

## 21 Terrestrial Vertebrate Ecotoxicity – Subclass 9.3

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### 21.1 Basic elements and general considerations

The basic elements to consider in determining hazard classifications under the Hazardous Substances and New Organisms Act 1996 (HSNO Act) for effects on terrestrial vertebrates are:

- acute mammalian toxicity (oral and dermal tests only);
- chronic mammalian toxicity;
- acute avian toxicity (oral gavage or short-term dietary test); and
- chronic avian toxicity.

See section 18.6 in [chapter 18](#) for definitions of the key terms used in this chapter.

### 21.1.1 Acute toxicity

Acute exposure to the substance is examined to determine the relationship between a single administered dose and the observed adverse effects to establish the substance's toxicity relative to other substances whose acute toxicity is known. By studying the effects, following administration by the most likely exposure routes (ingestion or absorption through the skin), the relative hazards of different pathways of exposure can be determined. Therefore, these studies identify highly toxic substances and provide information on the possible hazards that may occur where terrestrial organisms are exposed.

Ideally, acute toxicity data from both mammalian (oral and dermal tests) and avian sources (oral gavage or short-term dietary tests) will be available for classification purposes, with classification based on the most sensitive test result of either animal class.

### 21.1.2 Chronic toxicity

Chronic exposure to the substance is examined to determine the relationship between repeated administered doses and the observed long-term adverse effect to establish the substance's toxicity relative to other substances whose chronic toxicity is known. By studying the effects, following administration by the most likely exposure route (ingestion), the hazards can be determined. Therefore, these studies identify chronically toxic substances and provide information on the possible hazards that may occur where terrestrial organisms are exposed.

***Conversion of data: Values expressed as no observable effect level, no observable adverse effect level, or no observable effect concentration rather than a maximum acceptable toxicant concentration***

The maximum acceptable toxicant concentration (MATC) is the geometric mean of the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) that are derived from the same study.

- Qualitative prediction

As the NOEC gives a conservative estimate of the MATC, if the NOEC for a substance does not trigger the threshold, it can be assumed that the MATC will also not trigger the threshold.

- Quantitative prediction

The calculation of the MATC from a NOEC value can be problematic as none of the test guidelines requires doses to be in a specified concentration series. As a result, an up-front conversion factor cannot be used based on the maximum differences between the NOEC and LOEC.

***Maximum acceptable toxicant concentration values expressed in units of parts per million diet or milligrams per kilogram body weight, while chronic threshold is limited to parts per million diet***

The expression of the MATC in milligrams per kilogram (mg/kg) body weight is inconsistent with the chronic threshold (see 21.2.2 2(1)(c)(iii)). Equations to determine the average food intake per body weight for standard test species are provided by *Guidance Document on Risk Assessment for Birds and Mammals under Council Directive 91/414/EEC* (EC, 2002). To accurately determine the food intake for a species, the body weight and diet must be provided within the test report. Given these data, the units of dose can be converted from mg/kg body weight to ppm diet (mg/kg) following the equations, data tables, and examples found in [Appendix 21C](#).

### **21.1.3 Metabolites**

The substances may be transformed in the environment by abiotic or biotic processes. The potential hazards that these metabolites pose to terrestrial organisms must be evaluated when classifying the parent substance. An in-depth discussion of the classification of metabolites is in [chapter 18](#).

### **21.1.4 Weight of evidence**

The best quality data should be used as the fundamental basis for classification. Preferably, classification should be based on primary data sources. It is essential that test conditions be clearly and completely articulated.

Data from internationally harmonised test methods are preferred for classification under this subclass. Preferably, data should be derived using Organisation for Economic Co-operation and Development (OECD) test guidelines or equivalent, according to the principles of Good Laboratory Practice (GLP). Where such data are not available, classification should be based on the best available data using a weight-of-evidence approach.

See section 1.3 in [chapter 1](#) for information about assessing data quality.

See [Appendix 21B](#) for a detailed list of acceptable test methods for acute toxicity.

When experimental data for acute toxicity are available in several vertebrate species, scientific judgement should be used in selecting the most appropriate LD<sub>50</sub> or LC<sub>50</sub> value from among valid, well-performed tests.

## 21.2 Hazard thresholds and classification criteria for terrestrial vertebrate ecotoxicity

### 21.2.1 Thresholds

Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 states:

#### 2 Minimum degrees of hazard

(1) A substance with ecotoxic properties is not hazardous for the purposes of the Act unless—

...

(c) the substance is ecotoxic to terrestrial vertebrates because—

- (i) data for the substance indicates an acute avian or mammalian oral or dermal LD<sub>50</sub> of 2000 milligrams or less of the substance per kilogram of body weight, as a result of exposure to the substance; or
- (ii) data for the substance indicates an acute avian or mammalian LC<sub>50</sub> of 5000 parts or less of the substance per million in the diet, as a result of exposure to the substance; or
- (iii) data for the substance indicates a chronic avian or mammalian MATC of 100 parts or less of the substance per million in the diet, as a result of exposure to the substance.

