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Working Document

Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC

This document has been conceived as a working document of the Commission Services which was elaborated in co-operation with the Member States. It does not intend to produce legally binding effects and by its nature does not prejudice any measure taken by a Member State within the implementation prerogatives under Annex II, III and VI of Commission Directive 91/414/EEC, nor any case law developed with regard to this provision. This document also does not preclude the possibility that the European Court of Justice may give one or another provision direct effect in Member States.

1 Introduction

This document intends to provide guidance to notifiers and Member States on how to conduct a risk assessment for birds and mammals in the context of the review of active substances for inclusion in Annex I of Directive 91/414/EEC. The need for the development of such a consensus approach has become apparent in the Working Group Evaluation of the Standing Committee for Plant Health and it is hoped that this document will stand up to these expectations under practical conditions of its future application. The document should be understood as a working document. It will be updated to take on board advances in scientific understanding as the necessity arises.

Although the risk assessment for birds and mammals is an integral part of ecotoxicology this guidance is developed as a separate document due to certain constraints. It should be noted that relevant sections in the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002), be it general issues (e.g. on NOEL values) or specific vertebrate issues (data requirements) remain effective. It is envisaged to merge this document with SANCO/10329/2002 in the long run.

Annex VI of Directive 91/414/EEC provides uniform principles for evaluation and authorisation of plant protection products in the Member States. Although not applicable *per se*, these principles give useful guidance also during the review of active substances for inclusion in Annex I according to Article 5 (1b), (2c) and (3) of the Directive.

Annex VI reads (under point 2.5.2.1, decision making criteria): "Where there is a possibility of birds and other non-target terrestrial vertebrates being exposed, no authorisation shall be granted if the acute and short-term toxicity/exposure ratio for birds and other non-target terrestrial vertebrates is less than 10 on the basis of LD50 or the long-term toxicity/exposure ratio is less than 5, unless it is clearly established through an appropriate risk assessment that under field conditions no unacceptable impact occurs after use of the plant protection product under the proposed conditions of use."

The scope of the document is to elucidate in particular the "unless" clause of this provision and its application in the context of Annex I inclusion of active substances. To that end the procedures of risk characterisation are described according to the present state of knowledge, but the document does not deal with the regulatory decision proper. In this tiered assessment framework potential risk for birds and mammals is identified on the basis of responses of individual organisms observed in controlled laboratory experiments. However, ecological risk assessors have long argued that except in the case of threatened or endangered species, the abundance and persistence of populations of organisms are more relevant as endpoints for assessment than are responses of individual organisms. The proposed approach is justified on the ground that too little is known about the responses of populations to chemical exposure to support regulatory decisions on that basis. Furthermore, extrapolation tools aiming at bridging the gap between individuum level and population level by means of population modelling techniques are not yet satisfactory (Kendall and Lacher 1994). Nevertheless risk assessors have to consider, at least qualitatively, that if only a small fraction of a population is exposed (spatial scale) risks associated with the use of plant protection products may be small even if some individuals would be affected. That does not preclude, that appreciable mortality without population level consequences may be judged unacceptable.

The standard risk assessment is based on the field scale and not landscape scale, i.e. the risk to non-target birds and mammals frequenting the treated field is assessed. No consideration is made of the risk from applications of the same plant protection product to neighbouring fields. If concern is raised (i.e. TER is less than appropriate trigger value) then the risk can be refined appropriately (see chapter 5). When refining the risk it may be appropriate to consider such issues as suitability of the standard scenarios, scale of use and potential impact on populations. However, no deviations from worst case assumption should be made unless they are justified.

2 Principles of the risk assessment

2.1 Risk characterisation

In the framework of Directive 91/414/EEC risks arising from direct toxic effects are considered; secondary ecological effects e.g. due to decline in food resources are currently not evaluated, although they are within the scope of the Directive. The initial risk characterisation is done by means of toxicity-to-exposure ratios (TER). The Annexes III and VI of the Directive 91/414/EEC request for the calculation of the following TERs:

acute TER for birds and mammals:	TER_a, based on LD50
short-term TER for birds:	TER_{st}, based on LC50
short-term TER for mammals:	TER_{st}, based on NOEL
long-term TER for birds and mammals:	TER_{lt}, based on NOEL

Notes:

- The necessity of the long-term assessment depends on the exposure pattern.
- Recently the terms ”short term” / ”medium term” / ”long term” are often used to describe the three time scales; in this document the terms ”acute” / ”short term” / ”long term” are used for sake of consistency among the EU-documents.

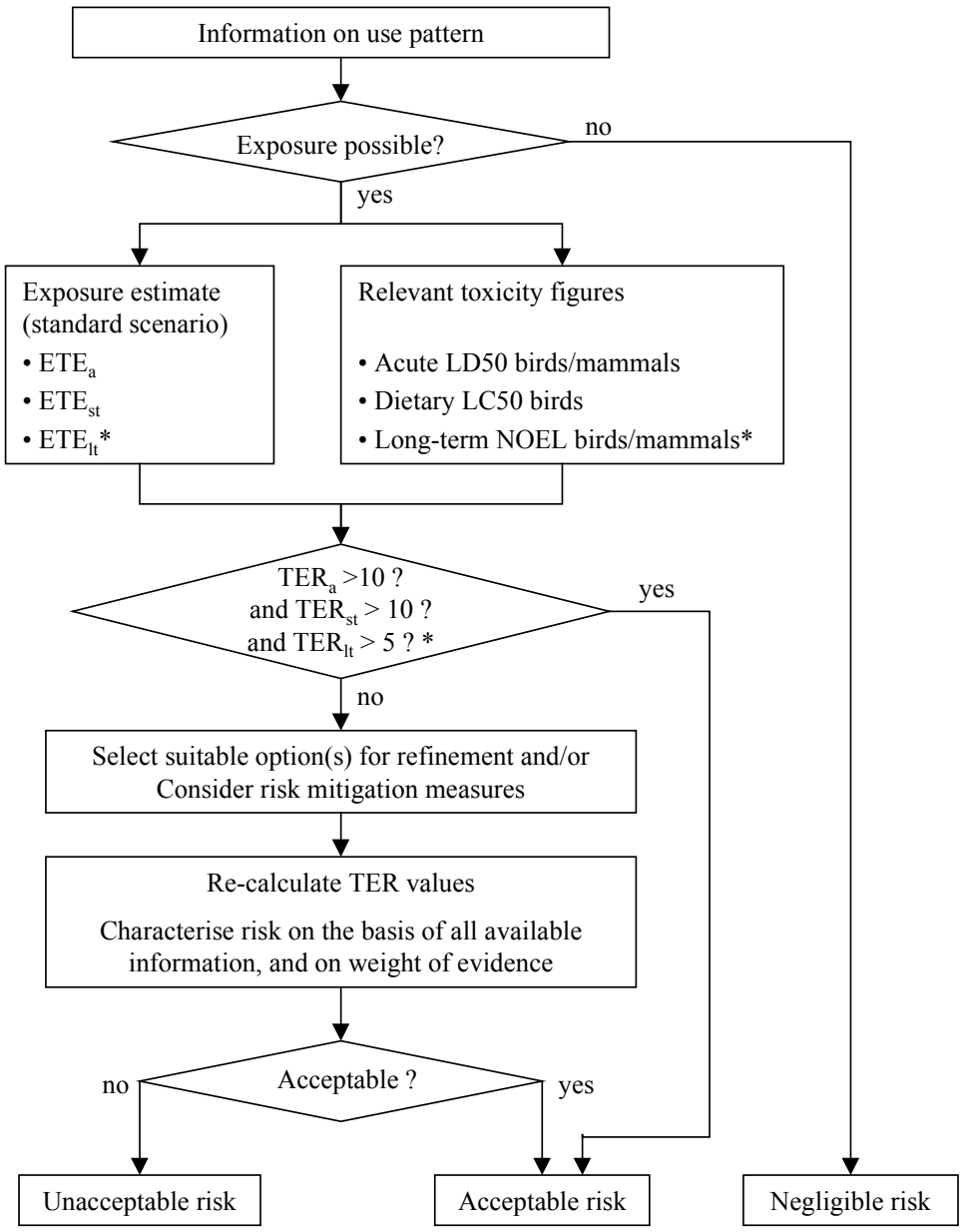
The distinction between short-term and long-term TER for mammals is poorly defined according to the toxicity input data; therefore these assessments should be combined to one which is termed ”long term” in this document. The resultant risk assessment addresses both the short-term as well as the long-term risk to mammals.

When toxicity figures and exposure estimates are put into a TER both figures have to match with regard to time scale and have the same unit, either daily dose or concentration. For the short-term and long-term assessments Annexes III and VI request comparisons based on concentration (mg/kg food). However, from scientific reasons exposure-to-toxicity ratios are better based on daily dose in order to avoid bias due to different food intake rates between lab and field. Therefore this approach is followed in the document here.

The TER values are compared with assessment factors (trigger values, levels of concern) which according to Annex VI are 10 for the acute and short-term scale and 5 for the long-term scale. If any of these triggers is not met further steps in the risk assessment are generally required.

From practical reasons it is useful to conduct the risk assessment in two tiers. Tier 1 contains simple procedures for the calculation of the TERs. These procedures involve standard

Risk Assessment Scheme for Terrestrial Vertebrates



* only necessary where long-term exposure or exposure during breeding season is possible

Figure 1: Risk assessment scheme

scenarios and default values for the exposure estimate which can be performed with a low input of effort. The Tier 1 standard scenarios include intake via feed and represent a realistic worst case assessment where the exposure scenarios are selected to reflect a situation where the total daily feed is contaminated. The aim is to exclude with sufficient certainty false negatives (= risk remains undetected).

If a potential risk is indicated in tier 1 then one or several refinement steps shall be considered. There are numerous options for the refinement and it depends on the specific case which of these are most appropriate (see chapter 5). Most often the refinement results in revised input data for the TER calculation (mainly exposure, but also toxicity). In those cases a loop goes back to the TER calculation and it is checked whether the revised TER is above the trigger. Some refinement options (e.g. higher tier tests) result in information that cannot be processed in terms of a TER; in line with the ”unless” clauses in the decision making criteria laid down in the Uniform Principles (Annex VI to Directive 91/414) such results are used to characterise the risk in a descriptive way. Such non-formalised procedures are briefly explained in chapter 5.10 (weight of evidence approach).

Refinement steps may also include probabilistic approaches, which may be useful to supplement and put into perspective the risk assessment (see chapter 5.8).

The refinement always needs additional data, either specific data on the product to be assessed or generic data. Some information may be available already in the dossier or can be produced by literature searches, other data have to be generated by new studies. As it is desirable to minimise animal testing other options for refinement should be explored first, where possible. In any case the assumptions and input data in the refinement steps should be fully justified. It should be noted that refinement reduces the uncertainty and produces a more precise characterisation of the risk, but additional data do not necessarily result in a risk level which is lower than previously expected.

Finally, risk management options are to be considered, which generally aim at a reduction of exposure. The possibilities of risk management very much depend on the type of product, the intended use, and specific conditions in the Member States (chapter 7). Usually this is the final step in the scheme, but often it may be useful to envisage risk mitigation measures before all possibilities of refinement are exhausted.

2.2 Toxicity figures

The relevant toxicity figures which are fed into the TER calculation are as follows:

Acute: Birds: LD50 from acute oral test
Mammals: LD50 from acute oral test

Short term: Birds: LC50 from 5-day-dietary test
Mammals: (This assessment is covered by acute and long-term assessment)

Long term: Birds: NOEL from avian reproduction study
Mammals: NOEL based on most sensitive endpoint of relevance for survival rate, reproduction rate and development of individuals, for example results from multi-generation studies or teratology studies on mammals (see chapter 5.7).

In each category the toxicity of the most sensitive test species is used.

With regard to the long-term scale in mammals the tier 1 assessment is conducted with a dose level that represents the no observed adverse effect level from a toxicological point of view. If the resulting TER falls short of the corresponding trigger then the ecological relevance of endpoints should be re-evaluated (see 5.7).

Daily dose

The results of dietary toxicity tests may be reported either as concentration (mg/kg diet also referred to as ppm) or as daily dose (mg/kg bw/d) or both. In mammalian toxicology the daily dose is nowadays the more relevant figure and thus reported routinely. In avian tests conversion from concentration to daily dose has not been common up to now. As explained above the daily dose is the preferred measure for the purpose of risk assessment and therefore the toxicity figures should be converted to daily dose based on average food intake rate during the exposure period of the test.

The general rule for the conversion is: Daily dose (mg/(kg bw/d) = Concentration in food (mg/kg) multiplied by daily food consumption (g per bird per day) divided by body weight (g).

Avian reproduction test:

- Food consumption: Data are reported on a weekly basis for pairs or groups. Food consumption usually is higher during egg-laying (to be attributed to the females), however, for the purpose here the average consumption over the entire exposure period is taken.
- Body weight: Take average body weight for both sexes over exposure period
- Convert each treatment group separately

5-day-dietary test:

- Food consumption: Usually group consumption rates (expressed as g per bird per day) are given in the report for the 5-day-exposure period and the 3-day-post exposure period; the former figure is needed here
- Body weight: Group means for day 0, 5, and 8 are reported. For the purpose here take the average of day 0 and day 5.
- The conversion from concentration to daily dose is not appropriate for those treatment groups where a strong food avoidance is obvious (in that case the average dose over 5 days is misleading) as well as for treatment groups with a high mortality (in that case data for the body weight at day 5 and for the food consumption have a poor quality or are missing at all).

  Case 1: LC50 is above top concentration
  Convert each treatment group separately (however, only the top level is needed for the risk assessment).

Case 2: LC50 is below top concentration, food consumption not affected
There are two possibilities:

a) convert concentration into achieved dose for each treatment group, and conduct a new probit analysis, this time using the daily-dose data
b) take the overall mean value for food consumption and body weight (mean from all dose groups where calculation is possible) and use these figures to convert the LC50 (this option is sensitive against concentration-dependent food avoidance).

If food consumption is slightly affected expert judjement is required to decide whether procedures according to case 3 should better be followed.

Case 3: LC50 below top concentration, distinct food avoidance well below the LC50:
Conversion from concentration to daily dose may be unreliable due to the low number of survivors on which food consumption is based; furthermore food consumption may change markedly from day to day. These problems alone should be no reason to repeat the study. Rather assessment should be conducted on a case-by-case basis (e.g. if the study delivers a NOEL then this could be a starting point as the converted LC50 must be well above this level); applicants should seek advice from the competent authority.

2.3 Exposure estimate

Exposure assessment for terrestrial vertebrates is a complex matter that not only encompasses concentrations in various environmental media (PECs) but also behavioural parameters and information on feeding ecology. Figure 2 summarises exposure routes that may become relevant according to the kind of product to be assessed (modified from ECOFRAM 1999).

For most situations, the principal risk is considered to arise through ingestion, and it is rarely necessary to consider other exposure routes in detail. However, identification of the most important route of exposure can only be made on a case by case basis, taking into account the mode of application of the product, crop specific conditions and environmental properties of the active substance (EPPO 1994). For example, if the ingestion rate of treated material is assumed to be low (from whatever reason) then the relative importance of other routes may increase (Driver et al. 1991). Unfortunately there are no validated exposure models for dermal uptake and inhalation under field conditions. Furthermore, dermal and inhalation toxicity tests are not required for birds. Dermal toxicity for mammals is of limited use as in these tests the test substance is applied on to the shaved skin. Therefore a quantitative risk assessment based on exposure and toxicity is impossible. (Note: This is a serious gap and all efforts should be made to develop models in the near future. It would be worthwhile to examine whether methods used to estimate operator exposure could be a starting point). If, however, field studies and biomarkers are employed in higher-tier testing then all exposure routes are integrated.

The standard scenarios described in chapter 3 cover the main routes of oral exposure. Exposure via drinking water, contact and inhalation is not done as part of routine assessment due to lack of knowledge regarding appropriate scenarios and in some cases appropriate toxicity endpoints. With regard to drinking water see chapter 4.3.

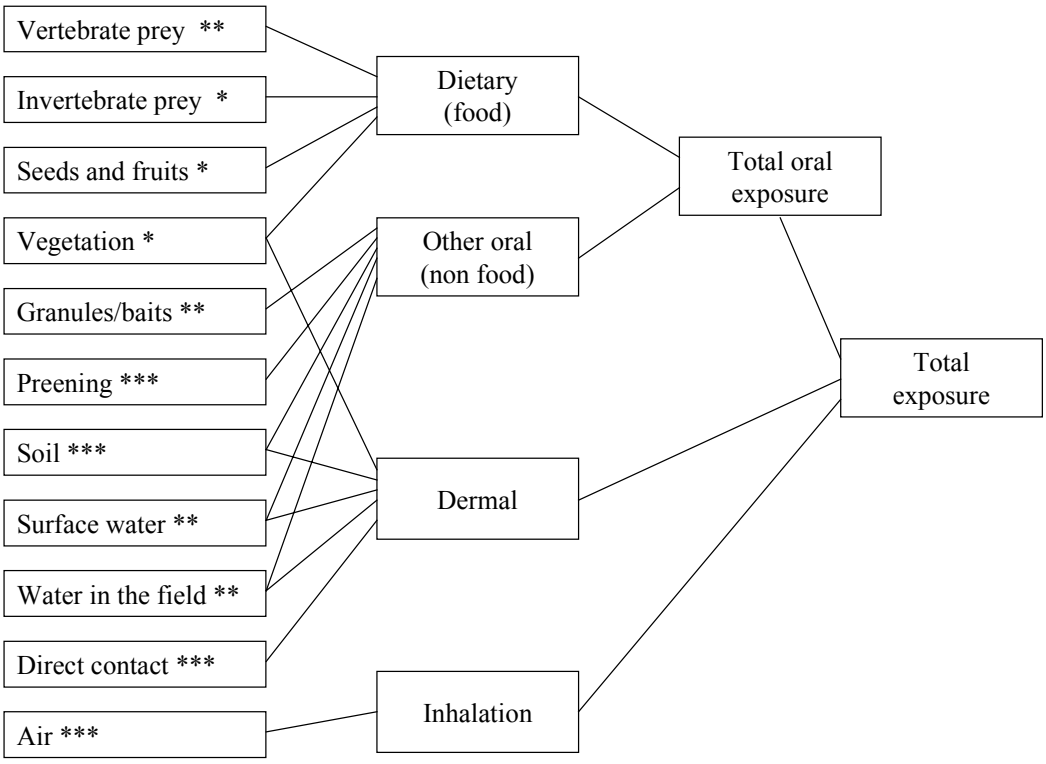


Figure 2: Exposure routes

* covered by the standard scenarios in chapter 3
** partly covered by non-standard scenarios in chapter 4
*** no guidance given in the present draft guidance document

3 Standard exposure scenarios for the tier-1 assessment

3.1 General approach and default values

The main scenarios and procedures are taken over from the EPPO risk assessment scheme for sprayed products (EPPO 2002), which covers intake via contaminated feed, generally considered to be the most important exposure route. In particular cases adaptations of the scheme or tailored procedures may be required some of which are described in chapter 4.

