, respectively.

Steps 3 and 4: Effects and risk assessment

At the onset of this exercise several issues related to exposure were discussed. They included factors such as variable agronomic practices, dietary choices of avian species, percent of diet from treated area, percent of time birds spend in the treated area, and residue decline over time. Although these topics were considered, they were relegated to a more detailed exposure assessment and not considered within the context of this case study.

Issue: Initial hazard assessment with regard to acute oral exposure
Reason: Determine if additional data are needed
Outcome: Initial acute exposure values were based on the highest potential residue data (the worst-case 95th percentile values) of Fletcher et al. (1994), determined for the maximum application rate for the pesticide, 2.4 kg/ha. N is the cumulative number of LD50 values used in the assessment. The LD50 row provides the actual LD50 for that species. The geometric-mean row provides the geometric mean of the LD50s as they become available. The safety factors (SFs) are those provided by Luttik and Aldenberg (1997). The estimated exposure values for the pheasant and house sparrow are from the initial data for the case study. Risk quotients (RQs) are the ratio of the estimated exposure over the chosen toxic endpoint (i.e., the predicted 5th percentile of the species sensitivity distribution). Table 4-7 illustrates this initial assessment.

Table 4-7 Risk quotient calculation, 1 species (Bobwhite)

N	1
Species	Bobwhite
LD50 (mg/kg)	31.6
Geometric mean (mg/kg)	32
SF - 5th percentile (median estimate)	5.7
SF - 5th percentile (left 95% CL)	33
Pred. 5th percentile - Median estimate (mg/kg)	5.5
Pred. 5th percentile - 95 % left CL (mg/kg)	1
Estimated exposure - Pheasant (mg/kg/day)	28
Estimated exposure - Sparrow (mg/kg/day)	58
RQ Pheasant - 5th percentile (median)	5.1
RQ Pheasant - 5th percentile (left 95 % CL)	28
RQ House Sparrow - 5th percentile (median)	10.5
RQ House Sparrow - 5th percentile (left 95 % CL)	58

CL = confidence limit

Interpretation of the RQ involves comparison with a level of concern (LOC) of 1; this value was arbitrarily chosen by the group for the purpose of the case study. This approach represents a move away from the traditional LOCs of 0.2 in the U.S. and 0.1 in Europe. It was chosen to illustrate that the uncertainty related to interspecies variability was accounted for with the use of the safety factors above. It should be pointed out, however, that we did not account for other sources of uncertainty, such as age or sex of the birds. Our choice of a LOC of 1 reflects the necessity of accounting for the dominant sources of variability in an explicit manner early on in the risk calculations and not leaving it to be dealt with by using 1 arbitrary factor at the very end.

Using data on 1 species the RQs indicate that exposure is expected to be well above the predicted 5th percentile of the species sensitivity distribution.

Step 5: Is risk acceptable?

The Phase I assessment indicated a potential for adverse effects. Hazard was judged to be unacceptable, and an analysis was conducted to select studies to reduce uncertainty in the evaluation. Several factors were identified, which, if addressed, could increase the confidence of the assessment. They are listed in Table 4-8.

Step 6: Select and conduct appropriate studies to reduce uncertainty

It was determined that an additional LD50 test, either an approximate lethal dose (ALD) or dose-response test, with a different species would provide the greatest benefit for further analysis.

Table 4-8 Sources of uncertainty identified for Step 5 of Case Study 1

Type of uncertainty	Relative importance	Options for refined assessment
Interspecific variability in sensitivity	High; represents a major source of variation. Specific concern for altricial vs. precocial species.	Request additional LD50 test(s) to decrease uncertainty.
Intraspecific variability in sensitivity (age, development stage)	Moderate to high; variability may be accounted for in the factor accounting for interspecific variability.	In this assessment, variability is presumed to be accounted for by use of Luttik and Aldenberg's safety factors.
Potential variability in individual sensitivity (slope of the response) might be greater or lesser in non-tested species	Moderate	In higher-tiered assessments with sufficient dose-response data, this variability can be accounted for by assessing the standard error of the slope.