### 21.2.2 Classification

Schedule 6 to the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 identifies three classification categories for substances that are ecotoxic to terrestrial vertebrates (subclass 9.3).

A subclass 9.3 classification and the subsequent category apply to any substance that meets the following criteria.

- *Category 9.3A – substances that are very ecotoxic to terrestrial vertebrates*
  - (a) A substance for which data indicate an acute avian or mammalian (oral or dermal) LD<sub>50</sub> ≤ 50 milligrams of the substance per kilogram of bodyweight; or
  - (b) A substance for which data indicate an acute avian or mammalian LC<sub>50</sub> ≤ 500 parts per million of the substance in the diet.
- *Category 9.3B – substances that are ecotoxic to terrestrial vertebrates*
  - (a) A substance for which data indicate an acute avian or mammalian (oral or dermal) LD<sub>50</sub> > 50 milligrams, but ≤ 500 milligrams, of the substance per kilogram of bodyweight; or
  - (b) A substance for which data indicate an acute avian or mammalian LC<sub>50</sub> > 500 parts per million, but ≤ 1,000 parts per million, of the substance in the diet.

- *Category 9.3C – substances that are harmful to terrestrial vertebrates*
  - (a) A substance for which data indicate an acute avian or mammalian (oral or dermal)  $LD_{50} > 500$  milligrams, but  $\leq 2,000$  milligrams, of the substance per kilogram of bodyweight; or
  - (b) A substance for which data indicate an acute avian or mammalian  $LC_{50} > 1,000$  parts per million, but  $\leq 5,000$  parts per million, in the diet; or
  - (c) A substance for which data indicate a chronic avian or mammalian  $MATC \leq 100$  parts per million of the substance in the diet, but that does not meet the criteria for hazard classification 9.3A or 9.3B.

Note that assignment to category 9.1D due solely to biocidal action is discussed in [chapter 23](#).

### 21.2.3 Classification of single components

The terrestrial classification criteria for single component substances are summarised in [Table 21.1](#) and [Figure 21.1](#). The application of the criteria to mixtures is set out in more detail in section [21.2](#).

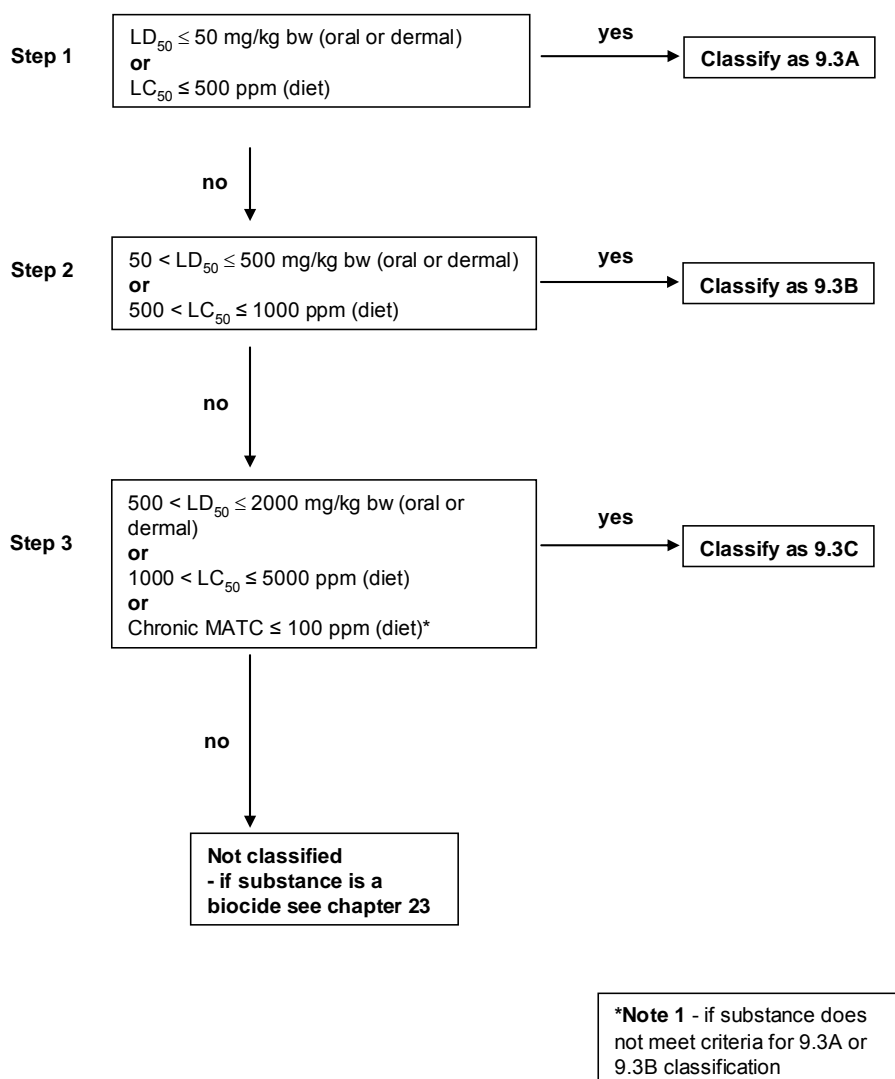
**Table 21.1:** Terrestrial vertebrate hazard classification of a single component substance