As explained in chapter 2 the exposure should be expressed as daily dose for all time scales. Thus the equations for acute, short-term and long-term exposure estimates are similar, but the assumptions for the input parameters may be different.

Basically the estimated daily uptake of a compound is given by the following equation:

ETE = (FIR / bw) * C * AV * PT * PD (mg/kg bw/d)

FIR Food intake rate of indicator species (gram fresh weight per day)

bw	Body weight (g)
C	Concentration of compound in fresh diet (mg/kg)
AV	Avoidance factor (1 = no avoidance, 0 = complete avoidance)
PT	Fraction of diet obtained in treated area (number between 0 and 1)
PD	Fraction of food type in diet (number between 0 and 1; one type or more types).

In case of multiple applications and/or long-term considerations the concentration C may be expressed as

$C = C_0 * MAF * f_{twa}$

C_0	Initial concentration after a single application
MAF	Multiple application factor (concentration immediately after the last application compared to a single application (Gonzales-Valero et al. 2000); see chapter 5.3)
f_{twa}	Time-weighted-average factor (average concentration during a certain time interval compared to the initial concentration after single resp. last application; see chapter 5.3)

$f_{twa} = (1-e^{-kt})/kt$

k	ln2/DT50 (velocity constant)
t	Averaging time

In the first tier it is assumed that

- the contaminated diet is not avoided
- animals satisfy their entire food demand in the treated area
- animals feed on a single food type

Thus the factors AV, PT and PD become 1 and can be omitted.

For food intake rate (FIR) and concentration default values are described below.

Food intake rate (FIR)

Data are derived from an extensive review by Crocker et al. (2002); the estimates of food intake are based on means of daily energy expenditure for free-ranging animals, energy content, moisture content and assimilation efficiencies. Equations and tables are found in Appendix I of this document.

Concentration (C)

a) Vegetation following spray applications:

Estimates are based on Fletcher et al. (1994); depending on the time scale either arithmetic means or 90th percentiles are used; the original figures were normalised to an application rate of 1 lb/acr; for the purpose here they are converted to 1 kg a.s./ha (residue per unit dose - RUD) and have to be multiplied by the actual application rate. (See tables 9 and 10 of Appendix II).

In the case of fungicides and insecticides applied in tall-growing crops such as orchards and vineyards it is assumed that a fraction of 60 % of the applied amount reaches the ground which is the maximum value applying to stages without leaves (FOCUS 2000); in later stages the interception is higher and accordingly the deposition lower; for refinement the deposition values given in FOCUS (2000) may be used:

- Vines: no leaves 60 %, first leaves 50 %, leaf development 40 %, flowering 30 %, ripening 15 %;
- Apples: no leaves 50 %, flowering 35 %, foliage development 30 %, full foliage 20 %;

b) Insects following spray application

There exists a generic data base collected by Fischer and Bowers (1997) which originates from 24 field studies. When normalised to an application rate of 1 kg a.s./ha the arithmetic mean residue is 5.1 mg/kg, the 90th percentile of the observed distribution is 14 mg/kg (see tables 4 and 5 of Appendix II). These figures have to multiplied by the actual application rate to give the concentration per wet weight. According to the opinion of the SCP (2002) these data should be applied for large insects only due to a bias caused by the sampling methods in the studies. For want of suitable data for small insects it is recommended to draw upon the surrogate values proposed by Kenaga (1973) which have been widely used in the past. Depending on the time scale either the "maximum value" should be used which is 52 mg/kg or the "typical value" which is 29 mg/kg (table 2 of Appendix II); again the figures have to multiplied by the actual application rate to give the concentration per wet weight. Small birds are assumed to prefer small insects, therefore the residues for small insects are the default values in the case of birds in order to cover the worst case. Insectivorous mammals always are assumed to eat large insects. In case of persistent and bioaccumulative substances residues in insects may increase over time, however such substances would be captured by the procedures described in chapter 4.2 (Bioaccumu-lation and food chain behaviour).
(Note: The residue estimate for small insects appears unsatisfactory, and as soon as better information becomes available this surrogate should be replaced. Research is highly desirable to develop more robust data for residues in insects, also with regard to the temporal pattern).

c) Seed treatments:

The concentration is based on the nominal seed treatment rate.

For background information on residues in food items for birds and mammals see Appendix II.

3.2 Establishment of scenarios

In the tier-1 assessment standardised realistic worst-case scenarios are considered. These involve generic indicator species designed according to various groups of mammals and birds. In each crop category several indicators with different feeding preferences may be relevant. For the tier-1 assessment, however, the number of scenarios has been restricted as far as possible. With mammals herbivorous species (if relevant) clearly represent the worst case, because, independent of their size, they receive higher doses than small omnivores (arthropods, seeds, vegetation = 1 : 1 :1), and insectivores. With birds the situation is somewhat different; as the exposure of insectivorous birds is based on residues in small insects (as opposed to large insects with mammals) the exposure is higher or close to herbivorous species; therefore two scenarios are proposed for some crops.

Table 1 shows which of the indicator species are considered in the various crops.

- "Grassland" includes pasture, lawn and turf; the vegetation in this group is represented by the category "short grass" in the database of Fletcher et al. (1994).

- "Cereals" are divided into early and late stages where "early" refers to a stage when the crop itself is likely to be grazed; in that case the category "short grass" is taken to estimate residues on the vegetation.
- "Leafy crops" form the bulk of the remainder of major field crops. The vegetation matches two groups of Fletcher‘s data base: forage crops (the data base includes alfalfa, clover, peas, beans) and leafy crops (data are said to cover all other dicotyledonous plants). Initial residues in both groups turned out to be similar and thus were merged in Appendix II to the group "Leaves etc.". Also maize and sweet corn should be added to this group (data on maize/corn were put to a separate category ("long grass") by Fletcher, but effectively the residues are similar to "leaves"). Tier-1 scenarios in this group of crops are based on herbivorous birds and mammals; however, many of these crops are not eaten by birds and mammals in late stages, so in cases where refinement becomes necessary the relevance of herbivores should be checked.
- For "orchard/vine/hops" it is assumed that these cultures have a ground vegetation which is represented by the category "short grass". In case of insecticides and fungicides, but not for herbicides, it is assumed that 40 % of the applied amount reaches the ground.

Table 1: Relevant indicator species according to crop and crop stage

Crop	Crop stage	Indicator species	Example
Grassland	-	Small herbivorous mammal - 25 g	Vole
		Large herbivorous bird - 3000 g	Goose
		Insectivorous bird - 10 g	Wren, tit
Cereals	Early	Small herbivorous mammal - 25 g	Vole
		Large herbivorous bird - 3000 g	Goose
		Insectivorous bird - 10 g	Wren, tit
	Late	Insectivorous mammal - 10 g	Shrew
		Insectivorous bird - 10 g	Wren, tit
Leafy crops	Early / late	Medium herbivorous mammal - 3000 g	Hare
		Medium herbivorous bird - 300 g	Partridge, pigeon
		Insectivorous bird - 10 g	Wren, tit
Orchard / vine / hops	Early / late	Small herbivorous mammal - 25 g	Vole
		Insectivorous bird - 10 g	Wren, tit
Seed treatment	-	Granivorous mammal - 25 g	Wood mouse
		Granivorous bird - 15 g	Linnet

In the case of herbicides applied to bare soil (with regard to crops and weeds) residues in vegetation may be negligible so the use of herbivores as indicators may not be relevant. However, if the active substance is systemic then the risk to herbivores should be assessed. It also should be assessed as to whether there is a risk from other routes of exposure (e.g. soil invertebrates and earthworms).

Table 2: Food intake rate (FIR) for indicator species with reference to Appendix-I data

Indicator species	Example	Body weight (g)	DEE (App I Tab 1/2)		Food characteristic (App I Tab 3)			Assimil. effic. (App I Tab 4/5)		FIR (fresh material) (g/day)	FIR / bw
			Equation	DEE (kJ/d)	Food type	Energy (kJ/g dry wgt)	Moisture (%)	Food type	%		
Small herbivorous mammal	Vole	25	Other eutherians	68	Grasses, cereal shoots	18	76.4	Grasses	46	34.8	1.39
Medium herbivorous mammal	Hare	3000	Other eutherians	1983	Non-grass herbs	18	82.1	General vegetation	74	832	0.28
Insectivorous mammal	Shrew	10	Other eutherians	36	Arthropods	21.9	70.5	Insects	88	6.3	0.63
Granivorous mammal	Mouse	25	Other eutherians	68	Cereal seeds	16.7	13.3	Nuts and seeds	83	5.7	0.23
Medium herbivorous bird	Partridge, pigeon	300	Other birds	389	Non-grass herbs	18	82.1	Herbage (Mean)	53	228	0.76
Large herbivorous bird	Goose	3000	Other birds	2302	Grasses, ceral shoots	18	76.4	Herbage (Ans)	41	1322	0.44
Insectivorous bird	Wren	10	Passerines	51	Arthropods	21.9	70.5	Animal (Pass)	76	10.4	1.04
Granivorous bird	Linnet	15	Passerines	67	Cereal seeds	16.7	13.3	Seeds (Pass)	80	5.8	0.38

These scenarios are designed for a generalised assessment of a substance intended for major crops or a broad spectrum of crops on EU level. The standard scenarios should fit in most cases, however if not applicable or if the use to be assessed is more specific with regard to crop, application technique, region, season, etc. then more tailored scenarios may be employed. The information necessary to construct non-standard scenarios may be taken from Appendix I of this document or the upcoming assessment scheme of the EPPO (EPPO 2002). Scenarios for plant protection products to be used in rice are under development in the EU (SANCO/1090/2000: Draft Guidance Document for Environmental Risk Assessment of Active Substances Used on Rice).

For all three time scales the same indicator species will be used. In order to estimate ETE the food demand needs to be known. In table 2 FIR is determined for all indicators according to the data from Appendix I.

3.3 Acute exposure

With regard to residues in vegetation and insects 90th percentiles of the initial concentration are used (small insects: " upper limit"). This figure has been chosen to give, along with the other settings, a reasonable and realistic worst case exposure for the first tier assessment. Multiple applications may cause sum-up of residues and therefore need considerations. In the case of vegetation a simple model based on first-order decline is used to calculate multiple-application factors (MAF) which give the ratio of the initial concentration after the last of n applications compared to the initial concentration after the first application. MAF is a function of the number of applications, interval, and DT50 (details see chapter 5.3). In the first tier a default value of 10 days for DT50 on vegetation is used (see chapter 5.3 for reasoning). However, ordinary MAF-values cannot be applied to upper percentiles because it is unlikely that each time the upper percentile is exceeded. Therefore special MAF factors have been calculated in order to predict the true 90th percentile of the peak after n applications based on the log distribution of the residue data (table 3). Note that these MAF_{90Fl} values contain specific variance information; they are only applicable on the 90th percentiles of these residue data, not on other data. In the case of insects little is known on time-course of contamination. However, it is expected that repeated applications do not cause appreciable accumulation of residues at least in foliage dwellers because in addition to other factors replacement of individuals due to migration and reproduction will contribute to the residue decline in the population. Therefore no MAF is applied for residues in insects.

Table 3: Multiple Applications Factors (MAF_{90Fl}) to be used in connection with 90th percentiles for residues on short grass and leafy crops according to Fletcher et al. (1994).

Interval (d)	Number of applications					
	2	3	4	5	6	8
7	1.4	1.7	1.8	1.9	1.9	2.0
10	1.3	1.5	1.6	1.6	1.6	1.6
14	1.2	1.3	1.4	1.4	1.4	1.4

Table 4 shows the standard residues (normalised to an application rate of 1 kg/ha) for the various scenarios. Calculation of ETE in terms of daily dose (mg/kg bw) is as follows:

- Spray application: Multiply relative daily intake (column 4) by RUD (column 6) and application rate (kg/ha); when applicable multiply also by MAF which is taken from table 3.
- Seed treatment: Multiply relative daily intake (column 4) by nominal seed treatment rate (mg/kg)

Table 4: Standard scenarios for the acute exposure estimate

1	2	3	4	5	6	7
Crop	**Crop stage**	**Indicator species**	**FIR / bw**	**Category**	**RUD (90 %)**	**MAF**
Grassland	-	Small herbivorous mammal	1.39	short grass	142	Table 3
		Large herbivorous bird	0.44	short grass	142	Table 3
		Insectivorous bird	1.04	small insects	52	n.a.
Cereals	Early	Small herbivorous mammal	1.39	short grass	142	Table 3
		Large herbivorous bird	0.44	short grass	142	Table 3
		Insectivorous bird	1.04	small insects	52	n.a.
	Late	Insectivorous mammal	0.63	large insects	14	n.a.
		Insectivorous bird	1.04	small insects	52	n.a.
Leafy crops	Early / late	Medium herbivorous mammal	0.28	leafy crops	87	Table 3
		Medium herbivorous bird	0.76	leafy crops	87	Table 3
		Insectivorous bird	1.04	small insects	52	n.a.
Orchard / vine / hops	Early / late	Small herbivorous mammal	1.39	short grass* I, F: IF=0.4	H: 142 I, F: 85	Table 3
		Insectivorous bird	1.04	small insects	52	n.a.
Seed treatment	-	Granivorous mammal	0.23	seeds	n.a.	n.a.
		Granivorous bird	0.38	seeds	n.a.	n.a.

*) For insecticides (I) and fungicides (F) but not for herbicides (H) an interception factor of 0.4 (deposition factor = 0.6) is assumed which applies to stages without leaves; in later stages deposition is lower (see chapter 3.1)

3.4 Short-term exposure

This assessment is conducted for birds only (see chapter 2.1); it aims at a time frame of a few days. Therefore initial residues are more appropriate than time-weighted averages. As usual in the first tier animals are assumed to feed on the treated field only (PT=1), however in the course of some days they will gather food in an area that is large compared to the spatial scale of residue variation. So averaging of residues is expected to occur and therefore arithmetic means are taken for residues in vegetation and insects (small insects: ” typical limit“).

Multiple applications are again considered. However, as residue estimates are based on arithmetic means now standard MAF values can be applied here (table 5).

Table 5: Standard Multiple Applications Factors (MAF) for residues in vegetation based on a DT50 of 10 days (equation and example calculations)

$MAF = (1-e^{-0.069ni})/(1-e^{-0.069i})$ i = interval; n = number of applications						
Interval (d)	Number of applications					
	2	3	4	5	6	8
7	1.6	2.0	2.2	2.4	2.5	2.5
10	1.5	1.8	1.9	1.9	2.0	2.0
14	1.4	1.5	1.6	1.6	1.6	1.6

Table 6 shows the standard residues (normalised to an application rate of 1 kg/ha) for the various scenarios. Calculation of ETE in terms of daily dose (mg/kg bw) is as follows:

- Spray application: Multiply relative daily intake (4) by RUD (6) and application rate (kg/ha); when applicable multiply also by MAF (7) which is taken from Table 5.
- Seed treatment: Multiply relative daily intake (4) by nominal seed treatment rate (mg/kg)

Table 6: Standard scenarios for the short-term exposure estimate

1	2	3	4	5	6	7
Crop	**Crop stage**	**Indicator species**	**FIR / bw**	**Category**	**RUD (mean)**	**MAF**
Grassland	-	Large herbivorous bird	0.44	short grass	76	Table 5
		Insectivorous bird	1.04	small insects	29	n.a.
Cereals	Early	Large herbivorous bird	0.44	short grass	76	Table 5
		Insectivorous bird	1.04	small insects	29	n.a.
	Late	Insectivorous bird	1.04	small insects	29	n.a.
Leafy crops	Early / late	Medium herbivorous bird	0.76	leafy crops	40	Table 5
		Insectivorous bird	1.04	small insects	29	n.a.
Orchard / vine / hops	Early / late	Insectivorous bird	1.04	small insects	29	n.a.
Seed treatment	-	Granivorous bird	0.38	seeds	n.a.	n.a.

3.5 Long-term exposure

The exposure estimate is very similar to the short-term assessment. Again residue estimates are based on arithmetic means, and for vegetation the same multiple application factors are employed.

In contrast to the short-term assessment time-weighted average (twa) residues are used here as these better reflect long-term exposure. It is obvious that a constant exposure level (if above the response threshold) will have more serious long-term effects than an exposure pattern

which starts with the same level and then rapidly declines, either due to accumulation of the substance (increase of body burden) or due to accumulation of effects. This has to be considered when relating toxicity (constant exposure level) to field exposure. Also, when assessing a persistent and a non-persistent substance the degradation rate in some way should be reflected in the exposure estimate and the risk indicator. An appropriate means to reduce such kind of bias is to average the exposure over a certain time interval. Unfortunately there is no sound scientific basis and no generally accepted rule on how long this interval should be; to simply take the study duration is disapproved by most experts. For the time being a period of 3 weeks is proposed as a convention, unless there are good reasons to take shorter or longer times. For example, cases where the effects data used are derived from a study with a shorter exposure period, or where a short delay between the onset of exposure and the onset of effects is observed, or where effects are to be ascribed to the exposure during a brief sensitive period would call for a shorter averaging time. With regard to residues on vegetation a simple twa-factor is used in the first tier which is based on the following default values:

- time window (averaging time) = 3 weeks
- DT50=10 days (for reasoning see chapter 5.3)

With these assumptions f_{twa} is 0.53; it means that over a period of 3 weeks the average concentration is about half the initial concentration. (Note: In case of repeated applications the maximum twa may be underestimated when the interval is shorter than the time window; with a time window of 3 weeks and a DT50 of 10 days the inaccuracy is small and the factor of 0.53 can be used uncorrected; however with parameters set to different values observe chapter 5.3.)

In the case of insects no default twa-factor is employed in the first tier as the time course of residue level is unknown.

