CHAPTER 5

Case Study 2: Seed Treatment

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Case Study 2 presented an evaluation of the risk an insecticide seed treatment poses to avian wildlife.

Basic Data

General information and use pattern

Function:	Insecticide seed treatment
Mode of action:	Acetylcholinesterase (AChE) inhibitor
Type and composition of formulation:	Cereal seed treatment with colored dye. Nominal concentration on seed is 1000 mg a.i./kg.
Crop and pest:	Control of wheat bulb fly in autumn/winter-sown cereals
Application:	Treated seed is drilled to a depth of approximately 2.5 cm at a sowing rate of approximately 180 kg seed/ha. Soil type, seedbed, and climatic conditions may influence the proportion of seeds left on the soil surface. The label is explicit about procedures to minimize seed left on the soil surface and collection and disposal of spillages.

Physical and chemical properties and environmental fate

Water solubility:	10 to 20 mg/L
K_{ow}:	~4
Degradation rate/seed:	DT50 under typical conditions about 20 days
Degradation rate/soil:	DT50 ~70 days; very dependent on soil condition

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Species of concern and bodyweight

Focal species potentially exposed are

- gamebirds (e.g., pheasant), 1000 g;
- pigeons (e.g., woodpigeon), 500 g; and
- passerines (e.g., skylark), 50 g.

Environmental concentration

Nominal concentration = 1000 mg a.i./kg seed.

Risk characterization

Preliminary toxicity exposure ratios (TERs and risk quotients[RQs]) are based on the assumption that the focal species consume treated grain.

Acute toxicity-exposure ratio/risk quotient

See Table 5-3.

Short-term toxicity-exposure ratio/risk quotient

LC50:	250 ppm
Concentration on seed:	1000 ppm
TER (LC50/concentration):	0.25
RQ (concentration/LC50):	4

Framework Analysis

Step 1: Problem formulation

After a review of the basic data package the following issues were raised, the rationale for raising the issue identified, and the outcome of the subsequent discussion recorded.

Issue: Is there a relevant exposure scenario?
Reason: To identify if there is a need for avian effects testing.

Table 5-3 Acute TER/RQ for 3 species in a worst-case estimate

Species	Weight (g)	LD50 (mg/kg)	Daily intake (g dw/kg)	Daily dose (mg/kg)	TER (LD50/DD)	RQ (DD/LD50)
Pheasant	1000	128	58	73	1.8	0.6
Woodpigeon	500	128	37	93	1.4	0.7
Skylark	50	128	8.3	207	0.6	1.7

Food consumption (dry matter) based on Nagy (1987) estimates
Grain = 80% dry-matter content
DD= daily dose

Outcome: The scenario of potential concern for this use pattern is dietary exposure.

Issue: What is the relevant timing and duration of exposure?
Reason: To identify the types of avian effects test required. This needs to be estimated after taking account of the application window and DT50 on seed.
Outcome: The relevant period of exposure was estimated to be 6 weeks and outside of the breeding season for temperate zone birds. As a consequence, there was a need to measure short- and medium-term effects. Defining the maximum exposure period was more useful than constraining it to general terms like "medium-term."

Issue: Does the case study need to consider other routes of exposure, e.g., dermal and inhalation?
Reason: To ensure critical routes of exposure are not overlooked.
Outcome: Dermal and inhalation exposure were considered negligible in this specific case.

Step 2: Obtain minimum dataset for initial assessment

Issue: Is there a need to test the acute oral toxicity of the compound?
Reason: There is a need to provide data on effects for short-term risk assessment and intrinsic toxicity.
Outcome: A test on a single species is required as the minimum.

Issue: What type of acute oral study is required?
Reason: To identify the level of precision required in order to optimize the use of test animals.
Outcome: Consider the mammalian toxicity data, and if the mammalian median lethal dose (MLD) is low, consider an approximate lethal dose (ALD) test. If the mammalian MLD is high, consider a limit test. The need for a dose-response test was not considered.

Issue: What type of study is required to measure effects over the relevant 6-week exposure period?
Reason: To ensure a study of relevant route and exposure is conducted.
Outcome: A study needs to be conducted which allows the determination of an incipient LC50 or a study on parental effects over a 6-week dietary exposure period. No data on reproductive parameters are required since the exposure period does not include the breeding season.

Outcome: Additional ALD acute toxicity data on other species was required.

Reasons: 1) The accuracy of the mallard duck LD50 dose-response test was in doubt due to the possible influence of regurgitation.
2) The need to measure sensitivity across a range of species was considered more important in this case than the need for a dose-response relationship for a single species.
3) Differences in sensitivity would be useful to help select candidate species for any avoidance tests.
4) The influence of age on differences in toxicity seen in quail and duck might be explained.

Additional Data I

Additional acute toxicity

Age: Full-grown
Test substance: Technical active substance
Birds per treatment: 2
Observation period: 14 days
Observations: Clinical signs at all doses with all species, except Starling at 10 mg/kg dose (see Table 5-5).
Deaths: All birds dying did so within 24 hours. See Table 5-6 for extrapolation.