Classification category	Measure
9.3A (very ecotoxic to terrestrial vertebrates)	(a) $LD_{50} \leq 50$ mg/kg bw (oral or dermal); or (b) $LC_{50} \leq 500$ ppm (diet)
9.3B (ecotoxic to terrestrial vertebrates)	(a) $50 < LD_{50} \leq 500$ mg/kg bw (oral or dermal); or (b) $500 < LC_{50} \leq 1,000$ ppm (diet)
9.3C (harmful to terrestrial vertebrates)	(a) $500 < LD_{50} \leq 2,000$ mg/kg bw (oral or dermal); or (b) $1000 < LC_{50} \leq 5,000$ ppm (diet); or (c) a chronic $MATC \leq 100$ ppm (diet), but which does not meet the criteria for classifications 9.3A or 9.3B.
Substance classified as non-hazardous*	(a) $> 2,000$ mg/kg bw (oral or dermal); or (b) $> 5,000$ ppm (diet); or (c) a chronic $MATC > 100$ ppm (diet).

Notes:  $LC_{50}$  = median lethal concentration;  $LD_{50}$  = median lethal dose;  $MATC$  = maximum acceptable toxicant concentration; ppm = parts per million.

\* Unless intended for biocidal use, in which case 9.1D applies (see [chapter 23](#)).

**Figure 21.1:** Terrestrial vertebrate hazard classification of a single component



Notes: LC<sub>50</sub> = median lethal concentration; LD<sub>50</sub> = median lethal dose; MATC = maximum acceptable toxicant concentration; ppm = parts per million.

### 21.3 Classification of mixtures

To make use of all available data for the purpose of classifying the terrestrial vertebrate hazards of a mixture, the following assumption has been made and is applied where appropriate.

The ‘relevant components’ of a mixture are those that are present in a concentration of 1% (by weight – w/w) or greater, unless there is a presumption (for example, in the case of highly toxic components) that a component present at less than 1% can still be relevant for classifying the mixture for terrestrial vertebrate hazards.

The approach for classifying terrestrial vertebrate hazards is tiered, and depends on the type of information available for the mixture itself and for its components. Elements of the tiered approach include classification based on:

- tested mixtures (see section [21.3.1](#));
- bridging principles (see section [21.3.2](#)); and
- a summation approach, using the classifications of the mixture components (see section [21.3.3](#)).

### 21.3.1 Tested mixtures

For terrestrial vertebrate hazard classification, the test data on the mixture can be used directly to assign a substance to a classification as indicated in [Table 21.2](#). Where components of the mixture are toxic, the concentrations of components with these properties are summed to determine the classification of the mixture. Where the sum of these components is  $\geq 25\%$ , the more conservative classification applies.

**Table 21.2:** Terrestrial vertebrate hazard classification of tested mixtures

Classification category	Acute L(D)C <sub>50</sub> of the tested mixture	Chronic maximum acceptable toxicant concentration (MATC) of the tested mixture
9.3A	(a) $\leq 50$ mg/kg bw (oral or dermal); or (b) $\leq 500$ ppm (diet)	
9.3B	(a) $50 < LD_{50} \leq 500$ mg/kg bw (oral or dermal); or (b) $500 < LC_{50} \leq 1,000$ ppm (diet)	
9.3C	(a) $500 < LD_{50} \leq 2,000$ mg/kg bw (oral or dermal); or (b) $1000 < LC_{50} \leq 5,000$ ppm (diet)	MATC $\leq 100$ ppm (diet) but which does not meet the criteria for hazard classification 9.3A or 9.3B
Non-hazardous*	(a) $LD_{50} > 2,000$ mg/kg bw (oral or dermal); or (b) $LC_{50} > 5,000$ ppm (diet)	MATC $> 100$ ppm (diet)

Notes: LC<sub>50</sub> = median lethal concentration; LD<sub>50</sub> = median lethal dose; L(D)C<sub>50</sub> = LD<sub>50</sub> or LC<sub>50</sub>; ppm = parts per million.

\* Unless intended for biocidal use, in which case 9.1D applies (see [chapter 23](#)).

### 21.3.2 Bridging principles

Guidance on the bridging principles for classifying mixtures without test data is in [chapter 18](#).

### 21.3.3 Classification of a mixture based on the classification of components: Summation approach

When test data of the mixture are not available and the bridging principles are not applicable, the summation approach is used to derive a terrestrial hazard classification for the mixture.