Many birds are extremely mobile and hence there may be the possibility of concurrent and repeated exposure in adjacent fields which is particularly an issue in long-term assessments. It is considered that in the standard procedure the risk from multi-field scenarios is addressed by the conservative assumption that one bird obtains all of its food all of the time from the treated area. However, care has to be taken when going to refine PT (see chapter 5.6).

Table 7 shows the standard residues (normalised to an application rate of 1 kg/ha) for the various scenarios. Calculation of ETE in terms of daily dose (mg/kg bw) is as follows:

- Spray application: Multiply relative daily intake (4) by RUD (6), twa-factor (7) and application rate (kg/ha); when applicable multiply also by MAF (8) which is taken from table 5
- Seed treatment: Multiply relative daily intake (4) by nominal seed treatment rate (mg/kg)

Note on seed treatments:
Due to the fact that there may be a long-term effect from short-term exposure, there is the need to assess the long-term risk from compounds of this type although the assessment is difficult as reproductive effects are only tested in studies with long exposure periods (6 weeks to 1 year). See chapter 5.7 for further guidance regarding the interpretation of long-term toxicity tests. Further considerations for refinement are: availability of seeds, palatability (chapters 5.4 and 6.1), degradation from seed surface. At least for some kinds of seed there may be information available on the proportion in the diet of mammals and birds. If the compound is systemic and exposure is considered likely via the consumption of treated vegetation then this should be assessed appropriately.

Table 7: Standard scenarios for the long-term exposure estimate

1	2	3	4	5	6	7	8
Crop	**Crop stage**	**Indicator species**	**FIR / bw**	**Category**	**RUD (mean)**	**f_{twa}**	**MAF**
Grassland	-	Small herbivorous mammal	1.39	short grass	76	0.53	Table 5
		Large herbivorous bird	0.44	short grass	76	0.53	Table 5
		Insectivorous bird	1.04	small insects	29	n.a.	n.a.
Cereals	Early	Small herbivorous mammal	1.39	short grass	76	0.53	Table 5
		Large herbivorous bird	0.44	short grass	76	0.53	Table 5
		Insectivorous bird	1.04	small insects	29	n.a.	n.a.
	Late	Insectivorous mammal	0.63	large insects	5.1	n.a.	n.a.
		Insectivorous bird	1.04	small insects	29	n.a.	n.a.
Leafy crops	Early / late	Medium herbivorous mammal	0.28	leafy crops	40	0.53	Table 5
		Medium herbivorous bird	0.76	leafy crops	40	0.53	Table 5
		Insectivorous bird	1.04	small insects	29	n.a.	n.a.
Orchard / vine / hops	Early / late	Small herbivorous mammal	1.39	short grass I, F: IF=0.4	H: 76 I, F: 46	0.53	Table 5
		Insectivorous bird	1.04	small insects	29	n.a.	n.a.
Seed treatment	-	Granivorous mammal	0.23	seeds	n.a.	n.a.	n.a.
		Granivorous bird	0.38	seeds	n.a.	n.a.	n.a.

*) For insecticides (I) and fungicides (F) but not for herbicides (H) an interception factor of 0.4 is assumed (deposition factor = 0.6) which applies to stages without leaves; in later stages deposition is lower (see chapter 3.1)

4 Non-standard exposure scenarios and special considerations

4.1 Exposure routes and exposure estimates in case of baits

Slug pellets

Slug pellets are based on organic material and thus have a nutritional value for mammals and birds. It is known that small rodents like wood mice as well as granivorous birds ingest slug pellets if available (birds may take them as feed or as grit). As a starting point it could be assumed that animals feed exclusively on pellets. Suitable indicator species are the granivorous bird and the granivorous mammal from the standard scenarios. If a risk is indicated then palatability studies would be the most logical way to proceed because experience has shown that the attractivity of the pellets usually is limited. The pellets are coloured, and that feature among others may deter birds to a certain degree (Mastrota and Mench 1994, Best et al. 1996, Gemmeke 1999). Palatability studies should be conducted with the formulated product.

Rodenticidal baits

Rodenticides are inevitably toxic to mammals and birds, thus the risk assessment usually is a challenging task (Luttik et al. 1999). Most rodenticides are anticoagulants which are far more toxic if consumed repeatedly over several days compared to a single dosing making a short-term assessment more relevant than the acute assessment. Especially for mammals it may be necessary to take into account other toxicity figures than in the standard assessment, e.g. a five-day LD50 which often is carried out for such substances.

Primary poisoning:

Rodenticidal baits consist of cereals, grease or wax; therefore direct exposure is relevant mainly for rodents and seed eating birds. As rodenticides inevitably are toxic to non-target species an exposure assessment that is based on exclusive feeding on the bait will always come to the conclusion of potential risk. Two refinement steps are obvious:

- Consider accessibility of baits:
  - Baits placed in rooms or other enclosed spaces usually are inaccessible
  - Baits placed in bait stations or are covered in some other way are fairly inaccessible to non-target species; *Apodemus* species might enter bait stations; occasionally small birds might have access to the bait if the quality of the cover is poor.
  - Baits placed sub-surface (burrow-baiting) are inaccessible to almost all non-target animals. The burrows of common voles or water voles are usually not used by other rodents.
  - Baits spread on surface are accessible to many non-target species depending on factors such as kind and height of vegetation.

  Accessibility might be reduced by requiring appropriate use instructions to be put on the label (see chapter 7).
- Consider attractivity: Rodenticidal baits are designed to be attractive for rodents, so avoidance should not be expected. Often a bitter agent is added which repels children and carnivores but is unable to deter non-target rodents and birds. Nevertheless, the bait could be unattractive to birds to a certain degree due to colour, consistency and other factors, but that has to be tested before avoidance can be considered in the exposure estimate. Such tests could be conducted with a dummy formulation (which contains no active substance but is equal in all other features).

Secondary poisoning

Indirect exposure (secondary poisoning) can only be ruled out completely when the rodenticide is used in fully enclosed spaces so that rodents cannot move to outdoor areas. For other situations a risk assessment for avian and mammalian predators and scavengers is necessary. In order to estimate the exposure quantitatively a model calculation could be conducted based on a nominal concentration of the active substance in/on bait, bait uptake rate of target rodent, and estimated time to death of target rodent. However, such an estimate usually is unrealistically high as no elimination is assumed. Fortunately, in nearly all cases measured residues in rodents are available from different sources (special laboratory studies, secondary poisoning studies, monitoring of rodent control operations). For the purpose of exposure assessment whole body residues are relevant (not liver residues). If a risk is indicated the following options for refinement are promising:

- Evaluate secondary poisoning studies which are already available for current rodenticides (Joermann 1998, EPPO 1995)

- Improve estimate of proportion of target rodent in the diet of predators; suitable information might already be available from literature on feeding ecology; otherwise data could be generated using a marker in the bait.
- Field studies, monitoring

4.2 Exposure routes and exposure estimates in case of granules

If granules are based on an organic carrier having a nutritional value then they may be taken by birds or mammals as food. In such cases exposure could be assessed in a similar way as for baits or treated seeds. Granules with an anorganic base could be ingested either incidentally as birds and mammals inevitably incorporate a certain amount of soil when gathering food or intentionally when birds search for grit. Suitable guidance to quantify exposure from these route is found in the upcoming assessment scheme of the EPPO (EPPO 2002). Apart from direct uptake of granules it may be necessary to assess exposure via residues in seedlings or earthworms.

4.3 Bioaccumulation and food chain behaviour

With organic chemicals a logPow > 3 is used to indicate that there might be a potential for bioaccumulation. If that condition is met the three issues described below should be considered. As bioaccumulation processes often are slow and substances could be persistent a long-term assessment is appropriate. Relevant metabolites also have to be considered. For background information with regard to food chain modelling see Romijn et al. (1993, 1994).

a) Food chain from earthworm to earthworm-eating birds and mammals

A simple worst case assessment can be conducted according to the following steps:

(1) Take PEC_{soil} (twa, 3 weeks) from environmental fate section

(2) Estimate BCF (C_{worm}/C_{soil}) according to the following equation:

$$BCF = (0.84 + 0.01\ K_{ow}) / f_{oc}\ K_{oc}$$

K_{oc} = Organic carbon adsorption coefficient (available from physical/chemical data)
f_{oc} = Organic carbon content of soil (take 0.02 as a default value)

The equation originates from works of Jager (1998); the BCF is defined as earthworm fresh weight to soil dry weight. The model is empirically based on non-ionised, organic chemicals in the $\log K_{ow}$-range from 1 to 8, and it should not be applied on other types of substances or highly reactive substances. If modelling seems inappropriate it may be necessary to determine bioaccumulation factors experimentally.

(3) Estimate residues in earthworms: $PEC_{worm} = PEC_{soil} * BCF$

(4) Convert residue (PEC_{worm}) to daily dose by multiplying with 1.4 (mammals) resp. 1.1 (birds) and compare with relevant long-term NOEL. Multiplicators are based on a 10-g mammal eating 14 g worms (fresh) per day, and a 100-g bird eating 113 g per day, according to Crocker et al. (2002) (see Appendix I).

If the trigger of 5 is not met a refinement of the assessment is necessary. To that end it should be checked which options given in chapter 5 are applicable.

b) Food chain from fish to fish-eating birds and mammals

A simple worst case assessment can be conducted according to the following steps:

(1) Take highest PEC_{water} (twa, 3 weeks) from environmental fate section
(2) Take whole-body BCF for fish from aquatic section
(3) Estimate residues in fish: $PEC_{fish} = PEC_{water} * BCF$
(4) Convert residue (PEC_{fish}) to daily dose by multiplying with 0.13 (mammals) resp. 0.21 (birds) and compare with relevant long-term NOEL. Multiplicators are based on a 3000-g mammal eating 390 g fresh fish per day, and a 1000-g bird eating 206 g per day, according to Crocker et al. (2002) (see Appendix I).

If the trigger of 5 is not met a refinement of the assessment is necessary. To that end it should be checked which options given in chapter 5 are applicable.

c) Biomagnification in terrestrial food chains

With regard to terrestrial food chains those substances are of concern which have a potential for biomagnification, i.e. where the whole-body residue in an animal at steady state is higher than the residue in its food (BAF>1). For substances with such a property exposure may increase along the food chain, and the top predators are particularly at risk. In Annex VI of 91/414/EEC a trigger value of 1 is provided for the BAF (not quite correctly termed ”BCF”) which is specified as related to fat tissue. This trigger implies some degree of precaution because with lipophilic organic chemicals the whole body residue is lower than the residue in fat tissue.

The following step-wise approach is proposed:

(1) Get the information from the toxicology section on the ADME studies (ADME = adsorption, distribution, metabolism, excretion) and from the residue section on the metabolism studies with livestock. A brief conclusion from these assessments with regard to bioaccumulation is reported in the List of Endpoints. If the bioaccumulation potential is stated as low then stop; else continue with (2).

(2) Estimate the food-to-organism bioaccumulation factor (BAF) according to the following equation:

$$BAF_{organisms,food} = \alpha \, F / k_2$$

Where

α	Fraction of ingested dose that is absorbed; available from toxicokinetic studies
F	Food ingestion rate relative to body weight (FIR to body weight ratio); see Table 2 in chapter 3.2 and Appendix I; for carnivorous/ictivorous species a value of 0.3 can be used as a default value which corresponds to the 50th percentile for all carnivorous/ictivorous species listed in Appendix I and the 90th percentile for those over 100 g body weight.

$k_2=\ln(2)/T_{1/2}$	Rate constant for depuration; should also be available from toxicokinetic studies

If the BAF according to this calculation is clearly below 1 then stop; else conduct a detailed food chain modelling according to Appendix III.

4.4 Exposure via drinking water

Species that frequent open water bodies are liable to ingest residues of active substances that reach water for example via spray drift from treated fields. The exposure concentration in this case is equal to $PEC_{surface\ water}$, obtained from the environmental fate section of the monograph.

In some situations, some species may obtain all their daily water demand directly from puddles of spray liquid or reservoirs held in the axils of leaves. This situation may be relevant for certain crops (e.g. vegetables) or growth stages and certain season (summer). For substances that are volatile and rapidly photolysed in sunlight this route can be considered to be less relevant. The exposure concentration can be calculated from the dilution used to prepare the product for spraying (this information may be obtained from section 2 in the monograph). Analysis has shown that initial concentration in such sources are in the range 5-20 % of the sprayed concentration, therefore a dilution factor of 5 is applied (EPPO 1994).

The daily water intake is calculated allometrically as follows (Calder and Braun 1983):

Birds: Total water ingestion rate (l/day) = $0.059W^{0.67}$
Mammals: Total water ingestion rate (l/day) = $0.099W^{0.90}$

Where W is the body weight in kg. Thus, the daily dose of active substance is calculated as ($PEC_{drinking\ water}$ * total water ingestion rate) / W

4.5 Endocrine effects

Endocrine disruption is to be viewed as one of the many existing modes of action of chemicals and thus can be assessed in the normal conceptual frame-work. The environmental assessment is based on the ecological relevance of the observed effects, independently on the mechanisms of action responsible for such effects. Therefore, the general procedure for risk assessment can also be used for endocrine disrupters. For example, the standard TERs are applicable, if endocrine-mediated effects on reproduction are included in the toxicological endpoints. However, endocrine disrupting chemicals typically affect certain phases during reproduction and development, so potential effects may remain undetected if a test covers only part of the reproductive cycle. In the case of mammals the multi-generation study does cover the entire cycle and therefore a risk assessment for mammals based on integrative endpoints from this test may be reliable enough with regard to endocrine disrupters.

In the avian one-generation study, however, ecologically relevant effects associated with endocrine disruption could remain undetected due to technical aspects of the current test design, although it is not known how large the deficiencies really are. A two-generation study is under development in the OECD programme but not expected to be available for some time. Therefore, it is proposed to consider whether there are any indications on endocrine disruption in the mammalian tests such as deviating male-female ratio, abnormal sexual

development, or increased incidence of gonadal tumors. In such cases the avian reproduction test should be re-evaluated and re-interpreted carefully. In exceptional cases, where the substance clearly shows an endocrine disrupting effect with a high potency, acting at doses well below the threshold for other endpoints then the necessity of a non-standard avian reproduction test should be considered. Draft protocols are discussed in the OECD expert group on endocrine disrupter testing in birds and the OECD validation management group on ecotoxicity test methods for endocrine disrupter testing.

5 Options for refinement

In this chapter different options for refinement are described. Some options can always be used, others may be applicable to birds or mammals or in a certain time-frame only. There are no general rules on which option(s) could be chosen in a specific case. It depends on the availability of data, the cost of generating the necessary data and the level of uncertainty. It is advisable to assess the importance of a particular parameter before carrying out expensive studies.

5.1 Uncertainty factors

Under 91/414/EC a fixed assessment factor of 10 for acute and short-term risk assessment is used, whereas for long-term risk assessment a factor of 5 is used. There is no explanation in Annex II, III or the Uniform Principles specifying what uncertainties are meant to be covered by these factors and what level of protection is aimed at. Anyway, a major disadvantage of a fixed assessment factor is that by reducing the uncertainty, i.e. when more data become available, no correction can be made for this higher level of certainty, although it is obvious that e.g. the lowest out of 6 LD50 values has a different quality than a single LD50 value.

Where additional acute, dietary and reproductive data are available the uncertainty with regard to toxicity decreases and therefore, in principle, it should be possible to reduce the assessment factor. The use of species sensitivity distribution as described by Luttik and Aldenberg (1997) is a method that incorporates the level of uncertainty in the extrapolation factors used. This extrapolation factor decreases when the number of toxicity data increases. The method is based on the assumption that the effects assessment should be based on the 5^{th} percentile of the species sensitivity distribution. With other words for 95 % of the species the compound is less toxic and for 5 % of the species more toxic. As Luttik and Aldenberg (1997) optionally make use of generic variances their method is not reliant on a high number of species but can be applied to any number of data points; for a description see also EPPO (2002). Although statistical approaches like these are not yet routine in regulatory procedures they are founded well enough so that their use is acceptable as a contribution for refinement provided that additional toxicity data are available. (Conducting new toxicity studies in order to refine the risk assessment is not considered appropriate due to concerns over animal welfare). Further background information on the use of species sensitivity distributions are presented in the report from the Avian Effects Workshop held in Woudschoten (Hart et al. 2001) and a new elaborate publication (Posthuma et al. 2002).

5.2 Measured residues

C in the exposure equation is the residue on or in plant material, insects or other food material. The concentration will vary depending upon several factors, e.g. application rate, volatilisation, depuration, uptake by plant etc. For the first tier assessment generic data are

used to determine C (Fletcher et al. 1994, Fischer and Bowers 1997). Refinement may be possible by making use of available residue data for the substance and conditions to be assessed or by obtaining more data on residues on food sources, e.g. vegetation, arthropods, earthworms.

If residue trials involve repeated applications of the product and sampling starts at the last application then sum up of residues is included and these data are not subject to an additional multiple application factor.

With regard to the distribution and time-course of measured residues generally the same considerations are applied as in the standard assessment:

- For the acute assessment: Take 90th percentile (or equivalent) of initial residues
- For the short-term assessment: Take arithmetic means of initial residues
- For the long-term assessment: Take mean time-weighted-average residues (averaging may be done parametrically with an estimated DT50 or by considering the observed area-under-curve).

Deviations from these rules may be necessary depending on number, quality and representativeness of data.

Outlined below are factors to consider when designing a study to determine more realistic residue levels on potential food items:

- The proposed treatment regime should be in line with the worst case 'good agricultural practice'. For example if the product is to be used at 1000 g/ha on cereals from Growth Stage 60 onwards, then the study should be carried out at that rate at GS 60.
- The sites and conditions should be representative of the proposed usage. Data from a field study conducted in a northern Member State should be used for a northern Member State risk assessment and vice versa. However, it may be possible to use data from one region to support uses in another region, if it is obvious that the conditions in the first region tend to be worse compared to the second region so that the risk will not be underestimated. The acceptability of this should be considered on a case-by-case basis.
- More than one site should be used as between site variations are likely to be greater than within site. The number of sites should cover an appropriate range of situations to ensure that the data are representative of the proposed uses. Also, statistical advice should be sought when establishing the number of sites and the sampling scheme.
- There should be sufficient sampling time points to enable the risk to be addressed, for example if a high acute risk has been predicted from the use of an insecticide, then sampling should occur immediately after application. The sampling regime or timetable should be designed to address the concerns highlighted in the initial assessment.
- If a short, or long-term risk has been identified then the number of sampling points should enable a DT50 to be determined. For example day 0, 1, 2, 5, 10 and 20. If there is evidence from the residues package that the compound is likely to have a short half-life, for example from the residues or fate and behaviour, then the number of sampling points may be reduced. It should be noted that the number of sampling points should be justified. If the compound is applied several times per season it is not always necessary to repeat sampling through the season. However, if the product is likely to accumulate then repeat sampling should be carried out.