Framework Analysis

Iteration 2

Issue: Evaluation of additional data
Outcome: Based on the regurgitation observed in the pigeon ALD study, doubts were raised about the reliability of an ALD at 32 mg/kg. For purposes of risk assessment the ALD was set on 18 mg/kg as the geometric mean between 10 mg/kg (mortality 0/2) and 32 mg/kg (lowest level at which regurgitation occurred). It was recognized that regurgitation is likely to occur frequently while performing ALD tests. This presents a source of uncertainty and may be corrected for as in the case presented here. The acute toxicity was higher than indicated by earlier mallard duck study. The recalculated TER using the HD5 value indicated the potential risk was greater.

Issue: What additional studies are to be performed to reduce uncertainty to an acceptable level?

Outcome: Data on typical feeding rates in the field for species of concern was required.
Reason: To identify species for avoidance test based on rapid feeding rate (higher risk) in conjunction with species sensitivity from ALD tests.

Table 5-5 Results of additional acute toxicity test by up-and-down procedure

Species	Doses tested (mg/kg)	Regurgitation	Mortality	ALD50 (mg/kg)
Bobwhite quail	100	no	2/2	10
	32	no	2/2	
	10	no	1/2	
	3.2	no	0/2	
Mallard	100	yes	2/2	18
	32	no	2/2	
	10	no	0/2	
Pigeon	100	yes	2/2	32
	32	yes	1/2	
	10	no	0/2	
Starling	100	yes	0/2	32
	32	no	1/2	
	10	no	0/2	
House sparrow	32	yes	2/2	5.6
	10	no	2/2	
	3.2	no	0/2	

Table 5-6 Extrapolation factor and HD5

No. of species (species used)	Extrapolation factor	95th percentile LD50 (HD5) (mg/kg)
1 (Duck)	5	2.0
2 (Duck, Quail)	4.6	3.0
3 (Duck, Quail, Pigeon)	4.3	4.7
4 (Duck, Quail, Pigeon, Sparrow)	3.9	4.2
5 (Duck, Quail, Pigeon, Sparrow, Starling)	None	4.0

HD5 estimated directly using 5 species.
Extrapolation factor applied to the mean LD50 for each group of species.

Table 5-11 Method for the examination of repellency effects on birds

Parameter	1 Species
Species	Japanese quail or other suitable species
Pre-treatment acclimation period	7 days (food consumption measured over last 3 days)
Fasting period	16 hours
Treatment period (feeding time - hours/day)	24 hours continuous
Post-treatment observation period	3 days
Birds/treatment	10
Birds/cage	1
Presentation	Food hopper
Choice	No
Endpoint: Food consumption	Yes
Endpoint: Mortality and clinical symptoms	Yes
Endpoint: Bodyweight	Yes
Endpoint: Feeding Rate	No

Framework Analysis

Iteration 4

Issue: Evaluation of additional data

Outcome: Avoidance reduced the expected level of mortality in pigeons. Avoidance reduced the consumption of test substance in quail substantially. The study provided did not follow the protocol preferred by the case study team, because feeding rates were not reported. The team considered that more emphasis should be given to feeding rates in the study matching feeding rates in the field. The case study author then provided additional (fictitious) data on feeding rate, to resolve the inadequacy of the study design. Feeding rates in the middle 2 groups in the study were stated to be 5 to 10 pecks per minute, similar to those observed in headlands in fields (see Additional Data II). As conditions used in the avoidance test are critical, contact between registrant and regulator on the test design, prior to initiation, is preferred.

Issue: Can a final assessment on the acceptability of risk be made after Iteration 3?

Reason: Major sources of uncertainty identified under Step 5 have been studied.

Outcome: The feeding rate, toxicity, and avoidance data clearly indicate high risk for pigeons. Mortalities in the field for pigeons are likely to occur. Since

no protection goals were set prior to initiating the effects-assessment scheme, a definitive decision on acceptability of the risk could not be taken. Acceptability is dependent on protection goals.

Issue: What are the protection goals and acceptability criteria?

Reason: To measure the severity and frequency of expected impact on birds and pigeons, as well as other species.

Outcome: Acceptability is related to species, populations, and other (political and social) factors.

Iteration 5

Issue: What additional studies are to be performed to reduce uncertainty to an acceptable level?

Outcome: Need for field studies or a probabilistic assessment.

Reason: Data on feeding rates and effects on other species are needed to define whether mortality was likely to occur in just 1 or a range of species to identify if protection goals had been reached or not.

Comment: For the specific situation in this case, the option of measuring effects directly in the field was not considered to be the most effective way forward. Any field studies, since they are to generate data on a suite of species under a wide range of field conditions, are likely to leave uncertainty on the effect side.

As the next possible step, a probabilistic model should be considered, with distribution data on the following variables:

- species sensitivity (using data from ALD studies),
- avoidance (need additional studies on avoidance in other relevant species),
- availability of seed on soil surface (under different agricultural practices),
- feeding rate information for the species at risk (use of generic data), and
- availability of alternative food items.

A possible outcome of the probabilistic model assessment could be that the product causes X% mortality in Y species with Z frequency.

All of these parameters present a source of uncertainty that would have to be addressed if a field effects study was conducted, along with quantifying deaths in the field. However, the option of measuring effects in the field was not dismissed by all group members in this case, because death would occur quickly following lethal exposure. There was still a belief that the field effects were necessary to confirm that the predicted outcome from the probabilistic assessment did indeed occur.

Issue: Could risk mitigation change the acceptability assessment for the case study?