***Rationale***

The toxicity criteria for the terrestrial classification categories differ by a factor of 10 in moving from higher to lower categories. Substances with a classification in a high toxicity band may, therefore, contribute to the classification of a mixture in a lower band. The calculation of these classification categories, therefore, needs to consider the contribution of all substances that are classified for terrestrial toxicity.

When components are classified as 9.3A and their acute toxicity is well below the cut-off value (median lethal dose (LD<sub>50</sub>) << 5 mg/kg bodyweight or median lethal concentration (LC<sub>50</sub>) << 50 parts per million (ppm) diet) they contribute to the toxicity of the mixture even if they are present at a low concentration. Under these circumstances the application of the normal cut-off values/concentration limits may lead to an ‘under-classification’ of the mixture. Therefore, multiplying factors are applied to account for highly toxic components.

***Classification procedure***

In general, a more severe classification for mixtures overrides a less severe classification, for example, a 9.3A classification overrides a 9.3B classification. The classification is complete as a more severe classification than 9.3A is not possible.

First, all components classified as 9.3A are considered. If the sum of these components is  $\geq 25\%$  the whole mixture is classified as 9.3A. If the result of the calculation is a classification of the mixture as 9.3A, the classification process is complete.

The steps to follow in applying the summation approach to terrestrial hazard classification are set out below and summarised in [Table 21.3](#) and [Figure 21.2](#).

***Mixtures with no highly toxic components***

The steps to follow in applying the summation approach to terrestrial hazard classification for mixtures with no highly toxic components are set out below.

- *Step 1: Consider all components classified as 9.3A.*

If:

$$\sum(9.3A)\% \geq 25\%$$

then the mixture is classified as 9.3A, and the classification process is complete.

- *Step 2: Consider all components classified as 9.3A and 9.3B.*

If:

$$(\sum(9.3A)\% \times 10) + \sum(9.3B)\% \geq 25\%$$

then the mixture is classified as 9.3B, and the classification process is complete.

- *Step 3: Consider all components classified as 9.3A, 9.3B, and 9.3C.*

If:

$$(\sum(9.3A)\% \times 100) + (\sum(9.3B)\% \times 10) + \sum(9.3C)\% \geq 25\%$$

then the mixture is classified as 9.3C, and the classification process is complete.

The exception to this is where the substance is used as a biocide. See [chapter 23](#) for further guidance.

**Table 21.3:** Classification of a mixture for terrestrial vertebrate ecotoxicity based on the summation of classified components

Process	Summation formulae	Cut-off	Classification
Step 1	9.3A x M	≥ 25%	9.3A
Step 2	(M x 10 x 9.3A) + 9.3B	≥ 25%	9.3B
Step 3	(M x 100 x 9.3A) + (10 x 9.3B) + 9.3C	≥ 25%	9.3C
Step 4	(M x 100 x 9.3A) + (10 x 9.3B) + 9.3C	< 25%	Not hazardous*

Notes: M = multiplying factor.

\* Unless intended for biocidal use, in which case 9.1D applies (see [chapter 23](#)).

#### *Mixtures with highly toxic components*

In applying the summation of classified components approach, more weight should be given to highly ecotoxic components. When a mixture contains components classified as 9.3A, the tiered approach described above should be applied using a weighted sum by multiplying the concentrations of 9.3A components by a factor, instead of merely adding up the percentages. The multiplying factors to be applied to the component are summarised in the [Table 21.4](#). Therefore, to classify a mixture containing highly toxic components, the classifier needs to apply the multiplying factor (M) in assigning a terrestrial hazard classification to the mixture.

The multiplying factors to be applied to highly toxic components are set out in [Table 21.4](#). See '[Mixtures with highly ecotoxic components \(multiplication factors\)](#)' and [Table 21.5](#) for a worked example.

**Table 21.4:** Multiplying factors for highly ecotoxic components of mixtures

LD <sub>50</sub> (mg/kg body weight)/LC <sub>50</sub> (ppm)	Multiplying factor (M)
5 < LD <sub>50</sub> ≤ 50 50 < LC <sub>50</sub> ≤ 500	1
0.5 < LD <sub>50</sub> ≤ 5 5 < LC <sub>50</sub> ≤ 50	10
0.05 < LD <sub>50</sub> ≤ 0.5 0.5 < LC <sub>50</sub> ≤ 5	100
0.005 < LD <sub>50</sub> ≤ 0.05 0.05 < LC <sub>50</sub> ≤ 0.5	1,000
0.0005 < LD <sub>50</sub> ≤ 0.005 0.005 < LC <sub>50</sub> ≤ 0.05	10,000
(continue in factor of 10 intervals)	

Note: LC<sub>50</sub> = median lethal concentration; LD<sub>50</sub> = median lethal dose; ppm = parts per million.

*Mixtures with highly ecotoxic components (multiplication factors)*

The steps to follow in applying the summation approach to terrestrial hazard classification for mixtures with highly ecotoxic components are set out below [Table 21.5](#).