- The number of samples collected at each time point should reflect the degree of precision required. It is recommended that a small number of samples is collected first. Once these are collected the TER should be calculated and it should then be decided whether more data are required. If more data are required then attention should be focused on to specific food types of concern. It should be noted that in many cases the initial phase may be enough to show either that the risk is acceptable or that the original worst case assumption was about right.

The result of a measurement program will be a distribution of residue data accompanied by descriptive statistics. Appropriate values for the risk assessment should be established in the same way as set out in chapter 3 for the generic data (90^{th} percentiles for the acute exposure assessment, arithmetic means for short-term and long-term exposure) provided that the parameters are reliable from a statistical point of view.

Vegetation

If the main route of exposure is via the consumption of treated vegetation, then data from the residues part of the dossier should be used first. For example, this part of the dossier may include information on day 0 residues as well as information on residue declines etc. These data may give a more realistic level on vegetation as well as providing sufficient information to enable appropriate time-weight average concentrations to be generated. However, it has to be observed whether the part of the plant which was analysed matches what is expected to be eaten by birds and mammals. It should be noted that the generic residue data set in Fletcher et al. (1994) has been determined from consolidating data on the residue levels from several active substances and uses. Therefore it is possible that data from additional field trials may not always reduce the residue level significantly. It should be noted that if data from the dossier are used then these should always be related to the proposed use and scenario being refined. If it is not then it may be necessary to request more appropriate data.

Insects

If the main route of exposure is via the consumption of treated insects, then, it may be beneficial to determine residue levels on appropriate insects etc.

Insects should be collected via appropriate means, for example sweep netting, ‘tree beating’ (i.e. hitting trees with a stick and collect insect that fall out of them), D-vac and pitfall traps. The choice of collection technique will depend upon the risk highlighted and the insects likely to be consumed.

It should be noted that insects collected should be those that birds and/or mammals may be consuming. Samples from different collection techniques should not be pooled but should be kept separate and analysed separately. Keeping samples separate will ensure a more accurate indication of the true level of exposure via that particular food source. Residues on different food levels can be combined when PD is refined.

5.3 Residue decline in plants

The experience has shown that the disappearance of residues from plant material is fairly rapid even in the case the substance is persistent in other environmental media. There are different routes of disappearance of a substance from vegetation:

- Volatilisation

- Wash-off

- Degradation
- Metabolisation

In addition there is a decline of residues due to dilution by growth. The integrated result of these processes is usually expressed as an initial rapid decline in surface residues followed by a slower phase (Willis and McDowell 1987). So the assumption of first-order kinetics may be inappropriate when long time-frames are considered. Useful information may be derived either from a general data base or from the substance under assessment.

Generic data

Willis and McDowell (1987) presented a review of about 450 DT50 values (81 chemicals) for a broad spectrum of vegetative plant materials (grass, cereals, forage crops, cotton, vegetables, tobacco, foliage of fruit trees). Mean DT50 values and standard deviations for total residues were as follows:

Organochlorines:	5.8 ± 6.0 d
Organophosphates	3.3 ± 2.6 d
Carbamates	2.7 ± 1.2 d
Pyrethroids	5.9 ± 5.0 d

Due to the time schedule of sampling in the original studies the authors expect that many of the half-lives may be overestimates. This bias in mind and taking into account that the data base includes very stable substances such as organochlorines it is reasonable to use a DT50 of 10 days as a default value if the DT50 comes into play in the exposure assessment.

Specific data

Often the residue chapter of the dossier contains suitable information on residue decline in plants. For the purpose of exposure assessment for wild birds and mammals the following points should be observed:

- There is an interest in disappearance under practical use conditions. Therefore data from field residue trials covering all routes of loss are more relevant than plant metabolism studies which are focussed at metabolisation.
- With regard to time there is a major interest in the first weeks after application. So DT50 values (or other descriptors) should be derived from this interval, not from later periods.

Refinement of twa factor

The long-term exposure assessment employs time-weighted-average residues rather than initial residues. If data show that the DT50 is shorter than 10 days which is used as a default value in tier 1 then f_{twa} should be recalculated. Assuming first-order kinetics it is:

$$f_{twa} = (1-e^{-kt})/kt$$

k	ln2/DT50 (velocity constant)
t	Averaging time

This equation is also used when an f_{twa} for an averaging time other than 3 weeks is needed. Note that in case of repeated applications the averaging time should not be longer than the interval.

Refinement of MAF

In case of repeated applications residues will accumulate if at the end of an interval there are still remains from the previous application. When the basic concentration estimate is based on a single application but the intended use involves multiple applications then a multiple application factor (MAF) is introduced to care for sum-up of residues. In the first tier MAF is based on a DT50 of 10 days. If data show that the disappearance is faster then the MAF should be recalculated. Assuming first-order kinetics it is:

$$MAF = (1-e^{-nki})/(1-e^{-ki})$$

k ln2/DT50 (velocity constant)
n Number of applications
i Interval between applications (d)

Note: This is the ordinary MAF factor used for short-term and long-term exposure. The special MAF factor for acute exposure cannot be calculated by a simple equation (see 3.3).

5.4 Avoidance

Avoidance may be a significant factor that reduces the exposure. It may be a chemically-mediated response to the active substance being a primary repellent, a secondary repellent or inducing anorexia. Indications may be seen in the dietary toxicity test (Luttik 1998). However, in the case of granules and treated seeds avoidance often is observed even if the active substance is not repellent which then is due to co-formulants, colour, shape, texture and other features of the material. To characterise avoidance it is usually necessary to carry out palatability tests (see 6.1). There is no consensus yet on which approaches should be recommended. The key issue is that the extent of avoidance (and hence its effectiveness in reducing risk) is dependent on many factors which may differ between lab and field. Therefore, assessors should select a study design which manipulates the most important factors in a realistic way for the case in hand. General guidance on the factors to consider may be found in the report of the Pensacola workshop (OECD 1996).

Studies in which food consumption is measured under appropriate conditions can be used to provide estimates of the avoidance factor (AV). For example, if consumption of treated material under appropriate conditions is 10 % of normal consumption, then AV can be estimated as 0.1 and used to calculate a revised TER. Other test designs are to be regarded as simulated field tests which quantify effects rather than consumption. The results from these tests cannot be interpreted in terms of a revised TER but give immediately an indication of the likelihood of a certain effect. For all these types of studies, however, it is essential to take careful account of the test conditions when interpreting the results.

In assessing the effect of avoidance on acute avian risks, the key factor is the rate at which test birds feed during their first few hours of exposure to treated food. This depends on the test conditions and on the way birds are prepared for the test (Fryday et al. 1998, Pascual et al. 1999b). If the feeding rate in the test is close to the maximum in the field, then the result of the test can be regarded as close to worst case. If the feeding rate is lower, then the test is likely to underestimate the potential for risk in the field; such a test is useful in indicating the potential for avoidance but should not be used for a definitive assessment of risk. If the feeding rate in the field is unknown, it will be difficult to interpret the result of a test unless the feeding rate in the test is close to the maximum the species can achieve (in which case the

test is worst case). In principle the same considerations apply to acute mammalian risks, although mammals may show less variation in feeding rate than birds.

In assessing the effect of avoidance on short and long-term risks, the key factors are probably the duration of the test and the availability of alternative food. A prolonged test with no access to untreated food will overestimate risk, but a short test with untreated food freely available will underestimate it. If the test duration is appropriate and the availability of treated and untreated food is realistic, then the results should give a reliable indication of consumption and/or effects in the field.

Species differences are also a key factor to consider when assessing avoidance, for all timescales. Avoidance factors (AV) measured for one species may not be applicable to others. Also, species differences in toxicity may affect the interpretation of test results in different ways, depending on the type of test.

- Tests where consumption is measured: avoidance is likely to be strongest when the exposure is life-threatening, so AV for an insensitive species is likely to underestimate AV for a more sensitive species.
- Tests where only effects are measured: lack of effects may simply mean the test species is insensitive, and tells nothing about the avoidance response of more sensitive species.

There is no established method to extrapolate avoidance between species, so it may be necessary to test several species. The choice of the appropriate species to test will depend on species sensitivity (LD50 or LC50), feeding guild and feeding rates.

Once all the above points have been considered, an assessment has to be made whether, given the proposed use of the product, there is likely to be adverse effect. If there is still concern, various risk management options can be considered (see chapter 7) as well as additional testing, for example pen, cage or field studies.

5.5 Dehusking

In the case of seed eating birds and mammals dehusking may reduce exposure. Regardless, whether seed treatment is the intended use of the product or weed seeds are contaminated during spraying, the substance will be mainly on the husk and therefore dehusking can remove the majority of the residue. This reduction can be as high as 85 % (see review in Appendix II 2.6). Small birds are more likely to dehusk seeds than large birds, but anyway it depends on the kind of seed, and even when dehusking occurs, only a proportion of seeds are dehusked. For further information see Prosser (2001).

5.6 Steps to refine PT and PD

PT is defined as the ‘proportion of diet obtained in treated area’ (ECOFRAM 1999), whilst PD is defined as the ‘proportion of different food types in the diet’ (ECOFRAM 1999). In the first tier very simple assumptions are made regarding PT and PD, however it is possible to refine these estimates if sufficient information is available. It should be appreciated from the outset that extensive information on the agroecosystem is required in order to refine these steps. Information is required both on which birds and/or mammals occur in which crops as well as what they eat. It is acknowledged that this information is not yet readily available for the majority of crops across the EU, however information is available on certain crops in

certain Member States. There is also extensive data in the public domain which may help in refining these steps.

PT and PD are very specific to both the crop and species chosen and therefore any resulting assessment will tend to be Member State specific. This approach should be adequate to indentifying whether there is one 'safe use' or not, comparable assessments will however need to be done at the individual Member State level. It may, however, be possible to read across to other Member States depending upon the similarity of the agroecosystem.

In refining PT and PD it is usual to focus on one or two key species that are considered to be of concern. Due to the specific nature of these refinement steps it is not possible to use the generic species approach used in Tier 1.

PT – Proportion of diet obtained in treated areas

PT is defined as the 'proportion of diet obtained in treated area' (ECOFRAM 1999). In the first tier of risk assessment, it is assumed that birds and mammals obtain all their food from the treated area and hence PT is set to 1. Whilst this assumption is appropriate for first-tier risk assessment, it may significantly overestimate exposure. Therefore, if a high risk has been predicted (i.e. TER_a and/or TER_{st} <10 or TER_{lt}<5), then it may be feasible to consider this factor further. As PT is defined as the proportion of diet obtained from the treated area, it is preferable to obtain data for PT directly from studies using birds and mammals in the field or treated area. These data are very difficult to obtain and therefore outlined below are possible ways to refine PT.

PT can be estimated from the time spent in the treated area. For example, if it is assumed that the time spent in a habitat is a reliable indicator of the measure of food obtained there, then observational data may be used. It should be noted that this relationship will not be reliable if areas are used for feeding whilst others are used for nesting or if feeding rate is highly variable from location to location. Also the feeding rate will change during the course of the day. However, bearing in mind this proviso, it is considered that data on PT can be obtained in the following way:

- **Radiotracking:** This is where an appropriate radio transmitter is attached to the bird or mammal in question and its activities are recorded, i.e. the amount of time it spent in the treated crop compared to untreated areas. If radiotracking is supported with visual observation then an indication of what an individual is doing is obtained.
- **Visual observations:** This is simply watching individuals and recording what they do in specific habitats etc. This method, however has substantial drawbacks, for example it is not always possible to determine whether the same individual is observed or several individuals. Uniquely marked individuals may address some of this concern, however it is often difficult to track individuals for prolonged periods of time without 'flushing' individuals. It should be noted that the visibility of individuals is often low and hence this will underestimate exposure. Visibility will also vary depending upon the bird/mammals as well as the habitat being studied and this will lead in biased data set.

Data from radiotracking studies is obviously the most useful, but it is also the most difficult and expensive to obtain. Data are currently available on the exposure of birds in UK orchards (Crocker et al. 1998). Similar data are being generated to the exposure of birds and mammals in UK arable fields. An example of how these exposure data can be used is described below.

The data presented in Crocker et al. (1998) are appropriate to orchards grown in the UK. They may also be relevant to other Member States providing that the horticultural practices, i.e. size of orchard, size of trees, range of species exposed are all similar. When data of this type are available there will be a range of exposure values, when ranges are available the range of TERs should be calculated.

Good quality data on the exposure of birds/mammals in the agricultural environment is also able to provide an indication of the local potential impact at the population level, for further details see Crocker et al (1998).

Example:

From Crocker et al. (1998), data for blue tits suggests that 95 % of the local population spent less than 61 % of potential foraging time among orchard trees. This figure may be incorporated in the standard calculations of exposure as in the following example (note: it is not meant that the 95th percentile always is the figure of choice in considerations like this).

In a hypothetical example the TER, assuming that all food is obtained from the treated area, is 5.69 i.e. the TER is less than the Annex trigger value of 10.

Radio-tracking data indicates that 95 % of blue-tits find 61 % or less of their food in orchards. Therefore the standard calculation above could be modified by assuming that a 10 g blue tit daily consumes no more than 13.3 x 0.61 = 8.11 g caterpillars. This would result in a TER of 9.32 bringing it closer to the Annex VI trigger of 10.

For the majority of situations PT data are not available, however it may still be possible to refine PT to a more realistic figure using available published data and outlined below is a way to refine the risk:

- Highlight key species that are at risk according to Tier 1. This should include a range of species to cover the main feeding guilds, eg small insectivore bird, small herbivore bird, worm-eating bird, large herbivore bird, large insectivore bird and similarly for mammals.
- Obtain relevant data on the ecology and behaviour of those species at risk in the agricultural environment. If data are not available for the species at risk, then it may be possible to 'model' time spent in the treated area using general ecological knowledge on the behaviour of suitable birds or mammals. One possible way is to divide PT in four parts each representing 25% of the time, then using general knowledge on the behaviour of the bird or mammals in question, apportion time appropriately. For example in Tier 1 it is assumed that small insect eating birds spend 100 % of their time in a treated crop, however if reliable evidence shows that they only spend 50 % of its time in the treated crop, then the TER can be amended appropriately. A worked example is provided in Appendix IV. It should be noted that the time spent in the treated crop needs to be justified. It is also essential that a range of PTs are calculated for each bird to determine whether this is a pivotal factor in reducing the risk. If this refinement step is deemed to be pivotal then depending upon the reliability of the exposure data used, further data may be necessary.

This type of assessment provides a qualitative indication of the likely risk to individuals from several different species. It should be noted that when using published data of this type, the assumptions must be fully justified. If a wide range of exposure data are obtained from published literature, a sensitivity analysis should be conducted to demonstrate the range of

possible times spent in the crop and hence provide an indication of the possible range of 'risks'. This could be via the use of recalculated TERs to demonstrate the potential risks. If the resulting range is large with several species with refined TERs less than 10, then this indicates that the risk is still uncertain and hence more appropriate data required. Therefore, the use of published data to refine PT is unlikely to be sufficiently reliable to indicate a safe use. The above procedure will however be adequate for indicating whether generating further information on PT is appropriate and if so which species it should focus on.

In conclusion PT may be refined, however it should always be considered that all assumptions must be fully justified and that refinement of PT may not always reduce the risk sufficiently.

PD - Proportion of different food types in the diet

PD is defined as the 'proportion of different food types in the diet' (ECOFRAM 1999). For Tier 1 it is assumed that PD consists entirely of one realistic food type with the highest likely residue, e.g. short grass or small insects. If concern is raised it may be possible to refine PD in order to provide a more realistic indication of the risk. In order to refine PD data on food consumption of birds and mammals is essential. Ideally these data should be relevant to the proposed use and especially the time of application. However these are rarely available, therefore it is considered feasible to use basic ecological knowledge on bird and/or mammal feeding behaviour together with the proposed use of the plant protection product to model consumption appropriately.

In refining PD, data from stomach contents, faecal analysis, and pellet analysis can be used to determine likely food consumption. Data of this type have been collated for a wide range of species that may be exposed in the UK arable environment. It is proposed that these data could be used to generate hypothetical model diets for several species and then used to produce refined TERs. It should be noted that any data on diet composition should be relevant to the proposed use, i.e. it should be relevant in terms of both habitat and time of application. It is not relevant to use data on the diet of birds from the arable environment to refine the risk to birds feeding in an orchard and vice versa. It should also be noted that such data may underestimate the proportion of easily digestible food items, e.g. aphids.

The above refinement should then be used to recalculate the TER using appropriate figures for FIR and C. In chapter 2.3 the following equation is used to calculate the ETE:

$$ETE = (FIR / bw) * C * AV * PT * PD \qquad (mg/kg\ bw/d)$$

For a scenario with mixed diet it is necessary to calculate partial ETE values for each food type and sum them up to get the overall ETE. This would formally be expressed in the following equation:

$$ETE = \Sigma ((FIR_i / bw) * C_i * AV_i * PT_i * PD_i) \qquad (mg/kg\ bw/d)$$

Note: FIR_i in this equation is the daily uptake of fresh material an animal would require if it were feeding exclusively on that type of diet. Nevertheless, the proportions (PD_i) are related to dry material. (If FIR_i is composed of DEE, energy content, moisture and assimilation efficiency then strictly speaking the PD values reflect the contribution of the food type to the total energy expenditure which, however, is close to the proportion of dry weight uptake of that food type).

