Birds and mammal risk assessment

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What to do today

Main topic of this session is to develope a risk assessment method for birds and mammals::

- Setting protection goals,
- Develop a risk assessment method
- Apply the method developed by
  - assessing the risk, and
  - assessing the uncertainty.

(Slides will be available after this meeting but handouts will be provided for the exercises)

Toxicity testing

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Standard toxicity tests (1) LD50

Results of a standard LD50 test

with Bobwhite, Japanese quail or Mallard duck

Dose mg/kg BW	Mortality %
0	0
79	0
100	0
126	20
159	50
200	60
251	80

Standard toxicity tests (2) LD50

- According to Annex II of Directive 91/414/EEC, the acute oral toxicity of an active substance must be determined to a quail species (Japanese quail, *Coturnix coturnix japonica* or Bobwhite quail, *Colinus virginianus*) or to Mallard duck (*Anas platyrhynchos*).
- The highest dose used in tests need not normally exceed 2000 mg/kg body weight.
- It is permissible to extrapolate an LD50 value upwards in cases where there is no mortality or a single mortality at a limit dose in an acute avian toxicity study.

Number of animals tested at limit dose	Extrapolation factor for no mortality at a limit dose	Extrapolation factor for a single mortality at a limit dose
5	1.614	1.228
10	1.888	1,518
15	2.051	1.685
20	2.167	1.802

LD50 = limit dose * extrapolation factor

Standard toxicity tests (3) LD50

- Due to issues of regurgitation it is recommended not to use the Mallard duck (EFSA, 2007). Where regurgitation or emesis occurs at doses used for risk assessment, additional information is essential to complete the risk assessment. The amount of regurgitated material should be assessed for determination of the ingested dose.
- In the absence of this information, the lowest overall no observed effect level (NOEL) must be used for risk assessment purposes.
- Where more than one study has been submitted, the study/studies where no regurgitation has occurred should be used. If, however, mortalities appear in the study in which regurgitation has occurred (at dose levels at or around the LD50 value for the non-regurgitation study), then it is proposed to use the NOEL (for regurgitation or mortality, whichever is lower) from the study where regurgitation has occurred.

Standard toxicity tests (4) LD50

- Avian acute oral LD50 studies generally are conducted with a minimum of 60 birds (OPPTS850.2100).
- Since a number of years there is a new Test Guideline available: OECD 223 on *Avian Acute Oral Toxicity.*
- If available data suggest that the LD50 may be greater than 2000 mg/kg, a single stage limit test may be performed using 5 birds at the limit concentration and 5 birds in the control group. If toxicity is expected, to be less than 2000 mg/kg a full dose response test is performed using 3 or 4 stages (24 or 34 bird, respectively – excluding controls).
- The performance of the design was evaluated through extensive computer simulations. These simulations indicated that draft TG223 would estimate the LD50 as well as the 60-bird design, but using substantially fewer animals, and improve the frequency with which a slope is estimated. The computer program that performed the simulations has been independently validated.
- Round robin testing showed that it is feasible to carry out the new test design.

Standard toxicity tests (5) LC50

The following short term dietary test method with birds is often available (LC50 mg/kg food):

OECD Test 205 (OECD, 1984): Avian dietary toxicity test

The GD does not routinely use output from this LC50 study in risk assessment, because of scientific limitations and welfare issues
It is recommended that it should be conducted only for those pesticides where the mode of action and/or results from mammalian studies indicate a potential for the dietary LD50 measured by the short term study to be lower than the LD50 based on an acute oral study (e.g. organochlorines or some of the rodenticides).
It can also provide an indication of whether avoidance is worth considering in higher tier assessment, but is not sufficient on its own to demonstrate that avoidance will prevent mortality.

Food avoidance behaviour (1)

Results of a standard LC50 test

with Bobwhite and parathion (after Bennett 1989)

Dietary concentration mg/kg food	Assumed DFI g/b.d	Assumed DCI mg/b.d	Mortality %
0	7.2	0	0
79	7.2	0.57	0
100	7.2	0.72	0
126	7.2	0.91	20
159	7.2	1.15	50
200	7.2	1.44	60
251	7.2	1.81	80

Food avoidance behaviour (2)