**Table 21.5:** Example of the summation approach for a mixture containing highly toxic components

Component	LD <sub>50</sub> (mg/kg bw)	Individual substance (100%) classification	Concentration in mixture (%)	Multiplying factor	Weighted concentration of individual substance in mixture (%)
B	55	9.3B	5	-	5
P	0.2	9.3A	0.05	100	5
Q	9	9.3A	1	-	1
T	1,000	9.3C	40	-	40
U	Not classified	Not classified	53.95	-	53.95

Note: bw = bodyweight; LD<sub>50</sub> = median lethal dose.

- *Step 1*

Component P is highly ecotoxic and attracts a multiplier of 100, resulting in a weighted concentration of that component of 5%.

Component Q, although classified as 9.3A is not given addition weighting, that is:

$$\Rightarrow (100 \times P) + Q$$

$$\Rightarrow (100 \times 0.05\%) + 1\% = 6\% , \text{ which is } < 25\%$$

so the mixture Z is not classified as 9.3A.

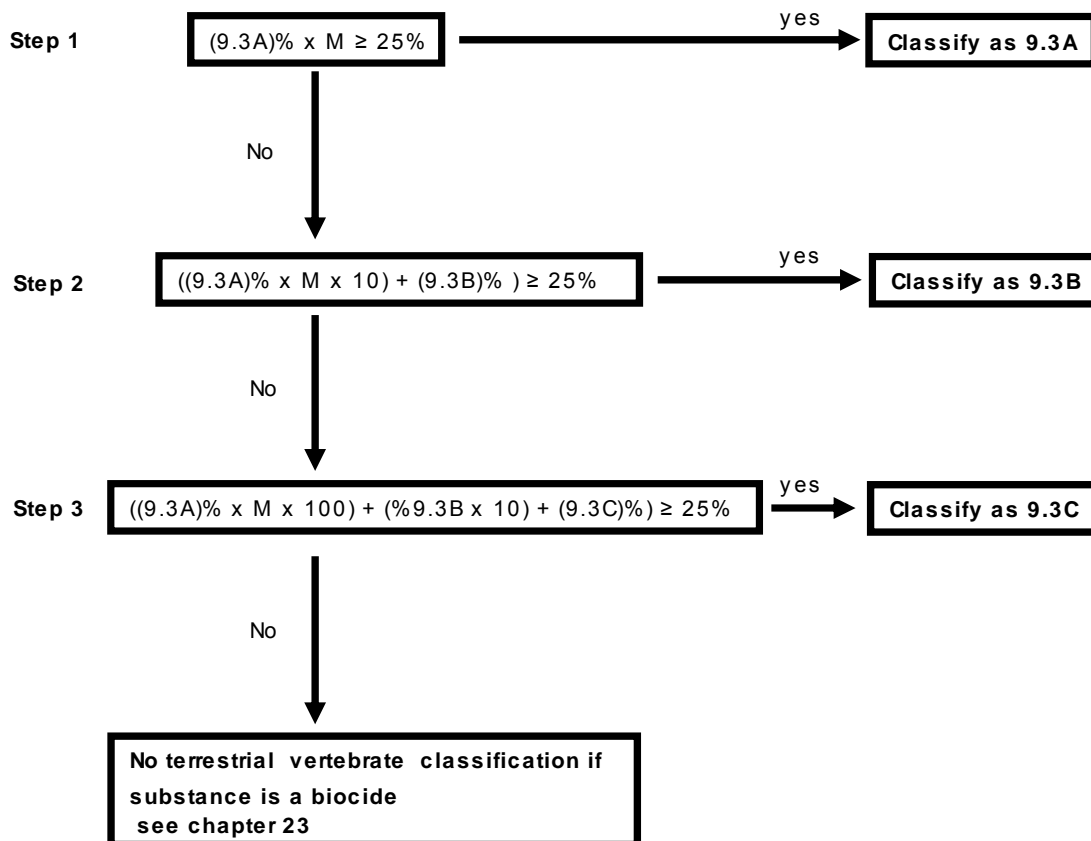
- *Step 2: Consider components classified as 9.3A and 9.3B.*

$$\Rightarrow 10((100 \times P) + Q) + B$$

$$\Rightarrow 10((100 \times 0.05\%) + 1\%) + 5\% = 60\% + 5\% = 65\%, \text{ which is } \geq 25\%$$

so the mixture is classified as 9.3B

**Figure 21.2:** Terrestrial vertebrate hazard classification of mixtures



## References

- EC 2002. *Guidance Document on Risk Assessment for Birds and Mammals under Council Directive 91/414/EEC*, SANCO/4145/2000 – final. European Commission.  
[http://ec.europa.eu/food/plant/protection/evaluation/guidance/wrkdoc19\\_en.pdf](http://ec.europa.eu/food/plant/protection/evaluation/guidance/wrkdoc19_en.pdf)

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## **Appendix 21A: Classification notes for avian studies**

### **Appendix contents**

#### **21A.1 Avian acute oral toxicity**

Regurgitation can substantially reduce the dose absorbed by birds in acute oral toxicity tests. Therefore, evaluation of avian acute oral tests should include whether regurgitation or emesis has occurred. If so, it may be appropriate to repeat the study using birds that do not regurgitate, in particular if a high-risk use such as seed treatment is being assessed.