Example:
Consider a 40-g bird feeding on vegetation (25 % on a dry weight basis), small insects (37.5 %), and large insects (37.5 %); using data from Appendix I it is estimated that a 40 g bird would require either 55 g vegetation or 28 g insects per day; assuming concentrations of 87, 52 and 14 mg/kg respectively for vegetation, small insects and large insects the calculation of the acute exposure would look like as follows:

Food	**bw**	**FIR**	**C**	**PD**	**ETE**
Vegetation	40	55	87	0.25	29.9
Small insects		28	52	0.375	13.7
Large insects		28	14	0.375	3.7
Σ					47.3

When refining PD the following should always be noted:

- Refinement of PD will not always result in an increase of the TER.
- Partial PD-values should always sum to 1.
- Assumptions behind diet composition should be fully justified.
- All food sources should contain appropriate residue levels.
- Data on dietary composition should be converted to dry weights before using them to estimate PD.
- if dietary composition differs between treated and untreated areas, PD should be based on the diet taken within the treated areas

5.7 Relevance of endpoints in long-term toxicity tests

One aim of the ecological risk assessment is to predict effects on the population level, although this is difficult or impossible to measure directly. The usual approach is based on the consideration that effects on populations will not occur if the survival rate, reproduction rate and development of individuals are not affected. Therefore, in principle, only endpoints in toxicity tests which are related to these key factors of population dynamics are ecotoxicologically relevant. By definition the NOEL is based on the most sensitive endpoint of the test, and that is used in the first tier. In a refined assessment it could become necessary to check the ecological relevance of the effects seen at doses above the NOEL.

Category of endpoint

Reproduction tests with mammals and birds include parental and reproductive endpoints. If the overall NOEL is based on a reproductive endpoint but exposure will be transiently outside the breeding season then the NOEL for parental effects would be more relevant. However, it has to be observed that in certain Member States mammals may breed all year round.

Sublethal parental endpoints

Mammalian tests are designed for human risk assessment. They contain a wide spectrum of sublethal endpoints some of which are ecologically not relevant. For instance some biochemical responses reflect the presence of the test substance in the body and even by toxicologists are not regarded as adverse effects. The relevance of other chemical and

haematological endpoints also is questionable, but before disregarding effects it should be borne in mind that the survival of an animal in the lab does not necessarily mean that it would also survive in the field. Pronounced effects on body weight and food consumption (if it is a toxic response and not caused by avoidance) may reduce the fitness of wild animals.

Reproductive endpoints

If not indicated otherwise by the overall toxicological data available, an endpoint relating to overall reproductive success should be selected to define the long term NOEC for birds and mammals. Depending from the individual case and the availability of data, this could be the reproduction rate, the survival or growth rate of the offspring, or behavioural parameters in adults or young.

Magnitude of effects

The NOEL is based on the lack of statistical significance and not on biological significance. In an avian reproduction test of high quality (low variation coefficient, high power) it may be possible to prove that a 5-percent deviation in hatchling weight is statistically significant, a difference that remains undetected in normal tests. If the chick weight at day 14 is normal such an effect should not be considered as biologically relevant. The critical magnitude depends on the endpoint; rules of thumb saying that 20 % or whatever difference to the control is relevant should be avoided. Conversely, in a test of poor quality statistically insignificant effects does not necessarily imply an absence of biologically significant effects.

Time course of effects

Sublethal effects that are transient or reversible after termination of exposure are less relevant than continuous or irreversible effects. If reproductive effects in a mammalian multi-generation study are more pronounced in the second generation whereas in practice exposure will be restricted to a short time period then the reproductive NOEC after the first generation should be used as a possible refinement step (unless in exceptional cases, e.g. with suspected endocrine disrupters, where effects in the second generation may be attributable to a brief exposure period in the first generation).

5.8 Probabilistic risk assessment

Probabilistic risk assessment (PRA) is a rapidly developing tool in ecological assessments. Although having certain shortcomings and being not yet established in regulatory procedures PRA offers promising possibilities and therefore could be envisaged for refinement steps. The potential of these methods including their strengths and weaknesses has been discussed at the European Workshop on Probabilistic Risk Assessment for the Environmental Impacts of Plant Protection Products (Hart 2001). See also EU Guidance Document on Terrestrial Ecotoxicology (Document SANCO/10329/2002).

5.9 Use of wildlife incident data

When reviewing a compound it may be possible to use data from incidents involving wildlife (see e.g. Fletcher and Grave 1992, Mineau et al. 1999). However, the probability that victims are noticed, collected, reported to an authority and identified as being affected by plant protection products depends on numerous factors:

- Large animals or a mass mortality of small animals are more conspicuous than single carcasses of small animals.
- Specimens with a high conservation interest are more likely to be reported than common species.
- Carcasses found on agricultural fields are more likely associated with plant protection products than carcasses found elsewhere causing a bias towards fast acting substances.
- Virtually nothing else than mortality is covered by wildlife incident schemes.
- The organisation of incident investigation scheme varies in different countries (deSnoo et al. 1999).

From these reasons the absence of incidents does not necessarily indicate no risk or impact. On the other hand, if incidents have been reported, then it confirms that effects occur at least under some circumstances; furthermore, the nature of the effects or circumstances may give some clues about exactly what the problem is and on practical options for mitigating the risk.

Apart from incident data there occasionally are results from monitoring programs that could contribute valuable information to the overall assessment. See for example Newton et al. (1999).

5.10 Weight of evidence

Several of the above refinement steps require quantitative data to refine the risk assessment appropriately. Other options, however, produce qualitative information. If there are several sources of qualitative information (which may include data and considerations from elsewhere in the dossier) a weight-of-evidence approach may be taken to draw conclusions from these pieces of information. To that end the supportive value of the individual evidence has to be judged carefully. For example, the lack of incidence data may or may not be meaningful, depending on the circumstances, but even in the best case the lack of incidence data will be not hard enough an argument to prove safety. But combined with a number of further arguments and considerations it may (or may not!) be sufficient to demonstrate that the risk is acceptable. It is more likely that such an approach is successful in borderline cases than in cases where the standard assessment indicates a high risk.

An example is included at Appendix VI. This case study indicates that whilst there is a ‘theoretical’ long-term risk to birds, on closer examination of the usage pattern and the possible exposure pattern, the risk is in fact low and hence acceptable.

It may be argued that the use of an active substance and associated product may, in certain situations, be considered to pose an overall low risk regardless of the ‘theoretical’ risk indicated by a TER. This may be due to the fact that birds and mammals may have restricted access to where the compound is being used, e.g. grain stores. Alternatively, it may be that the use pattern will limit exposure, for example spot treatment in an industrial setting.

The scale of use of a particular active substance and associated product may also play a part in the overall assessment of risk. Assume that acute mortality of small passerine birds is of concern, and field data suggest a certain mortality rate expressed as fatalities per 100 ha. It is obvious that the scale of use determines the total impact of the product. In using this factor, careful consideration is required to ensure that it is used properly. If as part of the overall assessment, the risk is deemed to be acceptable due to the use being limited, justification needs to be presented as to why the area is limited. For example, if it is limited due to the

occurrence of a specific pest/disease that is limited geographically, then this would carry more weight than simply stating that a crop is only grown on a limited area. This is due to the fact that the latter may change radically due to changes in market forces etc, whereas the former is unlikely to change significantly.

In conclusion, a weight of evidence approach is acceptable, however the arguments must be appropriate and substantiated.

6 Higher tier tests

Generally the methods of higher tier tests are not standardised. Therefore notifiers should consult regulatory authorities well in advance in order to discuss the usefulness of higher tier tests, appropriate choice of tests and details of the protocol, and also in order to minimise the unnecessary use of test animals and resources.

6.1 Avoidance / palatability tests

There are currently no internationally accepted guidelines for testing avoidance (repellency/palatability). Two national guidelines exist and a number of other protocols are under development (see below). Various other methods exist (for a discussion see OECD 1996), including some intended for testing the efficacy of avian repellents for protecting crops.

The main variables of the test design are:
- test substance: technical a.s. mixed into standard food or items really encountered in the field (granules, treated seed)
- size of test area: cage, small aviary, large aviary
- housing of birds: singly, in groups
- offering of test material: cups, trays or scattered on the ground
- alternative food: yes or no (choice, no-choice)
- kind of alternative food: similar or not similar to the test substance
- duration of exposure

There is no single test design that is optimal for all situations.

For some substances, a single bout of feeding can result in a lethal dose. Recent research has shown that survival in this situation is determined by whether the avoidance response (whatever its mechanism) sets in before the bird consumes a lethal dose, and therefore depends crucially on feeding rate. This implies that tests of avoidance for short-term exposures need to control feeding rate. Data on feeding rates of relevant species of seed-eating birds can help the assessor specify appropriate conditions for testing short-term avoidance, and also help in extrapolating the results to the field. In longer-term exposures, short-term feeding rate is less important and birds have greater opportunity to seek alternative food. Consequently, a realistic choice between treated and untreated food is probably the key factor in designing avoidance studies for longer-term exposures.

The French guideline (INRA 1990) measures consumption (on a daily basis) and effects in choice and no-choice conditions. Treated material is provided in pots or hoppers.

The German guideline (BBA 1993) is intended for use with granular formulations, treated seeds and baits. The emphasis of the test design is on presenting the treated and untreated

material in a realistic way, mixed together and spread on the floor of the test aviary. Feeding rate is not controlled. Two versions of the test are specified, with different proportions of treated and untreated food. This guideline is probably not worst case for granular formulations because the test material is placed on a substrate of sand, which provides an attractive and unrealistically abundant source of alternative grit. The primary endpoint is mortality and signs of intoxication. The test does not require measurement of consumption, but it may be advisable to add this to assist the interpretation of observed effects.

A proposal for a revised dietary test protocol provides a means of determining a no-repellency concentration (Luttik 1998). Feeding rate is not controlled and no untreated food is available.

Two draft guidelines are being developed by BIAC for submission to OECD. One (the repellency test) is intended to quantify the degree of repellency by measuring consumption in choice and no-choice conditions, and is similar to the INRA guideline (see above). The other (the avoidance test) is specifically designed for use with treated seeds and is more similar in concept to the BBA 25-1 guideline, with the emphasis on presenting the test diet in a realistic way. This test is designed to quantify effects rather than consumption. It is focussed on short-term exposures (<1 day): no untreated food is provided in most cases and control of feeding rate is included as option in the current draft. Consumption is measured in the pre-trial period to assess feeding rate, but it has yet to be decided whether consumption should be measured on the test day.

In longer-term exposures, birds will generally have to move to different habitats to find untreated food. Multiple habitats can be simulated in large pens but these do not require birds to move as far as they would in the field. An alternative option which has been explored is to offer a ‘choice in time’ - treated and untreated foods are provided at different times of day. This approach is more conservative than offering treated and untreated food side-by-side.

Current efforts to develop avoidance test methods are focussed on seed treatments. They should be applicable to bait formulations also, but are not immediately suitable for testing avoidance of granular formulations nor of food contaminated by spraying. Assessors may therefore need to develop special studies to deal with these situations. General recommendations on factors to take into account in designing avoidance studies are provided by OECD (1996).

If a chemical is not avoided effectively by birds, a high level of mortality may occur in the avoidance test. It may therefore be desirable to test groups of birds sequentially, rather than all at once, so that the study can be terminated early if high mortality occurs in the early groups.

6.2 Pen/cage studies

Pen tests are only rarely conducted with mammals and birds, and there is no recognised standard method. Principally pen tests follow a semi-field concept where the product is applied according to practical use conditions either by applying the substance within an aviary or pen or by setting up an open-bottom cage in the field after treatment. Evaluation is facilitated as replications are possible. Such a test design allows observations and measurements of individuals. In some regard the situation is severe as the animals are confined to the treated area, on the other hand, however, energy expenditure and feeding rate may be reduced. Therefore care is needed in interpreting the results appropriately.

6.3 Field tests

Field tests with mammals and birds are subject to the same characteristics as other field tests: The gain in ecological realism is accompanied by a loss in repeatability and uncertainties in extrapolation to other field situations. Usually field tests require a high expenditure of organisation, man-power and technical gear to produce useful results. Often the cost-benefit relation is poor, so field tests are not a simple routine option to solve problems.

There does not exist an internationally agreed standard protocol for avian and mammalian field studies, but recommendations on methodology originating from international workshop a decade ago are still valid (Greaves et al. 1988, Somerville and Walker 1990, Anonymous 1990); there also is a protocol by the U.S. EPA (OPTTS 850.2500 - Field testing of terrestrial wildlife). In contrast to laboratory tests rigid protocols are not desirable for field studies. The trial should rather be designed individually addressing the problems that have been identified.

In a first step the study objectives are to be determined. It is hopeless, however, to ask generally whether there is ”any effect on mammals or birds” resulting from the application of the test substance. Instead the study must aim at more specific questions, for example the rate of acute mortality in a certain species or quantitative information on a certain parameters that affect exposure. According to these objectives appropriate study sites and a set of methods have to be selected.

Over the years a wide suite of techniques have been employed successfully. Each has advantages and limitations depending on the case:

- Chemical analysis of potential feed
- Carcass searching
- Capture of animals for histological and biochemical investigations (biomarkers for exposure or effect)
- Investigation of the gut content of captured animal
- If product is a bait: Admixture of marker and analysis of feces
- Determination of abundance by census techniques
- Evaluation of breeding success
- Radio-tracking

With the study objectives in mind a field study is tailored by choosing the appropriate methods from the catalogue above (and may be some tools not mentioned). It would be ideal to employ as many methods as possible but the expenses soon become prohibitive. To get the best gain from limited resources two different strategies have been followed (Greig-Smith 1990). The ”extensive trial” approach relies on only a few simple techniques like carcass searching and census methods but employs a large number of sites thus covering a broad spectrum of use conditions. It provides true replicates for the statistical evaluation and thus allows for the evaluation of the probability of effects. The ”intensive trial” on the other hand is a very detailed study on a small number of sites, maybe only one. It puts more emphasis on mechanisms and cause-effect relationships by combining the results from different methods. Both approaches have advantages and weaknesses and none can be regarded as superior in general.

The kind of data that result from field testing is manifold according to the test design. Therefore no detailed guidance is possible for all cases on how to interpret the results and how to use the data for the risk assessment. Some general recommendations can be given:

- Observe time-scale and endpoints (carcass search addresses acute/short-term lethal risk only)
- Evaluate whether conditions during the study are representative for the situation(s) to be assessed; in case of deviations: are they likely to increase or decrease the risk?
- Judge whether the sensitivity and resolution meet the desired level (carcass search on a single 1-ha site is unable to detect reliably an average mortality rate of 2-4 animals per ha)

- Consider the information content of data (a biomarker of exposure, e.g. ChE-assay from a representative sample of individuals, would give more useful information than just the observation that the animals survived.
- Simple endpoints (e.g. chemical analysis of potential food, gut content) may be used to refine input parameters of the exposure model and thus will lead to a refined TER
- Integrated endpoints (breeding success, biomarker of effects) can be used together with the TER in a weight-of-evidence approach.

7 Risk management options

In the case at least one substantial area of use has been identified as acceptable in the risk assessment at the EU level, i.e. TER higher than the appropriate Annex VI trigger values, but high risk is still indicated for other areas of use, then it may be appropriate to consider risk mitigation options. These options are use specific and the feasability of them should be determined on a Member State basis. Outlined below are possible options which could be considered if a high risk is indicated:

Risk from seed treatments

If a high risk from a seed treatment is predicted a label instruction should require to remove spills immediately. Furthermore, it may be appropriate to consider that the seed be drilled or incorporated immediately after application. If seed is incorporated availability to birds and mammals will be reduced and hence if an acute risk has been highlighted then this will be reduced as birds and mammals will take longer to find and consume treated seed. It has to be assessed, of course, whether consumption is reduced enough to conclude that the risk is acceptable. In considering such an option agronomic practices should be considered, for example: Will the seed still germinate? Will the seed treatment be effective if the seed is incorporated? This risk management option has been considered in detail by Pascual et al. (1999b) and further information regarding risk management options for cereal seed is presented in Pascual et al. (1999a and b).

Risk from granules

If a high risk from granules has been highlighted, again removal of spills should be required and the feasibility of incorporating them at the time of application be considered in order to reduce the availability to birds. As for seed treatment, agronomic implications should be considered when assessing this as a risk management option.

Risk from spray application

If a risk to birds and mammals has been indicated from the use of a spray, then the risk may be reduced by decreasing application rate and/or application frequency, however this may significantly affect the efficacy of the product. Alternatively spot or row treatment may be

appropriate depending upon the pest or disease being treated. Changing the method of application from spray to a more target approach, e.g. bait or paste/paint may reduce the risk to birds and mammals, however the success of this approach will depend upon the disease or pest being treated. If a reproductive risk to birds or mammals has been highlighted, then it may be appropriate to restrict the time of application when birds or mammals are not breeding, or to limit the number of applications and hence reduce exposure.

Risk from rodenticides

The availability of bait to non-target birds and mammals can be reduced by prescribing burrow-baiting or the use of bait stations. When surface spreading is necessary then application should be on vegetation rather than on bare soil. As far as rodent control in and around buildings and similar premises is concerned removal of dead and moribund rodents and removal of bait remains after completion of the control operation should be regarded as routine safety measures.

What ever risk management option is chosen, the practicality should be assessed fully to ensure that it does not reduce the effectiveness and usefulness of the product.