Additional Data I

Acute oral toxicity

Method: USEPA 71-1
Species: House Sparrow
Age: Adult
Birds per treatment: 5 males + 5 females
Test material: Technical grade of a.i. (97.8%)
Observations: (See Table 4-9) Signs of toxicity such as ataxia and wing droop were observed within 30 minutes to 1 hour following dosing 2 females in the 19 mg/kg dose group. The birds recovered by day 1 and remained so for the duration of the study. Three birds in the 38 mg/kg dose group showed signs of intoxication within 30 minutes of dosing. All birds were normal by day 1 and remained so for the remainder of the study. All birds at the 75 mg/kg dose group showed signs of toxicity within 10 minutes of dosing. Surviving birds were normal in appearance and behavior by day 2. Birds in the 150 and 300 mg/kg dose groups showed signs of toxicity within 10 to 12 minutes of dosing. Mortality occurred within 4 hours of dosing with the exception of 1 bird at the 300 mg/kg dose group. All surviving birds were normal in appearance and behavior by day. There was no apparent difference in weight between the controls and surviving birds.
LD50: 120 mg/kg (25 to 40)
Probit slope: 2.3

Table 4-9 Mortality in acute oral toxicity test on House sparrow

Dose level (mg/kg)	Cumulative mortality on days after dosing											
	0	1	2	3	4	5	6	8	10	12	14	Total
0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
38	1	1	1	1	1	1	1	1	1	1	1	1
75	4	4	4	4	4	4	4	4	4	4	4	4
150	7	7	7	7	7	7	7	7	7	7	7	7
300	6	7	7	7	7	7	7	7	7	7	7	7

Framework Analysis, Round 2

Step 3 & 4: Revision of effects and risk assessment

Issue: Will additional data provide a satisfactory assessment of hazard?
Outcome: An assessment was conducted as above using 62 mg/kg as the effect value. This was calculated as the geometric mean of the bobwhite LD50 (36.1 mg/kg) and the sparrow LD50 (120 mg/kg). Table 4-10 provides more details.

Using data for 2 species, the RQs suggest that exposure is still expected to be greater than the predicted 5th percentile of the species sensitivity distribution. However, there was a significant narrowing of the gap between the median estimate and the 95% left confidence on the estimate.

Table 4-10 Risk quotient calculation, 2 species (Bobwhite and House sparrow)

Parameter	1 Species	2 Species
Species	Bobwhite	Bobwhite / House sparrow
LD50 (mg/kg)	31.6	31.6 / 120
Geometric mean (mg/kg)	32	62
SF - 5th percentile (median estimate)	5.7	5.7
SF - 5th percentile (left 95% CL)	33	19
Pred. 5th percentile - Median estimate (mg/kg)	5.5	10.8
Pred. 5th percentile - 95 % left CL (mg/kg)	1	3.2
Estimated Exposure - Pheasant (mg/kg/day)	28	28
Estimated Exposure - Sparrow (mg/kg/day)	58	58
RQ Pheasant - 5th percentile (median)	5.1	2.6
RQ Pheasant - 5th percentile (left 95 % CL)	28	8.8
RQ House Sparrow - 5th percentile (median)	10.5	5.4
RQ House Sparrow - 5th percentile (left 95 % CL)	58	18

CL = confidence limit

Step 5: Is risk acceptable?

The addition of another species decreased the RQ values but did not eliminate the presumption of risk.

Step 6: Select and conduct appropriate studies to reduce uncertainty.

It was determined that an additional LD50 test, either an ALD or dose-response test, with a different species would provide the greatest benefit for further analysis.

Additional Data II

Mallard LD50 75 mg/kg (2-to 4-month-old birds) with standard test methodology; no additional information was provided.

Framework Analysis, Round 3

Steps 3 and 4: Revision of effects and risk assessment

The additional LD50 value allowed the calculation of a new geometric mean and the use of a different safety factor for estimating the LD50 for left 95% confidence interval (CI) of the 95th percentile from the distribution of Luttik and Aldenberg (1997). The calculation of the RQ values is presented in Table 4-11.