For example, if regurgitation is observed in an acute oral toxicity test at 500, 1,000, and 2,000 mg active substance/kg body weight (bw), but not at 200 mg a.s./kg bw, and if there is no mortality at 200 mg a.s./kg bw, then the conclusion is valid that the median lethal dose (LD<sub>50</sub>) is > 200 mg/kg bw. Although this figure cannot be used for classification purposes it may be used in the initial risk assessment. If this assessment raises concern, then either an acute or a dietary study would be requested using a bird species that does not regurgitate. If the initial assessment does not raise concern, no further data will be requested. Sometimes regurgitation may occur in all doses while mortality occurs only in the top doses, that is, regurgitation is not sufficient to protect birds. Also, in this situation, a further study with a non-regurgitating species would be required.

#### **21A.2 Avian short-term dietary toxicity**

When the test diet has been analysed the results should be reported in the monograph. According to OECD guideline 205, a deviation up to 20% between measured feed concentrations and nominal values is considered acceptable. In the case of larger deviations, toxicity figures should be recalculated using effective concentrations.

#### **21A.3 Avian reproduction**

It should be noted that low acute and dietary avian toxicity are not sufficient to indicate a low reproductive toxicity. A reproductive toxicity study should always be conducted unless it can be demonstrated that the exposure of birds (adults and young) does not occur during the breeding season. When all relevant species are considered, the breeding season could be rather long and even short exposure periods may give rise to concern about potential reproductive effects. Thus, in the case of foliar applications during the breeding season, for example, the test should normally be required even if only one treatment per season is intended.

Reproductive data are always required for substances that are generally persistent or have a bio-accumulation potential.

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## Appendix 21B: Acceptable test methods for terrestrial vertebrate toxicity

### 21B.1 Introduction

Most of the guidelines mentioned in this appendix are found in compilations from the organisation issuing them. The main references to international guidelines referred to in the tables in this appendix are as follows.

- European Commission (EC) guidelines:  
European Commission 1997. *Classification, Packaging and Labelling of Dangerous Substances in the European Union. Part 2 – Testing Methods*. <http://ecb.jrc.it/testing-methods> Retrieved 18 September 2007.
- International Organization for Standardization (ISO) guidelines:  
Guidelines are available from the national standardisation organisations or the ISO website (<http://www.iso.ch> Retrieved 18 September 2007).
- Organisation for Economic Co-operation and Development (OECD) guidelines:  
OECD 1993. *OECD Guidelines for the Testing of Chemicals*. Organisation for Economic Co-operation and Development, Paris, with regular updates. [http://www.oecd.org/document/40/0,3343,en\\_2649\\_34377\\_37051368\\_1\\_1\\_1\\_1,00.html](http://www.oecd.org/document/40/0,3343,en_2649_34377_37051368_1_1_1_1,00.html) Retrieved 18 September 2007.
- United States Environmental Protection Agency (USEPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) guidelines:  
USEPA 2007. *Harmonized Test Guidelines*. United States Environmental Protection Agency. <http://www.epa.gov/opptsfrs/home/guidelin.htm> Retrieved 18 September 2007.
- ASTM International (ASTM) guidelines are available from the ASTM homepage (<http://www.astm.org> search on 'standards').

### 21B.2 Terrestrial vertebrate toxicity test guidelines

The guidelines in [Table 21B.1](#) are primarily relevant to substances that are, or solely contain, chemical substances. However, the Hazardous Substances and New Organisms Act 1996 (HSNO Act) also covers biopesticides that include micro-organisms. More specialised test methods may be required to adequately characterise the potential effects of biopesticides in the terrestrial environment.

For tests specific to the testing of microbial biopesticides, see:

- USEPA 2007. *OPPTS Harmonized Test Guidelines: Series 885 Microbial Pesticide Test Guidelines – Final Guidelines*. Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency. [http://www.epa.gov/opptsfrs/publications/OPPTS\\_Harmonized/885\\_Microbial\\_Pesticide\\_Test\\_Guidelines/Series](http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/885_Microbial_Pesticide_Test_Guidelines/Series) Retrieved 18 September 2007.

See also [Table 21B.2](#).