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9 Terms and Abbreviations

The following list contains technical terms used in the main document

AV	avoidance factor; dimensionless, between 0 (complete avoidance) and 1 (no avoidance)
BAF	Bioaccumulation factor; generally used for net accumulation from all exposure routes; in this document: ratio of concentration in body or organs related to concentration in food
BCF	Bioconcentration factor; ratio of concentration in body or organs related to concentration in media (e.g. soil, water)
bw	body weight
C	Concentration; here: concentration of a substance in food or other material which is ingested by birds or mammals (mg/kg)
DEE	Daily energy expenditure (kJ/d)
ETE	Estimated theoretical exposure; in this document defined as dose (mg/kg bw) or daily dose (mg/kg bw/d)
FIR	food intake rate (mass per unit time (g/d)); in this document it relates to fresh material if not otherwise stated
FIR/bw	food intake rate related to body weight; e.g. 0.25 means that an animal takes 25 % of its body weight per day; multiplying FIR/bw by concentration results in daily dose
f_{twa}	time-weighted-average factor; average concentration during a certain time period compared to the initial concentration
MAF	Multiple application factor; exposure level after the last of n applications compared to a single application
PEC	Predicted environmental concentration; concentration of a substance in environmental media
PD	Fraction of food type in diet; dimensionless (between 0 and 1)
PT	Fraction of diet obtained in treated area; dimensionless (between 0 and 1)
RUD	Residue per unit dose; generic residue data which are proportional to the applied dose; in this document RUD is related to kg/ha; actual residue estimates result from multiplying RUD by dose (kg/ha)
TER	Toxicity-to-exposure ratio

Appendix I: Daily food intake of wild birds and mammals

The tables in this appendix are taken from the report: Estimating daily food intake of wild birds and mammals, by D.R. Crocker, A. Hart, J. Gurney and C. McCoy. Available from www.pesticides.gov.uk/general/ResearchReports/index.htm

Table 1. Relationship between body weight and Daily Energy Expenditure (DEE) in birds for five groups of avian species. The general form of equation is: Log(DEE) = log a + b × (log Body weight). Insert log a and b from the table to obtain the specific equation for the relevant species group. Also shown are the standard errors for a and b (SE), the number of species in each group (N), and the proportion of variation explained by each equation (r^2). (DEE in kJ, body weight in g)

Group	log a	SE Log a	b	SE b	N	r^2
Desert	0.6107	0.1727	0.7299	0.0663	7	0.95
Hummingbirds	0.7495	0.0822	1.2064	0.1090	5	0.97
Other	0.6768	0.1896	0.7723	0.0861	11	0.89
Passerine*	1.0017	0.0647	0.7034	0.0503	38	0.84
Seabird	1.1482	0.1022	0.6521	0.0356	35	0.91
all birds	1.0220	0.0392	0.6745	0.0180	96	0.94

*excluding marine and desert passerines

Table 2. Relationship between body weight and Daily Energy Expenditure (DEE) in mammals for five groups of mammalian species. The general form of equation is: Log(DEE) = log a + b × (log Body weight). Insert log a and b from the table to obtain the specific equation for the relevant species group. Also shown are the standard errors for a and b (SE), the number of species in each group (N), and the proportion of variation explained by each equation (r^2). (DEE in kJ, body weight in g)

Group	log a	SE Log a	b	SE b	N	r^2
Non-eutherians	1.0232	0.0749	0.5814	0.0251	19	0.97
All eutherians	0.6794	0.0445	0.7646	0.0173	54	0.97
Desert eutherians	0.5120	0.0625	0.7843	0.0290	18	0.98
Marine eutherians	2.4203	0.7592	0.4266	0.1567	6	0.56
Other eutherians*	0.8459	0.0526	0.7050	0.0250	30	0.96
All mammals	0.7401	0.0467	0.7204	0.0174	73	0.96

* excluding desert and marine eutherians

Table 3. Energy and moisture contents for 15 general categories of food type, based on a total of 1783 reported values for energy and 761 for moisture.

Food type	Energy content (Kj/g dry weight)	Moisture content (%)
Dicotyledenous crop leaves	11.2	88.6
Grasses and cereal shoots	18.0	76.4
Non-grass herbs	18.0	82.1
Tree leaves	20.7	51.4
Orchard topfruit	11.6	83.7
Cereal seeds	16.7	13.3
Weed seeds	21.0	11.9
Small mammals	21.7	68.6
Bird and mammal carrion	22.6	68.8
Arthropods	21.9	70.5
Caterpillars	21.7	79.4
Soil invertebrates	19.3	84.6
Fish	20.7	71.1
Aquatic invertebrates	19.6	77.3
Aquatic vegetation	15.0	81.4

Table 4. Assimilation efficiencies (%) for mammals, based on 91 published studies. For details of underlying data and sample sizes (where known).

Mammal group	Food type	Mean	Standard deviation
Shrews and bats	Insects	88	5.9
Carnivores	Vertebrates	85	5.8
Squirrels	Nuts	85	7.5
Small mammals	Nuts and seeds	83	8.5
Small mammals	Grasses	46	10.7
Small mammals	Crops, forbs, mixed vegetation	74	12.3
Lagomorphs	General vegetation	74	13.5
White tailed deer	Tree browse	32	8.4
Ruminants	Hay and browse	80	2.8

Table 5. Assimilation efficiencies for birds, from Baerlein (1999). Standard deviations and sample sizes for individual order/food type combinations are included in the original reference and in the spreadsheet accompanying this report. N species = number of species, N cases = number of studies.

		N Species	N cases			Food typ
Order				animal	fruits	herbage
Struthioniformes	Ostriches	2	6			36
Gruiformes	Cranes, coots, rails	1	5	34	45	59
Ralliformes	Coots, rails	1	1			
Charadriiformes	Gulls, waders	7	19	69		
Lariformes	Gulls, terns	1	3	79		
Alciformes	Auks	1	2	76		
Sphenisiciformes	Penguins	7	26	75		
Procellariformes	Petrels	2	3	87		
Pelecaniformes	Pelicans, gannets, cormorants	4	8	80	76	
Columbiformes	Pigeons	4	36			
Psittaciiformes	Parrots	1	4			
Strigiformes	Owls	6	45	77		
Falconiformes	Eagles, falcons	4	12	84		
Accipitriformes	Hawks	11	22	82		
Ciconiiformes	Herons, storks	4	8	80		
Anseriformes	Ducks, geese	22	98	87		41
Galliformes	Fowl	18	184	70	57	42
Opisthocomiformes	Hoatzin (S. America)	1	2			
Trochiliformes	Hummingbirds	7	16			
Coliiformes	Mousebirds (Africa)	4	15		56	
Piciformes	Woodpeckers	1	14	64		61
Passerriformes	Passerines	67	441	76	67	76

Appendix II: Residues of plant protection products on food items

R. Luttik

(Reproduction of a RIVM Fact Sheet)

1. Introduction

In 1992 a RIVM-report (Luttik, 1992) was published in which a hazard/risk assessment method of the use of plant protection products for birds and mammals was described. This method was thereafter, beside some small changes, used in the process of placing plants protection products on the Dutch market (Handleiding voor de toelating van Bestrijdingsmiddelen van het CTB).
In the report of 1992 a method was described for estimating the residues on food items for birds and mammals due to spraying of plant protection products. The method is based on research carried out by Hoerger and Kenaga in 1972 in which they analysed data on residues of 28 plant protection products on 60 different crops. They provided maximum and "typical" values (the typical values are the mean values of the maximum for each crop/pesticide combination) that can be expected immediately after spraying on the vegetation (see Table 2).
In 1973 Kenaga proposed, for lack of measurements, to use the residue data of forage crops and cereals for small and large insects, respectively. Based on a smaller data base (27 plant protection products and 36 crops) the so called "nomograms of Kenaga" were developed by the U.S.EPA (Urban and Cook, 1986). This nomogram is still playing an important role in the first tier hazard/risk assessment in the USA.
Premises are that the residues that one can expect are not the result of the compound but of the crop and that the initial concentration increases proportional with increasing dose.

Table 2 Relationship between "typical" and maximum residue concentrations on plants or parts of plants (in mg/kg fresh weight) and the dosage (D) of plant protection products (in kg active ingredient per hectare) immediately after spraying (according to nomogram of Kenaga).

Plant/plant parts	Typical values	Maximum values
Short grass	112 * D	214 * D
Long grass	82 * D	98 * D
Leaves and leafy crops	31 * D	112 * D
Small seeds / forage crops[1] /small insects	29 * D	52 * D
Pods	2.7 * D	11 * D
Cereals / large insects	2.7 * D	8.9 * D
Fruit	1.3 * D	6.3 * D

[1] In the Hoerger and Kenaga paper the fourth category (29*D/52*D) is termed "forage crops" (based exclusively on alfalfa and clover); the sixth category is termed "grain" (mainly based on cereal grain, but also on cotton and soybeans).

Recently several studies have been carried out; in the first place to check whether nowadays the results of the research of 1972 are still valid (different compounds, low volumes, etc.) and in second place to provide better data for small and large insects:

- residues on plants by Fletcher et al. (1994) and Pfleeger et al. (1996),
- residues on invertebrates by Fischer and Bowers (1997), Brewer et al. (1997) and Joermann (1998), and
- Residues on weed seeds by Edwards et al. (1998).

In this factsheet a summary of the results of this research will be given and a proposal how to use these new data in the hazard/risk assessment for birds and mammals will presented.

2. Summary of new residue literature

2.1 Fletcher et al. (1994)

This study re-examines the Kenaga nomogram using information compiled at the University of Oklahoma. The database has 42000 individual records pertaining to over 1000 different organic chemicals, 65% of which are plant protection products. There are data for more than 400 species of plants, representing 95 plant families and all major crops.
Pesticide residue levels on days 0 and 1 following application were examined for 72 plant species and 68 chemicals. Most residue data pertained to leaves and leafy crops, legume foliage, and fruit. In Table 3 the maximum and typical data of Kenaga are presented, the percentage of measurements found by Fletcher that were higher than the values of Kenaga (% of exceeding), the mean values found by Fletcher and the 95th percentile values, estimated as the mean plus 1.6 times the standard deviation.
Fletcher et al. propose to use higher maximum values for small seeds/forage crops and fruit, 121 instead of 52 and 13 instead of 6.3, respectively. They propose to combine two categories pods/large seeds and fruit to one with a maximum value of 13 and one category for leaves/leafy crops and forage crops/small seeds with a maximum value of 121.
The percentage of exceeding is low for the categories of short grass, long grass, leaves and leafy crops, but considerable for forage crops/small seeds and for the category of fruit.
The linear relationship that the Kenaga nomogram has between application rate and residue amounts is consistent with the findings of Fletcher et al.
No indications were found to treat one particular compound group differently from the others. No correlation was found for morphological differences (e.g. surface texture, leaf shape).

2.2 Pfleeger et al. (1996)

The objective of this study was to evaluate the nomogram using field data. Six plant protection products (azinphos-methyl, dimethoate, disulfoton, esfenvalerate, endosulfan en chlorobenzilate) were tested on 15 different plant species with application rates ranging between 0.06 to 2.8 kg/ha.
The percentages of measurements above the maximum values of Kenaga for the categories short grass, long grass, forage crops/small seeds, pods/large seeds and fruit are 0, 16, 3, 17, 21 and 0%, respectively. These data indicate that three of the nomogram categories need to be altered: long grass, forage and pods. In the case of wheat (large seeds), the exceeding values may be an artefact of the sampling design (entire heads and not just grain). No higher values

Table 3 Residue values (normalised for an application rate of 1 kg active ingredient per ha); typical and maximum values according to Kenaga, mean and 95th percentile values according to Fletcher et al. and the percentage of values found by Fletcher et al. above the maximum values of Kenaga (% of exceeding).

Plants/plant parts	Kenaga "typical"	Kenaga Maximum	Fletcher Mean	Fletcher 95th percentile	Fletcher % exceeding Kenaga
Short grass	112	214	76	164	0 (0)
Long Grass	82	98	32	92	4 (2)
Leaves and Leafy crops	31	112	31	98	3 (0)
Forage crops/ small seeds	29	52	40	121	22 (9)
Pods, large seeds (cereals)	2.7	11	4	13	8 (4)
Fruit	1.3	6.3	5	20	19 (7)

were found for the category short grass. Probably the reason that the value of 214 in the nomogram of Kenaga was not exceeded is that the values (gallons per acre) from the original literature source were mistakenly made equivalent to pounds per acre by Kenaga and Hoerger. A statistical analysis carried out by Pfleeger et al. showed that it is not necessary to distinguish between the two categories forage crops/small seeds and leaves/leafy crops. The models developed in this experiment are quadratic, suggesting that the assumption that a linear relationship between application rate and residue level is not necessarily true.

2.3 Fisher and Bowers (1997)

Fischer and Bowers (1997) compiled measurements made in terrestrial field studies conducted by industry in the late 1980's and early 1990's (see also ECOFRAM, 1999, chapter 3.10.6.3). This data base included measurements made within 24 h of 175 foliar applications and 56 soil applications to actual field study sites. Descriptive statistics (mean, standard deviation, etc.) of these data sets are given in Table 4 and in Table 5 for selected percent exceedence probability levels. Measurements at foliar sites were close to the Fletcher nomogram model estimates for fruits which EPA has assumed are a surrogate for large insects, but much less than the corresponding nomogram values for forage crops which EPA has assumed are a surrogate for small insects. For example, Fletcher et al. (1994) reported a mean and standard deviation residue level per 1 kg/ha applied in/on fruits of 4.8 and 8.8 mg/kg respectively. The comparative values measured by Fischer and Bowers for invertebrates were 5.1 and 8.2 mg/kg, respectively. Measured residues in invertebrates at sites where applications to the soil were made were much lower with the mean in these cases being <1 mg/kg. It is not surprising that these levels were lower since incorporation of the chemical into the soil mechanically, or via watering, ”dilutes” the amount of residue that is likely to contact invertebrates crawling on or in the soil at these sites. The invertebrates in these studies were mostly collected in pitfall traps set immediately after application and retrieved the next morning, or by sweep netting the top of the treated vegetation a few hours after application. These collection methods have

Table 4 Residue levels (mg/kg wet weight) for insects/invertebrates as a result of an application rate of 1 kg active substance per hectare (according to Fischer and Bowers, 1997).

Application type	Mean		Maximum	Minimum
	Normal	Geometric		
Foliar	5.1	1.9	48	0.036
Soil-incorporated	0.53	0.036	23	0

Table 5 Residue levels (mg/kg wet weight) for insects/invertebrates as a result of an application rate of 1 kg active substance per hectare for selected percent exceedence probability levels (according to Fischer and Bowers, 1997).

Application type	Calculation method	Exceedence probability level			
		50%	20%	10%	5%
Foliar	Observed data	1.5	7.8	14.3	20.8
	Regression model	1.9	6.8	13.3	23.0
Soil-incorporated	Observed data	0.026	0.20	0.44	1.2
	Regression model	0.036	0.20	0.54	1.3

potential biases. For example, a net swept against the surface of treated vegetation is likely to remove dislodgeable residues and these residues may in turn adsorb to the surface of insects caught in the net. Thus, these insect samples might have artificially inflated pesticide concentrations. On the other hand, an opposite bias may be associated with pitfall trap samples. This is because although some individuals falling into the traps ”rain down” from the vegetation upon death after an insecticide application, most animals probably fall in while walking across the ground. In the case of insecticide applications, which represent the vast majority of samples in Fischer and Bower’s data set, the most highly exposed individuals are expected to become immobilised and therefore have a lower chance of encountering and falling into a pitfall trap. If this is true, the residue levels in pitfall trap samples might by biased on the low side. The following study (Brewer et al., 1997) has been conducted that controls for these sources of bias and allows one to judge their likely significance in the Fischer and Bowers data set.

2.4 Brewer et al. (1997)

Brewer et al. (1997) conducted small plot residue trials with several compounds specifically to obtain measurements of residues in invertebrates (see also ECOFRAM, 1999, chapter 3.10.6.3). In these trials, adult insects (crickets and/or beetles) and ”wormy” larvae (beet armyworms and/or beetle larvae) were placed just prior to application on the ground or on vegetation within a spray swath and confined there until they were collected several hours later. Mobile individuals (i.e., adults) were confined to the spray path by pinning them to vegetation or placing them in enclosures. Residue levels (see Table 6) in these samples fell well within the range of observations in the Fischer and Bowers data set. The average values as a result of an application rate of 1 kg active substance per hectare for both adult insects (3.3 mg/kg) and larvae (2.1 mg/kg) were below the average of the Fischer and Bowers data set (5.1

mg/kg). This finding is inconsistent with the potential concern that Fischer and Bowers data are biased on the low side due to the use of pitfall traps as a collection method.

Table 6 Pesticides residue levels per unit dose of 1 kg/ha measured in adult and larval insects confined to the spray swap during foliar applications to experimental field plots (according to Brewer et al., 1997).

Insect type	n	Mean		Maximum	Minimum
		Normal	Geometric		
Adult crickets and beetles	5	3.3	2.4	4.8	0.34
Larval armyworms and beetle larvae	5	2.1	6.4	0.29	0.29

2.5 Joermann (personal communication, E-mail dd. 18 February 1998)

G. Joermann of the Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA) in Braunschweig (Germany) has carried out a small literature research concerning residue levels on arthropods as a result of spraying. In Table 7 the results of this research are presented. Most of the data are within the range presented by Fischer and Bowers. In a few cases higher values are found than the concentration presented for 5% percent exceedence probability level.

2.6 Edwards et al. (1998)

Little published data are available for weed seeds. An important food item for many wildlife. Edwards et al. conducted two residue trials. In study 1 the lentil crop and surrounding natural vegetation were aerially sprayed at a rate of 550 g a.i./ha and in study 2 weed plots and wheat stubble were ground sprayed at 1000 g a.i./ha. Differentiation was made between whole seeds sprayed on plants, whole seeds sprayed on ground, seeds sprayed on plant in pod and seed sprayed on plant then dehusked. Residue values were adjusted for unit dose (1 kg a.i./ha) and for low interception on artificial soil surface (x 0.6).
The results of these residue trials are presented in Table 8. According to these results there is no need to discriminate between seeds sprayed on the plant or on the ground because residues are similar. Mean residue levels on weed seeds (= small seeds) is 43 mg a.i./kg (fresh weight). There is no need to discriminate between the large seed category of Hoerger and Kenaga (1972) and seeds in pods because residue levels are similar. There is a need to discriminate between birds which do and do not dehusk seeds before consumption; about 80-95% of the spray residue is on the husk. For birds with a body weight smaller than 50 g a dehusking factor of 0.13 should be used (43 ➔ 5.6 mg a.i./kg (fresh weight).

Table 7 Measured residue levels on arthropods. Application rate in kg active ingredient/ha. Residue levels in mg /kg wet weight, standard residue levels normalised for an application rate of 1 kg active ingredient/ha (after Joermann).