Table 4-11 Risk quotient calculation, 3 species (Bobwhite, Sparrow, Mallard)

Parameter	1	2	3
Species	Bobwhite	Bobwhite / Sparrow	Bobwhite / Sparrow / Mallard
LD50 (mg/kg)	31.6	31.6 / 120	31.6 / 120 / 75
Geometric mean (mg/kg)	32	62	66
SF - 5th percentile (median estimate)	5.7	5.7	5.7
SF - 5th percentile (left 95% CL)	33	19	15.6
Pred. 5th percentile - Median estimate (mg/kg)	5.5	10.8	11.5
Pred. 5th percentile - 95 % left CL (mg/kg)	1	3.2	4.2
Estimated exposure - Pheasant (mg/kg/day)	28	28	28
Estimated exposure - Sparrow (mg/kg/day)	58	58	58
RQ Pheasant - 5th percentile (median)	5.1	2.6	2.4
RQ Pheasant - 5th percentile (left 95 % CL)	28	8.8	6.6
RQ House Sparrow - 5th percentile (median)	10.5	5.4	5
RQ House Sparrow - 5th percentile (left 95 % CL)	58	18	13.8

Step 5: Is risk acceptable?

The addition of an additional species resulted in no significant change in the RQs. The group thought that, given animal welfare considerations, we would choose to delay further acute testing and recommend a detailed analysis of exposure in order to move away from worst-case-exposure scenarios. Exposure estimates used in the above calculation were based on worst-case application of 2.4 kg/ha (typically 10% of use frequency).

Subchronic Exposure: Scenario I

It was determined in the initial assessment of this compound that a subchronic exposure scenario was plausible and an effects assessment was needed.

Step 2: Obtain minimum dataset for initial assessment

The base dataset included both a mallard and bobwhite LC50 study. Examination of the data indicated a significant reduction of food consumption at several dietary concentrations and a concomitant loss of weight of surviving birds. There was also early observance of adverse clinical signs of toxicity. The group questioned whether the LC50 values represented actual toxicity or starvation resulting from avoidance of the treated diet. However, it was decided to move forward with the effects assessment with an understanding of the inadequacy of the data.

Steps 3 and 4: Effects and risk assessment

The initial assessment used the geometric mean of the Mallard and Bobwhite LC50 values provided in the base dataset and the maximum residue values (Fletcher et al. 1994) based on the maximum application rate. The safety factors of Luttik and Aldenberg (1997) were used with the recognition that they are based on an evaluation of LD50 values; however, the group felt that at this time they represent the best choice to account for interspecific variation. See Table 4-12 for the assessment.

Step 5: Is risk acceptable?

The RQs in this assessment are based on a simplistic relationship between the LC50 and worst-case exposure values. The results suggest that birds may be at risk from subchronic exposure. However, the group viewed this procedure with caution because of the conservative nature of the assessment of exposure and the lack of knowledge of actual exposure in the LC50 tests due to food avoidance.

It was concluded that, due to apparent diet avoidance, there was a high level of uncertainty associated with the calculated LC50 value. Because a full accounting of food consumption data was unavailable, the group recommended that a second assessment be considered using the lowest NOEC or the geometric mean of the

Table 4-12 Sub-chronic exposure RQ using LC50 values for Mallard and Bobwhite with Luttik and Aldenberg (1997) safety factors

Parameter	2 Species
Species	Bobwhite / Mallard
LC50 (ppm)	364 / 342
geometric mean of LC50 (ppm)	353
SF - 5th percentile (median estimate)	5.7
SF - 5th percentile (left 95% CL)	19
Pred. 5th percentile - Median estimate (ppm)	62
Pred. 5th percentile - 95 % left CL (ppm)	18.5
Estimated Exposure (mg/kg in food)*	576
RQ - 5th percentile (median)	9.3
RQ - 5th percentile (left 95 % CL)	31

* Worst-case exposure based on an application rate of 2.4 kg/ha and 240 mg/kg on short grass per kg/ha (Fletcher et al. 1994)

NOEC and lowest-observed-effect concentration (LOEC) as the effects criterion. However, it was quickly recognized that using the NOEC or LOEC values (values lower than the LC50) without a refinement of exposure would result in a greater perception of risk and added uncertainty. Table 4-13 shows the sources of uncertainty about which the group was concerned.

Step 6: Select and conduct appropriate studies to reduce uncertainty

Because of the uncertainty associated with food avoidance and the daily dose consumed in the dietary tests, the group recommended additional tests be conducted, specifically a dietary toxicity test, using an experimental design that will provide a better estimate of daily dietary intake and an avoidance test to more clearly define the extent and magnitude of avoidance.