**Table 21B.1:** Terrestrial vertebrate toxicity test guidelines for chemicals, including mixtures

Species	Test guideline number		
	OECD	EC	USEPA OPPTS
<i>Mammalian acute</i>			
Acute oral toxicity	401: Acute Oral Toxicity *	None	870.11001
	420: Acute Oral Toxicity – Fixed Dose Procedure	EC Method B.1 bis	None
	423: Acute Oral Toxicity – Acute Toxic Class Method	EC Method B.1 tris	None
	425: Acute Oral Toxicity – Up and Down Procedure	None	None
Acute dermal toxicity	402: Acute Dermal Toxicity	EC Method B.3	870.1200
<i>Avian acute</i>			
Acute oral toxicity	None	None	850.2100
Acute dietary toxicity	205: Avian Dietary Toxicity Test	None	850.2200
<i>Mammalian chronic</i>			
Rodent sub-chronic oral toxicity	408: Repeated Dose 90-Day Oral Toxicity Study in Rodents	EC Method B.26	870.3100
Non-rodent sub-chronic oral toxicity	409: Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents	EC Method B.27	870.3150
<i>Avian chronic</i>			
Reproduction Test	206: Avian Reproduction Test	None	850.2300

## Note

- \* OECD Test Guideline 401 was deleted from the OECD manual of internationally accepted test guidelines on 17 December 2002. Acute oral toxicity studies conducted after this date should now adhere to one of the three alternative methods (OECD Codes 420, 423 and 425).

**Table 21B.2:** Terrestrial vertebrate toxicity test guidelines for microbial biopesticides

USEPA OPPTS guidelines
885.0001 Overview for microbial pest control agents
885.3050 Acute oral toxicity/pathogenicity
885.3100 Acute dermal toxicity/pathology
885.3150 Acute pulmonary toxicity/pathogenicity
885.3550 Acute toxicology, Tier II
885.3600 Subchronic toxicity/pathogenicity
885.4000 Background for non-target organism testing of microbial pest control agents
885.4050 Avian oral, Tier I
885.4100 Avian inhalation test, Tier I
885.4150 Wild mammal testing, Tier I
885.4600 Avian chronic pathogenicity and reproduction test, Tier III
885.5000 Background for microbial pesticides testing
885.5200 Expression in a terrestrial environment

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## Appendix 21C: Maximum acceptable toxicant concentration conversion from milligrams per kilogram bodyweight to parts per million diet (milligrams per kilogram diet)

### 21C.1 Estimation of average daily food intake

For a species of a given weight, allometric equations can be used to predict its daily energy expenditure. (See Crocker et al, 2002.) Knowing the energy value and moisture content of the diet, and the efficiency with which the species digests the diet, we may calculate the average amount of food the organism is likely to eat in a day, using:

Daily food intake (wet g) =

$$\frac{\text{Daily energy expenditure (kJ)}}{\text{Energy in food (kJ/g dry)} \times (1 - \text{moisture in food}) \times \text{assimilation efficiency}}$$

where moisture and assimilation efficiency are proportions between 0 and 1.

The equation for daily energy expenditure (DEE) is:

$$\text{Log}_{10} (\text{DEE}) = \text{Log}_{10} a + (b \times (\text{Log}_{10} \text{ body weight (g)})).$$

Where a and b are given in [Table 21C.1](#) for birds and [Table 21C.2](#) for mammals.

For both birds and mammals, a strong relationship exists between body weight and DEE. In addition, there are significant differences between taxonomic groups and between species occupying different habitats (Nagy, 1987; Nagy et al, 1999). Therefore, separate equations are calculated for passerines (perching birds), sea birds, desert birds, hummingbirds, and others. Placental mammals (eutherians) are similarly divided into non-eutherians, desert eutherians, sea eutherians, and terrestrial eutherians. These equations are presented in [Table 21C.1](#) and [Table 21C.2](#). Also shown are the standard errors (SE) for a and b, the number of species in each group (N), and the proportion of variation explained by each equation.

**Table 21C.1:** Relationship between body weight and daily energy expenditure in birds for five groups of avian species

Group	Log <sub>10</sub> a	SE Log <sub>10</sub> a	b	SE b	N	r <sup>2</sup>
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

Notes: Variables a and b are given in [Table 21C.1](#) (birds) and [Table 21C.2](#) (mammals); N = number of species in each group; r<sup>2</sup> = proportion of variation explained by each equation; SE = standard error.

\* Excluding marine and desert passerines.

**Table 21C.2:** Relationship between body weight and daily energy expenditure DEE in mammals for five groups of mammalian species

Group	Log <sub>10</sub> a	SE Log <sub>10</sub> a	b	SE b	N	r <sup>2</sup>
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	0.0250	0.0174	73	0.96

Notes: N = number of species in each group; r<sup>2</sup> = proportion of variation explained by each equation; SE = standard errors.

\* Excluding marine and desert eutherians.

## 21C.2 Moisture and energy content of foods

The means for 15 major groups of food types are in [Table 21C.3](#).

**Table 21C.3:** Energy and moisture contents for 15 general categories of food type

Food type	Energy content (kJ/g dry weight)	Moisture content (%)
	n = 1,783	n = 761
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

## 21C.3 Assimilation efficiency

The main categories used to calculate the daily food intake are listed in [Table 21C.4](#) (for birds) and [Table 21C.5](#) (for mammals).