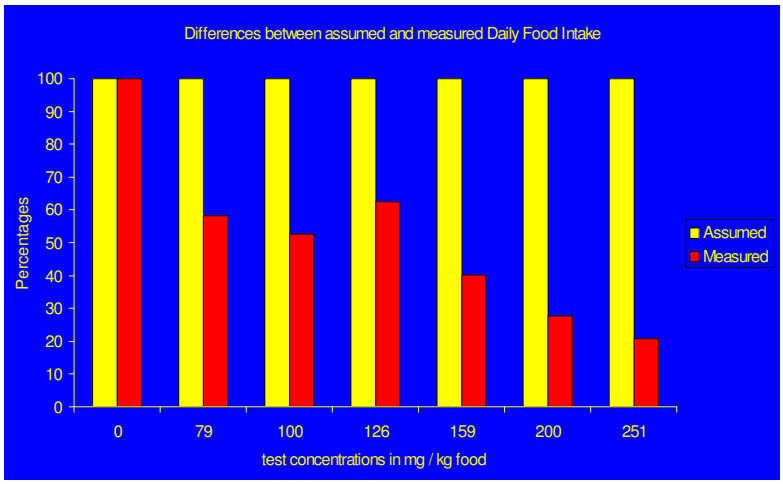
Difference between assumed and measured daily food intake (DFI)

and daily chemical intake (DCI) in a LC50 test with Bobwhite and parathion

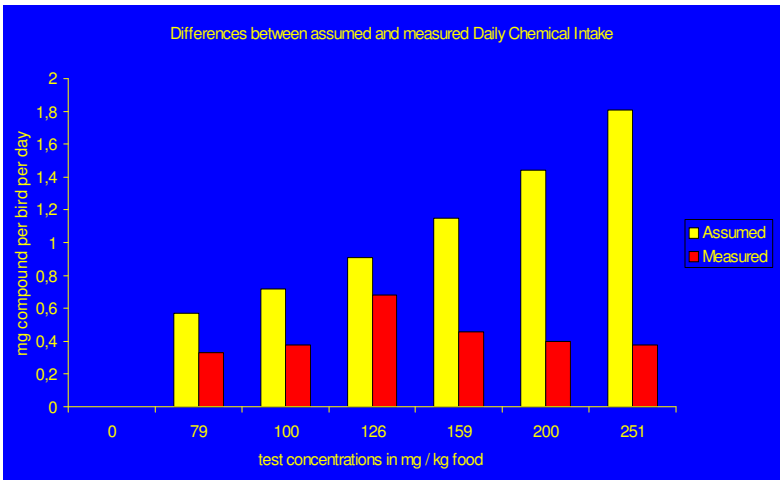
(after Bennett 1989)

Dietary concentration mg/kg food	Assumed DFI g/b.d	Measured DFI	Assumed DCI mg/b.d	Measured DCI mg/b.d	Mortality %
0	7.2	7.2	0	0	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	1.44	0.4	60
251	7.2	1.5	1.81	0.38	80

Food avoidance behaviour (3)



Food avoidance behaviour (4)



Standard toxicity tests (6) NOEC

A test for effects on reproduction in birds is currently requested if birds are likely to be exposed during the breeding season.

There are two standard studies, OECD Test 206 (avian reproduction study; OECD, 1993) and the US EPA 71.4 study (US EPA, 1996).

The US EPA protocol recommends that tests be carried out on first-time breeders of an upland game species, preferably the northern bobwhite quail (*Colinus virginianus*), and a wild waterfowl species, preferably the mallard duck (*Anas platyrhynchos*).

The OECD version states that the Japanese quail (*Coturnix coturnix japonica*), preferably experienced breeders, is also acceptable.

However, there are concerns regarding the appropriateness of this species due to its greater sensitivity and ability to attain breeding readiness under short daylight conditions.

Standard toxicity tests (7) NOEC

Birds are acclimated to laboratory conditions. The substance to be tested is mixed into the diet. The birds are fed *ad libitum* for a recommended period of 10 weeks before they begin laying in response to a change in photoperiod. The egg-laying period should last 8 - 10 weeks. Eggs are removed from the adults the day they are laid, stored and then artificially incubated.

Variables recorded during the study include:

- Adult body weight and food consumption;
- The number of eggs laid per hen;
- The mean eggshell thickness;
- The proportion of eggs set (placed in the incubator) that are fertile at 11 (bobwhite) or 14 days (mallard);
- The proportion of fertile eggs containing viable embryos one week later (i.e. days 18 and 21, respectively);
- The proportion of eggs that hatch and produce chicks;
- The survival of the chicks at 1 and 14 days of age.

Standard toxicity tests (8) NOEC

OECD Test 416 (OECD, 2001c) – Two-generation reproduction toxicity study (adopted 22 January, 2001).

Endpoints: fertility, litter size and survival, gross necropsy and pathology of the reproductive tract and histopathology. This study allows also an examination of the full growth, development, sexual maturation and reproduction of the F1.

OECD Test 414 (OECD, 2001d) – Prenatal developmental toxicity study (adopted 22 January, 2001).

OECD Test 407 (OECD, 1998a) – Repeated dose 28-day oral toxicity in rodents (adopted 27 July, 1995).

OECD Test 408 (OECD, 1998b) – Subchronic oral toxicity – rodent 90 day study (adopted 21 September, 1998).