These tests would allow a more thorough and accurate assessment of effects and potential exposure through food consumption. Of course this conclusion assumes that avoidance in the field will be similar to that observed in the laboratory. If avoidance in the field is more pronounced than in the laboratory, potential exposure in the field will be less than predicted. However, if avoidance is less pronounced in the field hazard would be underestimated.

Reproductive Effects: Scenario II

Steps 2 and 3: Obtain minimum data and conduct effects assessment

Review of the reproductive tests showed that in the bobwhite test there were no effects at the highest concentration tested, 125 ppm. In the mallard test, however, there were clear sublethal effects on the adults that were accompanied by apparent secondary effects on reproductive endpoints such as eggs laid and 14-day-old

Table 4-13 Sources of uncertainty identified for the case study scenario

Type of uncertainty	Relative importance	Option for refined assessment
Interspecific variability in sensitivity	High; represents a major source of variation. Specific concern for altricial vs. precocial nestlings	Apply Luttik and Aldenberg's safety factor (1997) to account for variability.
Intraspecific variability in sensitivity (age, development stage)	Moderate to high; variability may be accounted for (nested within) the factor accounting for interspecific variability	This variability was assumed to be accounted for here by use of Luttik and Aldenberg's safety factors.
Variability in sensitivity of the test population	Moderate	Higher-tiered assessments may have sufficient dose-response data to account for this variability by assessing the standard error of the slope.
Avoidance of diet	High; lack of confidence in the LC50/NOEC	Request an avoidance study; consider using the NOEC as interim effect value in the assessment.
Exposure inaccurately measured due to group housing and lack of accurate food consumption data	High	Request an additional dietary study conducted using a design to address this uncertainty.
Toxicity of metabolites	Unknown	Testing of metabolites may be required in a higher-level assessment.

survivors at 75 and 125 ppm. It was concluded that reproductive effects were most likely a consequence of parental toxicity, and it was appreciated that, in this case, the reproductive tests may simply represent long-term dietary tests.

Step 4: Risk assessment

The preliminary analysis indicated potential for reproductive effects. The initial assessment of reproductive hazard used the NOEC from the mallard study (25 ppm) which was compared to the highest potential residue value (576 ppm on short grass). To account for interspecies variability, unacceptable risk was assumed if the Exposure/NOEC >1. The RQ in this case was 576/25, providing a value of 23.

However, a more definitive review of the tests was not possible due to the lack of precision in establishing dietary intake levels. Due to the mode of action of the pesticide there is a need to factually establish the dose levels required for maximum potency and the timescale of biological effects. Nevertheless, the tests were judged

to provide relevant information to assess potential effects on fecundity under certain exposure conditions and could be used for the initial assessment.

Step 5: Is risk acceptable?

Because reproductive effects appear only within a concentration range that results in parental toxicity, it was recommended that either a longer-term dietary test (for example, 28 days) or an abbreviated reproduction test should be conducted to verify longer-term effects on adults or actual reproductive toxicity.

Conclusion

The nature of the data available was insufficient to establish the safety of this pesticide. Preliminary analyses indicated that birds may be at risk from acute dietary exposure but there is apparently less risk from short- to mid-term exposure. Potential for reproductive effects requires additional evaluation. The analyses were confounded by the lack of information on interspecific variation in sensitivity, the lack of confidence in the calculated LC50 values, and the general lack of information on magnitude and duration of exposure. The group concluded that additional exposure and effects data are needed, and that a more detailed probabilistic risk assessment would be required to quantify potential risk.

Recommendations/Issues to be Addressed

Research should be conducted to determine the effect of using safety factors on a limit dose. Will the use of safety factors always trigger additional testing? What is the influence of application rate? Is a limit of 5X the application rate a more appropriate limit dose?

Analysis of existing data should be conducted to judge whether applying safety factors based on LD50 data to LC50 study results is justified.

The usefulness of mammalian dermal toxicity data in predicting avian dermal toxicity should be evaluated.

- If the acceptability of the RQ value is changed to 1 when safety factors are applied to effects values, it must be recognized that the factors are based on interspecies variability in acute oral toxicity and ALD tests.
- Potential endocrine effects should be addressed by analysis of mammalian data to indicate possible concerns.