**Table 21C.4:** Assimilation efficiencies for birds

Order	Bird	No. of species	No. of cases	Food type					
				Animal	Fruits	Herb- age	Seeds	Sugar	Arti- ficial
Struthioniformes	Ostriches	2	6			36			
Gruiformes	Cranes, coots, rails	1	5	34		59			69
Ralliformes	Coots, rails	1	1						
Charadriiformes	Gulls, waders	7	19	69					74
Lariformes	Gulls, terns	1	3	79					
Alciformes	Auks	1	2	76					
Sphenisciformes	Penguins	7	26	75					
Procellariiformes	Petrels	2	3	87					
Pelecaniformes	Pelicans, gannets, cormorants	4	8	80	76				
Columbiformes	Pigeons	4	36						76
Psittaciiformes	Parrots	1	4					96	
Strigiformes	Owls	6	45	77					
Falconiformes	Eagles, falcons	4	12	84					
Accipitriformes	Hawks	11	22	82					
Ciconiiformes	Hérons, storks	4	8	80					
Anseriformes	Ducks, geese	22	98	87		41	83		74
Galliformes	Fowl	18	184	70	57	42	65		67
Opisthocomi- formes	Hoatzin (S. America)	1	2						74
Trochiliformes	Hummingbirds	7	16					98	
Coliiformes	Mousebirds (Africa)	4	15		56				73
Piciformes	Woodpeckers	1	14	64		61			80
Passeriformes	Passerines	67	441	76	67	76	80	90	72

Source: Bairlein (1999).

**Table 21C.5:** Assimilation efficiencies for mammals, based on 91 published examples

Mammal group	Food type	No. of studies	Mean	Standard deviation
Shrews and bats	Insects	8	88	5.9
Carnivores	Vertebrates	16	85	5.8
Squirrels	Nuts	10	85	7.5
Small mammals	Nuts and seeds	11	83	8.5
Small mammals	Grasses	15	46	10.7
Small mammals	Crops, forbs, mixed vegetation	17	74	12.3
Lagomorphs	General vegetation	4	74	13.5
White tailed deer	Tree browse	7	32	8.4
Ruminants	Hay and browse	3	80	2.8

**Example**

Conversion of a maximum acceptable toxicant concentration (MATC) of 40 mg a.i./kg body weight per day from OECD 408 (average rodent body weight taken as 0.250 kg) to parts per million (ppm) diet:

- *Step 1: Convert milligrams per kilogram body weight per day (mg/kg bw/day) to exposure as milligrams per day (mg/day):*

$$\begin{aligned} &= \text{mg chemical/kg bw/day} \times \text{bw (kg)} \\ &= 40 \text{ mg a.i./kg bw/day} \times 0.250 \text{ kg} \\ &= 10 \text{ mg/day} \end{aligned}$$

- *Step 2: Calculate rodent daily energy expenditure (DEE):*

$$\begin{aligned} \text{Log}_{10} (\text{DEE}) &= \text{Log}_{10} a + (b \times (\text{Log}_{10} \text{bw (g)})) \\ \text{Log}_{10} (\text{DEE}) &= 0.6794 + (0.7646 \times (\text{Log}_{10} 250))^* \\ \text{Log}_{10} (\text{DEE}) &= 0.6794 + (0.7646 \times 2.398) \\ \text{Log}_{10} (\text{DEE}) &= 2.513 \\ \text{DEE} &= 325.77 \text{ kJ} \end{aligned}$$

\* Values for  $\text{Log}_{10} a$  and  $b$  taken from [Table 21C.2](#), ‘All eutherians’.

- *Step 3: Calculate rodent daily food intake (kJ):*

Daily Food Intake (wet g) =

$$\frac{\text{Daily energy expenditure (kJ)}}{\text{Energy in food (kJ/g dry)} \times (1 - \text{moisture in food}) \times \text{assimilation efficiency}}$$

where moisture and assimilation efficiency are proportions between 0 and 1.

$$\text{Daily food intake} = 325.77 / (16.7 \times (1 - 0.133) \times 0.83)$$

$$\text{Daily food intake} = 325.77 / 12.02$$

$$\text{Daily food intake} = 27.10 \text{ wet g}$$

Where:

$$\text{Energy in food} = 16.7^{**} \text{ (Table 21C.3, food type = cereal seeds)}$$

$$\text{Moisture in food} = 13.3\%^{**} \text{ (Table 21C.3, food type = cereal seeds)}$$

$$\text{Assimilation efficiency} = 83\%^{**} \text{ (Table 21C.5, food type: nuts and seeds)}$$

\*\* Assumption: Closest match to diet of laboratory animals.

- *Step 3: Convert milligrams per day to ppm (as milligrams substance per kilogram diet (mg/kg diet)*  
= (mg/day) / (kg diet/day)  
= (10 mg/day) / (0.0271 kg diet/day)  
= 368.97 mg/kg diet<sup>-1</sup> (ppm).

Therefore, an MATC of 40 mg/kg body weight for 0.250 kg rodents can be considered equivalent to 369 ppm diet.

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