Active Substance	Appl. Rate	Crop	Arthropod	Measured Residues	Standard Residue	Author
Acephate	0.61	Rangeland	Grasshopper	10.8-14*	18-23	Stromborg et al. 1984
Carbaryl	0.50	Rangeland	Grasshopper	17	34	Fair et al. 1995
Carbofuran	0.14	Pasture	Grasshopper	2.2-3.9 max 5.7	16-28 max 41	Forsyth and Westcott 1994
Carbofuran	0.13	Laboratory	Grasshopper	2.1-2.9	22	Forsyth and Westcott 1994
Carbofuran	0.13	Laboratory	Grasshopper	6.1-6.8	46-51	Martin et al. 1996
Carbofuran	0.53	Laboratory	Grasshopper	35-38	66-72	Martin et al. 1996
Chlorpyrifos	0.72	Pasture	Leatherjacket	Max 1.2	max 1.7	Clements et al. 1988
Chlorpyrifos	0.28	Laboratory	Grasshopper	16-19	57-68	Martin et al. 1996
Chlorpyrifos	1.12	Laboratory	Grasshopper	73-83	65-74	Martin et al. 1996
Diazinon	0.84	Tobacco	Hornworm	Nd-2.5	nd-3.0	Stromborg et al. 1982
Diflubenzuron	0.81	Trees	Caterpillars	78	96	deReede 1982
Diflubenzuron	0.3	Trees	Caterpillars	2	7	deReede 1982
Diflubenzuron	0.08	Trees	Caterpillars	3-11	37-137	deReede 1982
Dimethoate	0.21	Laboratory	Grasshopper	4.0-4.1	19	Martin et al. 1996
Dimethoate	0.85	Laboratory	Grasshopper	13.4-16.4	16-19	Martin et al. 1996
Fenitrothion	0.3	Forestry	Caterpillar	1.3-2.7	4.3-9.0	Hamilton et al. 1981
Fenitrothion	0.3	Forestry	Different Invertebrates	0.5-25	1.7-83	Hamilton et al. 1981
Fenitrothion	0.21	Forestry	Budworm	0.7-1.2	3.3-5.7	Forsyth and Martin 1993
Fenthion	0.052	Grassland	Different Invertebrates.	0.28	5.4	Powell 1984
Fenvalerate	0.112	Cotton	Grasshopper	0.18-0.24	2.2	Bennett et al. 1983
Fenvalerate	0.112	Cotton	Ground beetle	0.55	4.9	Bennett et al. 1983
Fenvalerate	0.112	Grassland	Grasshopper	0.03-0.33	0.3-2.9	Bennett et al. 1986
Fenvalerate	0.112	Grassland	Ground beetle	Nd-0.15	nd-1.3	Bennett et al. 1986
Fenvalerate	0.112	Grassland	Crickets	Nd-0.1	nd-0.9	Bennett et al. 1986
Isazofos	5.0	Turf	Mole cricket	0.06-1.3	0.01-0.3	Brewer et al. 1988
Malathion	0.61	Rangeland	Grasshopper	1.4-2.8	2.3-4.6	Stromborg et al. 1984

* Acephate + Methamidophos; nd = not detectable

Table 8 Measured residue levels on weed seeds

Description	Mean		Minimum value	Maximum value	No. of samples	No. of species
	Normal	Geometric				
Whole seed sprayed on plant	42	37	17	76	6	5
Whole seed sprayed on ground	45	44	31	52	4	4
Whole seed sprayed on plant and ground	43	40	17	76	10	6
Seed sprayed on plant then dehusked	6.5	5.4	2.1	14	5	4
Seed sprayed on plant in pod	1.8	1.4	0.7	3.8	3	3

3. Conclusions

The maximum residue per unit dose values found by Hoerger and Kenaga on long grass and leaves/leafy crops (98 and 112 mg/kg wet weight) are comparable with the 95th percentile data of Fletcher et al. (92 and 98 mg/kg ww).

The maximum residue per unit dose values found by Fletcher et al. for small seeds/forage crops and for fruit, respectively 121 and 20 mg/kg ww, are higher than the data provided by Hoerger and Kenaga (52 and 6 mg/kg ww).

To adjust the forage crops/ small seeds category from 52 to 121 mg/kg ww the number of exceeding values on day 0 would be reduced from 22% to 5%.

To adjust the category of fruit from 6 to 13 mg/kg ww the number of exceeding values on day 0 would be reduced from 19% to 8%.

The maximum residue per unit dose values for short grass given by Hoerger and Kenaga are probably based on a mistake.

Because the residue per unit dose value of 121 for forage crops/small seeds found by Fletcher et al. is close to the existing residue per unit dose value of 112 for leaves and leafy crops, it would be appropriate to combine the two categories. This was also affirmed by the research of Pfleeger et al.

In a similar fashion, fruit (new residue per unit dose value 13) and pods/large seeds (old residue per unit dose value 11) could be placed in a single category.

The research carried out by Fisher and Bowers, Brewer et al. and Joermann showed that the residue levels proposed in earlier days for small and large insects by Kenaga, and still used nowadays in the hazard/risk assessment, are in most cases too high.

Although only a small number of initial residue trials have been carried out with small seeds the results found by Edwards et al. do not give an indication for changing the values proposed by Hoerger and Kenaga and by Fletcher et al. for the categories of small seeds and large seeds or pods.

4. Recommendations

Because the database used in the Fletcher et al. research is much larger and more a reflection of the state of the art than the one used by Hoerger and Kenaga, preference is given to the Fletcher et al. database.

It is recommended to use four plant categories and two insect categories:

- short grass,
- long grass,
- leaves, leafy crops, forage crops and small seeds
- fruit, pods and large seeds,
- insects (foliar application), and
- insects (soil incorporation).

In Table 9 the proposed values for the "mean" situation are given. Multiplying the arithmetic mean by the application rate (in kg/ha) of the compound of concern gives the residue on the food item in mg/kg food.

The ECOFRAM report (1999) suggests that the data probably are lognormally distributed. Fletcher et al. (1994) give the percentage of values that exceed the upper Kenaga limit, and indeed these percentages better match the lognormal parameters than the linear parameters (pers. comm. G. Joermann). Therefore, the lognormal transformed mean and standard deviation are also presented in Table 9. The arithmetic mean and standard deviation are lognormal transformed using the following two formulas:

$$s_y^2 = \ln[1 + (s_x^2 / m_x^2)]$$

$$m_y = \ln m_x - 0.5 * \ln[1 + (s_x^2 / m_x^2)]$$

Table 9 Mean data and standard deviations according to Fletcher et al. (1994) and Fischer and Bowers (1997) for six types of food (normalised for an application rate of 1 kg active ingredient/ha).

	Arithmetic		lognormal transformed		
Description	Mean	Std	Mean	std	n
Short grass	75.7	53.8	4.12	0.64	18
Long grass	32.1	36.3	3.06	0.91	46
Leaves etc	40.2	50.6	3.22	0.98	96
Fruit etc.	4.8	8.8	0.84	1.21	108
Insects foliar	5.1	8.2*	0.99	1.13	175
Insects soil	0.5	3.0*	-2.37	1.87	56

* Data according to ECOFRAM (1999) page 3-88

Based on the lognormal transformed data for four percentiles (50, 90, 95 and 99) at three levels of confidence (95, 50, and 5%) values have been calculated according to the method described by Aldenberg and Jaworska (2000). This method takes the samples sizes into account. The calculated values are presented in Table 10.

In chapter 3.3 to 3.5 it is recommended to use the following input data for assessing the residue levels on food for birds and mammals in the reasonable worst case situation:

Acute exposure: 90th percentile of the initial concentrations

Short-term exposure: arithmic means of initial residue levels and no adjustment for degradation (no time weighted averages)

Long-term exposure: arithmic means and adjustment for degradation in time.

Table 10 Residue values (normalised for an application rate of 1 kg/ha) for four different percentiles (50, 90, 95 and 99) at three levels of confidence (5, 50 and 95%).

Food type	50th percentile			90th percentile			Sample Size (n)
	Lower	Median	Upper	Lower	Median	Upper	
Short grass	47.2	61.6	80.2	105	142	218	18
Long grass	17.0	21.3	26.8	52	69	96	46
Leaves etc.	21.2	25.0	29.6	71	87	111	96
Fruit etc.	1.9	2.3	2.8	9	11	15	108
Insects foliar	2.3	2.7	3.1	10	11	14	175
Insects soil	0.1	0.1	0.1	1	1	2	56
	95th percentile			99th percentile			n
	Lower	Median	Upper	Lower	Median	Upper	
Short grass	129	180	296	186	281	533	18
Long grass	70	96	141	121	178	292	46
Leaves etc.	99	125	164	181	244	345	96
Fruit etc.	13	17	24	28	39	58	108
Insects foliar	14	17	22	29	38	50	175
Insects soil	1	2	4	4	7	18	56

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Appendix III: Bioaccumulation of chemicals in terrestrial vertebrates

PROPOSAL TO ESTABLISH AN INITIAL RISK ASSESSMENT OF TERRESTRIAL VERTEBRATES FOR THE ESTIMATION OF PESTICIDES WITH BIOMAGNIFICATION POTENTIAL

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PROPOSAL TO ESTABLISH AN INITIAL RISK ASSESSMENT OF TERRESTRIAL VERTEBRATES FOR THE ESTIMATION OF PESTICIDES WITH BIOMAGNIFICATION POTENTIAL

MV Pablos, C. Ramos, M.M. Vega, and J.V. Tarazona.

The current model assesses the potential for consumption of sprayed items and bioaccumulation of pesticides. Other hazards, such as biomagnification are not taken into account in this evaluation, but are important aspects for the protection of top predators. If the substance is persistent and bioaccumulable (on the aquatic and/or terrestrial compartment), it would be necessary to apply an additional biomagnification model. For this assessment, it is necessary to calculate for each trophic level, the percentage of the total intake that is retained by the organism. These data can be obtained from the studies of both, metabolism on mammals and bioaccumulation on fish. Therefore an initial biomagnification assessment can be easily done with the available information.

This proposal presents a simplified model to assess the potential for biomagnification through the food chain.

BIOACCUMULATION OF CHEMICALS IN TERRESTRIAL VERTEBRATES

The bioaccumulation of pesticides in terrestrial vertebrates is estimated from the food-organism bioaccumulation factor (BAF):

$$BAF = C\ organisms/C\ food$$

where C organisms and C food represent the steady-state concentrations of the chemical in the organism and the food respectively.

The BAF can be directly obtained from experimental assays or estimated from a combination of default values and the available data on the toxicokinetics of the pesticide in mammals.

The following equation is proposed for the estimation of the BAF:

$$BAF_{organisms,food} = \alpha F/k_2$$

This is a modification of the typical equation ssBCF= k_1/ k_2 where the uptake rate is represented by the product of the assimilation efficiency (α) and the feeding rate (F) while k_2 represents the depuration rate.

The assimilation efficiency (α) represents the ratio between the amount of chemical existing in the food and the amount of chemical absorbed by the organisms. This information is generally available in the toxicokinetic studies on mammals.

The feeding rate (F) represents the Food intake rate related to body weight (FIR/bw) Appendix I offers estimated values for several bird and mammal species. The following table covers predators and top-predators.

Table 1: Food intake rate (FIR) and Food intake rate related to body weight (F) for predator and top-predator species with reference to Appendix-I-data

Indicator species	Example	Body weight	DEE (App I Tab 1/2)		Food characteristic (App I Tab 3)			Assimil. effic. (App I Tab 4/5)		FIR (fresh material)	FIR / bw
		(g)	Equation	DEE (kJ/d)	Food type	Energy (kJ/g dry wgt)	Moisture (%)	Food type	%	(g/day)	
Predator bird	Peregrine falcon	1000	Other birds	986	Bird and mammal	22.6	68.8	Animal	84	166	0.17
Top predator bird	Golden eagle	5000	Other birds	3416	Bird and mammal	22.6	68.8	Animal	84	577	0.12
Predator mammal	Fox	8000	Other mammals	4911	Bird and mammal	22.6	68.8	Vertebrate	85	819	0.10
Top predator mammal	Linx	20000	Other mammals	9965	Bird and mammal	22.6	68.8	Vertebrate	85	1663	0.08
Top predator mammal	Wolf	40000	Other mammals	17020	Bird and mammal	22.6	68.8	Vertebrate	85	2840	0.07

The depuration rate (k_2) is obtained from the metabolism studies in mammals, using the elimination half-life $T_{1/2}$ in the following equation:

$$k_2 = \ln (2) / T_{1/2}$$

For a first Tier assessment the estimation could consider a steady state concentration, estimated as:

$$\text{ss PEC organisms} = (\alpha F / \ln(2) / T_{1/2}) \text{ PEC food}$$

where PEC food is estimated from the application rate and the RUD (90th percentiles)

For the refinement, the dissipation of the pesticide in the environment can be incorporated, assuming first order kinetics, by a slightly modified equation frequently used for oral exposures (i.e Fisk et al., 1998):

$$PEC_{organisms} = (\alpha F / \ln(2) / T_{1/2}) PEC_{food} (1 - e^{-(\ln(2)/DT50)\, t})$$

A SIMPLIFIED SCHEME FOR FOOD-CHAIN RELATIONSHIPS

Ecosystems are constructed by a set of assembled food chains producing very complex structures. For the inclusion of biomagnification in the environmental risk assessment of pesticides, these structures must be simplified to workable schemes.

Tables 2 and 3 describe the different links of the food-chain considered in the proposal for birds and mammals respectively.

Table 2. Characteristics of selected birds.

Diet	Food issues	Body size
Insectivore	100 % contaminated insects 100 % contaminated soil-dwelling invertebrates	Medium & Small
Herbivore	100 % contaminated plants	Medium & Large
Omnivore	33 % contaminated invertebrates, 33% contaminated seeds, 33 % contaminated plants	Small
Carnivore	100 % contaminated birds and mammals	Medium
Carnivore/Piscivore	50 % contaminated birds and mammals 50 % contaminated fish	Large & Medium
Piscivore	100 % contaminated fish	Medium & Large
Aquatic herbivore/insectivore	50 % contaminated aquatic invertebrates 50 % contaminated aquatic plants	Medium

Table 3. Characteristics of Selected mammals

Diet	Food issues	Body size
Insectivore	100 % contaminated insects	Small
Herbivore	100 % contaminated plants	Small & Medium
Omnivore	33 % contaminated invertebrates, 33% contaminated seeds, 33 % contaminated plants	Medium
Carnivore	100 % contaminated mammals	Medium
Piscivore	100 % contaminated fish	Medium

ESTIMATION OF PEC FOR THE DIFFERENT FOOD CHAIN LEVELS.

The simplified proposal can be easily quantified using the equations described previously. For steady state conditions, each trophic level is considered to feed exclusively on contaminated food, corresponding to the previous trophic level.

The initial assessment, to quantify the concentration in the food items for intermediate consumers (birds and mammals) considers the consumption of sprayed food items, fish from contaminated waters and earthworms from contaminated soils.

The steady state concentration for the intermediate consumers is therefore calculated by:

$$\text{PEC intermediate consumers} = (\alpha F/ k_2)\ (ETE) = (\alpha * F * T_{1/2} * \text{application rate} * RUD / \ln(2))$$

In the case of omnivores the estimation assumes that the feeding of the animal is distributed proportionally between leaves, grass and insects; therefore, the estimation is:

$$\text{PEC intermediate consumers (omnivores)} = (\alpha F/ \ln(2)/ T_{1/2})\ (\text{application rate} * R_p)$$

The R_p is the averaged coefficient assuming the different proportions of the animal diet. This R_p is estimated as:

$$R_p = (\sum^n_i\ RUD_i/P_i)/n$$

The steady state concentration for predators is estimated assuming that contaminated intermediate consumers constitute 100% of their diet; the equations are different depending on the predators are piscivores, insectivores or carnivores. PECs can be estimated as:

$$\text{PEC predator (piscivores)} = (\alpha F/\ln(2)/ T_{1/2})\ PEC_{sw} * BCF$$

$$\text{PEC predator (insectivores)} = (\alpha F/\ln(2)/ T_{1/2})\ \text{PEC soil} * \text{BAF soil-earthworms}$$

$$\text{PEC predator (carnivores)} = (\alpha F/\ln(2)/ T_{1/2})\ \text{PECintermediate consumers (omnivores)} =$$
$$=(\alpha F/\ln(2)/ T_{1/2})[(\alpha F/\ln(2)/ T_{1/2})\ (\text{application rate} * R_p)]$$

The same values for α and k_2 than those used for intermediary consumers can be used for the preliminary assessment. Only those insectivore species feeding on soil dwelling organisms are considered in this assessment as those feeding on foliar insects have been already covered as intermediate consumers. Earthworms are suggested as model as QSARs for soil bioaccumulation are available. Other soil-dwelling organisms can also be considered.

Similarly, the steady state concentration for top predators is estimated assuming that contaminated predators constitute 100% of their diet:

$$\text{PECtop predators} = (\alpha F/\ln(2)/ T_{1/2})\ \ \text{PEC mammals and birds}$$

Depending on the relevant compartment, the equations are:

$$\text{PEC top predators} = (\alpha F/\ln(2)/ T_{1/2})\ \text{PEC predator (piscivores)} =$$
$$= (\alpha F/\ln(2)/ T_{1/2})\ (\alpha F/\ln(2)/ T_{1/2})\ PEC_{sw} * BCF$$

$$\text{PEC top predators} = (\alpha F/\ln(2)/ T_{1/2})\ \text{PEC predator (carnivores)} =$$
$$=(\alpha F/\ln(2)/ T_{1/2})\ (\alpha F/\ln(2)/ T_{1/2})[(\alpha F/\ln(2)/ T_{1/2})\ (\text{application rate} * R_p)]$$

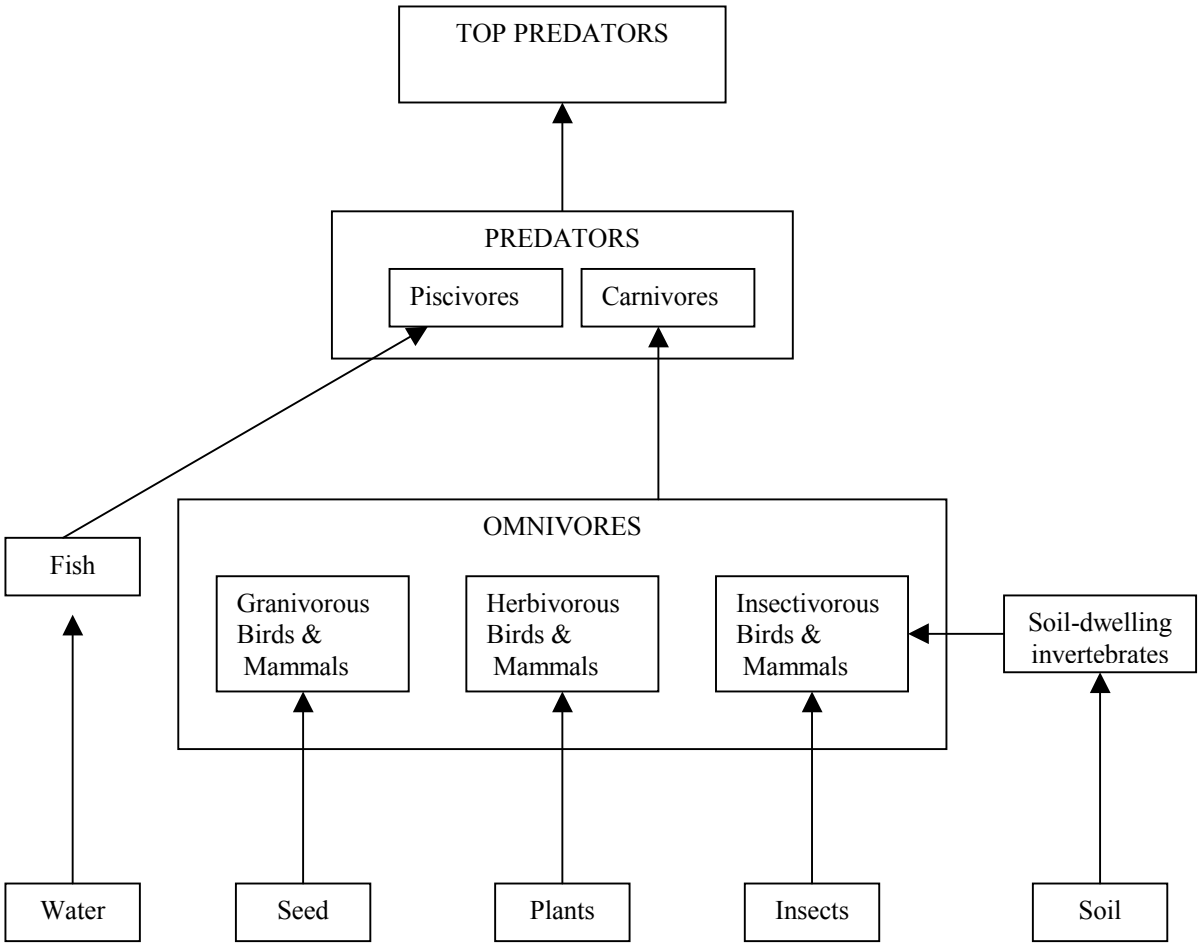
For episodic or intermittent exposures, the steady state calculations are not appropriate and the equations must be substituted by the kinetic equations. These equations can be modelled as combinations of two additive components, the chemical remaining from previous exposures and the newly absorbed chemical. Selecting Δt

values much lower than the $T_{1/2}$, the elimination component for the newly absorbed chemical becomes negligible, and the concentration in the organisms at time t, assuming first order dissipation kinetics, is represented by:

$$PEC_{organisms,t} = PEC_{organisms,(t-1)}(e^{-k2\Delta t}) + [(\alpha F)\ PEC_{food,t})\ \Delta t]$$

Finally, the following scheme (figure 1) summarises the links assumed in this proposal.

Figure 1.



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Appendix IV: Worked example - Standard assessment and refinement

This example describes the risk to birds from a fictitious substance for all three time scales. It starts with the tier-1 assessment and then procedes with the exploration of several refinement options. It is provided purely to demonstrate how the different refinement steps can be pieced together and does not imply that the assumptions or data used are either appropriate or acceptable to use in refining a risk assessment.

Key endpoints

Avian acute oral toxicity	lowest LD50 = 38 mg a.s.kg bw
Avian dietary toxicity	lowest LC50 = 160 ppm a.s. equivalent to 40 mg a.s./kg bw/d
Avian reproduction toxicity	NOEC = 55 ppm a.s. equivalent to 6.6 mg a.s./kg bw/d based on significant reduction in 14 day mallard duck hatchlings at 75 ppm.
logKow	less than 3
Use	Cereal herbicide applied post-emergence between growth stage 11 and 21 (early growth stage). Applied once at a rate of 200 g a.s./ha Approximate time of application would be in early spring.

First tier risk assessment

From chapter 3 Tables 1 and 2 the indicator species are a 3000 g herbivorous bird with a food intake rate of 1322 g/day wet weight and a 10 g insectivorous bird with a food intake rate of 10.4 g/day wet weight. With regard to the latter the standard scenario assumes small insects as a food source. However, in very early growth stages of cereals the abundance of small insects is considered to be relatively small whereas larger insects could form a diet for birds. Therefore in the standard scenarios according to tables 4, 6 and 7 the residue estimates for small insects are replaced by those for large insects. For the indicator species the ETE-values and the resulting TER-values are as follows:

Endpoint	**Toxicity**	**Exposure**						**TER**
		FIR/bw	RUD	Appl. rate	MAF	f_{twa}	**ETE**	
Herbivorous birds								
Acute	**38**	0.44	142	0.2	1	-	**12.5**	**3.0**
Short term	**40**	0.44	76	0.2	1	-	**6.7**	**6.0**
Long term	**6.6**	0.44	76	0.2	1	0.53	**3.5**	**1.9**
Insectivorous birds								
Acute	**38**	1.04	14	0.2	-	-	**2.9**	**13**
Short term	**40**	1.04	5.1	0.2	-	-	**1.1**	**36**
Long term	**6.6**	1.04	5.1	0.2	-	-	**1.1**	**6**

With regard to insectivorous birds all TER-values are above the relevant trigger values whereas with herbivorous birds all TER-values are below the trigger. Therefore a refined assessment is required for herbivorous birds.

Refined risk assessment

In the above risk assessment, the default values for 'food intake', 'body weight', 'concentration', 'avoidance', 'Proportion of diet obtained in treated area' and 'Proportion of different food types in the diet' have been used. In the following refined risk assessment some of these factors will be considered for refinement.

Concentration

In the above first tier risk assessment it was assumed that the product was applied once and hence the concentration on treated food, in this case short cereal shoots, was taken from Fletcher et al. (1994) In the acute risk assessment 90th percentile data had been used, whereas for the short term the arithmetic mean had been used. For the long-term risk assessment mean data had been used together with a generic DT50 of 10 days and a time window of 3 weeks.

In trying to refine the concentration estimates, it is possible to examine the residues data submitted as part of the dossier. This is usually presented in Section 4 of the dossier and section 6 of the monograph. For the above assessment, data are required on the initial residues immediately after application, unfortunately for this type of application, these type of data are rarely available for new products with the above use pattern. It may be possible that residue decline data are available and - providing that this is on the correct crop etc - it may be appropriate. Turning to this example the Notifier has generated some real residue data in line with the guidance outlined at Section 5.2. In producing these data the crop was sampled immediately after application (D0) and on three separate occasions, i.e. D2, D5, D7. These sample dates produced the following results: D0 average residue is 12 mg/kg (90th percentile residue is 20 mg/kg), D2 average residue figure is 6 mg/kg, D5 average residue figure is 1.5 mg/kg and D7 is 0.1 mg/kg. From these data the DT50 was calculated to approximately 2 days.

From these data exposure estimates and resulting TER values are as follows:

Endpoint	**Toxicity**	**Exposure**						**TER**
		FIR/bw	init. Conc	f_{twa}	PD	PT	**ETE**	
Acute	**38**	0.44	20	-	1	1	**8.8**	**4.3**
Short term	**40**	0.44	12	-	1	1	**5.3**	**7.6**
Long term	**6.6**	0.44	12	0.14	1	1	**0.74**	**8.9**

In conclusion, there is still concern regarding the acute and short-term risk. The long-term risk is adequately addressed However, the acute and short-term risk, i.e. where birds obtain their food in a relatively short period of time (minutes to hours and hours to days), has not been adequately addressed by the refined residue data. Therefore, further refinement is required.

Avoidance

No data are generally available on the avoidance, palatability or attractiveness of treated food, however it may be possible to design a suitable protocol to address this type of issue. However, considering that this is a spray application and that the test substance was not avoided at relevant concentrations neither in the 5-day-dietary test nor in the reproduction test avoidance doesn't appear as a promising factor for refinement.

Proportion of diet obtained in treated area (PT)

In the first tier risk assessment it is assumed that individuals obtain all their dietary requirements from the treated area. In reality it would extremely rare if this was always the case. Using information outlined in Chapter 5.6, it may be possible to reduce the default value of 1 to a more realistic figure. In order to do this data from radiotracking studies may, if they are available, help, however it is appreciated that these will be rarely available. Therefore, an alternative option would be to carry out an appropriate literature search to try and determine the proportion of the diet that may be obtained from the treated area. However, before doing this, key species that may be exposed should be identified. With the above scenario the major types of birds of concern are geese.

There is much evidence of geese grazing short grass and cereal shoots, for example Greylag goose, Brent goose and Canada goose are identified as an appropriate indicator species. However, there is currently a lack of information to indicate the proportion of diet obtained from the treated area. It is known that the main areas that the product will be used is predominantly coastal and where geese feed on arable field they only do so for the part of the day when the tide is in. There is no quantitative way to indicate the exact time that this is, however it is estimated that out of a maximum 8 hour feeding period, geese are only on arable crops for 4 hours. This means that PT, i.e. proportion of diet obtained from treated cereal fields, can be reduced from 1 to 0.5.

It should be noted that this example is provided for illustrative purposes **only** and for any real example the reduction in PT would need to be fully justified as outlined in Section 5.6.

Proportion of different food types in the diet (PD)

In the first tier it is assumed that all the food consumed by the bird is young cereal shoots. In order to refine this factor, it would be ideal to have data on the likely composition of birds in the field when the product was being applied. Unfortunately specific data of this type is rarely available, however, data are available on what birds eat at different times of the year.

Data from the public domain on the Brent goose indicated that it will eat grass and maintain it body weight, however this information came from a study where Brent geese had been kept on pasture so therefore it is of limited use for refining the risk assessment. Data on Canada geese indicated that between the months of October and March grasses made up 33 % of the composition of the gizzards of Canada geese. It should be noted that this was 33 % by volume and that the study was conducted in the USA. Data on the Greylag goose indicated that grass occurred in 73 % of the stomachs of greylag geese sampled between November and February. Further data indicated that between the months of March and May, grass made up 96 % of the stomach contents of sampled geese. Between the months of September and November grass and cereal seedlings made up 17 % of the stomach contents, whilst between December and

February grass made up 60 %. The remainder of the diet consists of food obtained from the intertidal zone. These studies were carried out in the UK.

Please note that in a real assessment all assumptions would have to be fully justified as outlined in Section 5.6.

From the above, it can be seen that accurate refinement of PD is difficult and the published data available is only likely to help on a qualitative basis. For example, the above data indicate that grass (and it is assumed cereal shoots) will be consumed by geese and it will make up a significant proportion of the diet. From the data presented on the greylag goose, it can tentatively be concluded that for the time period of interest (i.e. February to April) grass made up between 60 and 96 % of the diet. The remainder of the diet is made up of food from the intertidal zone. These food items are assumed to have no residues.

Revision of PD and PT leads to the following exposure estimates and TER-values:

Endpoint	**Toxicity**	**Exposure**						**TER**
		FIR/bw	init. Conc	f_{twa}	PD	PT	**ETE**	
Acute	**38**	0.44	20	-	0.6-0.96	0.5	**2.7-4.2**	**9.0-14.4**
Short term	**40**	0.44	12	-	0.6-0.96	0.5	**1.6-2.5**	**16-25**

It should be noted that in the above worked example only one estimate of PT has been used, given the guidance in Section 5.6 and the proximity of the resulting TER_a to the Annex VI trigger value further assessments should be carried out to determine the importance of this factor. Such work could include work on the behaviour of geese in the intertidal area.

The above refined risk assessment indicates that the TER_a lies between 9.0 and 14.4 whilst the TER_{st} lies between 16-25.

Appendix V: Worked example - Bioaccumulation issues

This example describes the risk to birds and mammals arising from bioaccumulation potential of a fictitious substance. It is assumed that the standard tier 1 assessment has been completed.

Key endpoints

long-term NOEL mammals	50 mg/kg bw/d
long-term NOEL birds	20 mg/kg bw/d
BCF (fish)	640
Adsorption, distribution, excretion and metabolism in mammals	Rate and extent of excretion: >95 % after 7 days Potential for bioaccumulation: none
K_{ow}	20000 ($\log K_{ow}$ = 4.3)
K_{oc}	6200
PEC_{soil}	1.4 mg/kg (3-week average)
$PEC_{surface\ water}$	0.001 mg/l (3-week average)

Initial trigger

It is noted that $\log K_{ow}$ is greater than 3 thus making necessary the considerations outlined in chapter 4.3

Food chain from earthworms to earthworm-eating birds and mammals

Measured residues in earthworms are not available, nor experimentally determined bioconcentration factor for worms. Therefore the model calculation is applied.

- PECsoil = 1.4 mg/kg
- The BCF for worms is estimated as BCF = (0.84 + 0.01 K_{ow}) / f_{oc} K_{oc} with K_{ow} = 20000, K_{oc} = 3200, and f_{oc} = 0.02 (default value) the resulting BCF is 1.6
- The estimated concentration in worm (PEC_{worm}) is PEC_{soil} * BCF, i.e. 1.4 * 1.6 = 2.2 mg/kg
- The daily dose for mammals is 2.2 * 1.4 = 3.1 mg/kg bw/d, and for birds it is 2.2 * 1.1 = 2.4 mg/kg bw/d

The long-term TER-values are 50/3.1 = 16 for mammals and 20/2.4 = 8.3 for birds, and therefore the risk is acceptable.

Food chain from fish to fish-eating birds and mammals

A model calculation is applied using the PEC for surface water and the experimentally determined BCF for fish.

- PEC_{sw} = 0.001 mg/l
- The estimated concentration in fish (PEC_{fish}) is PEC_{sw}* BCF, i.e. 0.001 * 640 = 0.64 mg/kg
- The daily dose for mammals is 0.64 * 0.13 = 0.08 mg/kg bw/d, and for birds it is 0.64 * 0.21 = 0.13 mg/kg bw/d

The long-term TER-values are 50/0.08=625 for mammals and 20/0.21=154 for birds, and therefore the risk is acceptable.

Biomagnification in terrestrial food chains

As the evaluation of the toxicokinetic studies in the toxicology section concluded that the potential for bioaccumulation is low it can be assumed that there is no biomagnification along the food chain.

Appendix VI: Worked example - Weight-of-evidence approach

Problem

This worked example applies to a case where the long-term risk to birds is of concern.

Use pattern

Function:	Herbicide
Kind of application:	spray
Frequency:	one application per season
Application rate:	3.2 kg a.s./ha

Crop and season:

- Autumnal use on established perennial and biannual weeds in orchards and vineyards
- Winter use on annual and biannual weeds seedling in orchards and vineyards. According to the intended use applications will not be made after February.

Relevant toxicity data

Avian reproduction toxicity:

- Mallard duck: NOEC = 100 ppm equivalent to 11 mg/kg/d
  Treatment-related reductions in male body weight and feed consumption, and a statistically significant effect upon egg production were observed in groups receiving dietary concentrations of 350 and 1 250 ppm a.s over 17 weeks. However, clear recovery was observed when birds in the 350 and 1 225 ppm a.s. treatment groups were switched to untreated basal ration at the beginning of Week 18. Feed consumption increased during Week 19 and were comparable to the control group during Weeks 19 through 22. Approximately two weeks after beginning the withdrawal period a rise in egg production was noted in both the 350 ppm and 1 225 ppm a.s. groups, with 7 and 11 hens laying apparently normal eggs, respectively. After five weeks of recovery, 12 and 14 hens were laying in the 350 and 1 225 ppm a.s. groups, respectively, and the number of eggs laid was comparable to the number of eggs laid in the control.
- Bobwhite quail: NOEC = 350 ppm equivalent to 44 mg/kg/d
  At 1250 ppm the body weight of males and females was reduced; other endpoints were not affected.

Standard evaluation

Exposure estimates and TER values according to the standard scenario:

- Large herbivorous bird (grassland): ETE_{lt} = 57 mg/kg/d TER_{lt} = 0.19
- Insectivorous bird (grassland): ETE_{lt} = 97 mg/kg/d TER_{lt} = 0.11

From this standard first tier approach a potential long term risk for birds is identified.

Refined exposure assessment

The standard scenario is usually considered as a realistic worst case scenario, however, in this case the standard scenario is considered conservative and not totally appropriate due to the recommended GAPs.

No additional studies are available to refine the above assessment and remove the uncertainty associated with

- the dissipation of residues of the active substance on food items
- the diets leading up to and during the breeding season (spring and summer)
- the proportion of the diet that comes from treated fields/crop.

Thus, calculations represent only a tentative estimate of exposure and some additional information have to be considered for the final decision.

Considering the time of application in the autumn or winter long term exposure of wild birds from the consumption of contaminated insects is not expected. This is due to rapid elimination of dead and living insects under field conditions. In addition, a high density of insects on soil/grass is not expected in autumn/winter.

Consumption of contaminated vegetation is not expected as

- Full weed destruction occurs within one month after treatment, thus, it is considered unlikely that the DT50 on food items would be greater than 14 days (see trigger in Terrestrial Guidance Document (SANCO/10329/2002)).
- The active substance is highly soluble in water and rapid dissipation is expected under the prevailing weather conditions. The active substance dissipates from soil with a comparatively short DT50 of 14 days. Considering that dissipation from vegetation usually is clearly more rapid than from soil the default DT50 for vegetation of 10 days is a conservative estimate in this case.
- Combining the estimated DT50 of 10 d in/on the food items and the availability of the food itself, the exposure is unlikely to be over a long period especially considering that application is at least 60 days before breeding starts.

Refined Effects Assessment

Parental effects are restricted to body weight and feed consumption. Considering that the magnitude of this effect was slight and effects were reversible it is unlikely that exposed birds are affected to a degree that would lower the survival rate.

Reproductive effects, namely reduced egg production are likewise of transient nature. It is unlikely that any exposure during autumn or winter would affect the reproductive performance of the birds in spring.

Conclusion

The calculations of TER_{lt} based on the standard scenario (worst case approach) lead to the conclusion that there is a potential risk for wild birds. However, it is concluded that this assessment is not appropriate for the substance under consideration.

On the basis of

- exclusive use in autumn and winter, outside of breeding periods
- the full recovery of contaminated birds and
- the rapid dissipation of the active substance

there is circumstantial evidence that the observed reproductive effects in test animals are unlikely to pose a risk to wild birds under practical conditions.

It should be noted that the above approach may not always be sufficient to demonstrate ‘one safe’ use, however it is useful in demonstrating where the uncertainties lie and hence where further work may be better focussed.