European Food Safety Authority

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SCIENTIFIC OPINION

Guidance for establishing the safety of additives for the consumer[1†]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

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This document provides guidance on how to conduct studies concerning safety for the consumer.

The assessment of the safety of the use of the additives, related to consumer exposure to food products derived from animals given feed or water containing or treated with the additive and containing residues of the additive or its metabolites, is based on:

i) experimental studies that investigate:
   - the metabolic fate and residues of the additive in the target species and laboratory animals,
   - the potential toxicity of the additive in *in vitro* biological systems and laboratory animals from which an acceptable daily intake (ADI) for the consumer can be derived,

ii) the evaluation of the theoretical (worse case scenario) consumer exposure resulting from the consumption of products derived from animals administered the maximum dose of the additive proposed for use, and considering different withdrawal time points of the additive,

iii) the proposal for maximum residue limits (MRLs) and withdrawal periods, as management tools.

The rationale of this integrated approach is summarized in Figure 1. The different steps are examined successively in this document.

Where an additive has multiple active components, each should be separately assessed for safety for consumers and then consideration given to additivity (exclusion of interactions). Alternatively, when the components of a mixture cannot be fully separated (e.g., a plant extract), the complete mixture should be assessed.

Studies must be carried out, where possible, using internationally validated test methods and should be done in accordance with European legislation in force or OECD Guidelines for methodological details and according to the principles of Good Laboratory Practices (GLP).

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[1] On request from EFSA, Question No EFSA-Q-2010-01161, adopted on 14 December 2011.

[†] This guidance document replaces the previous Technical Guidance for establishing the safety of additives for the consumer, adopted in September 2008 (EFSA-Q-2008-406). The following sections have been updated: 2, 3, 4, 5 and an appendix introduced.

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The studies should respect the rules on animal welfare laid down by Community legislation and they should not be repeated if not necessary.

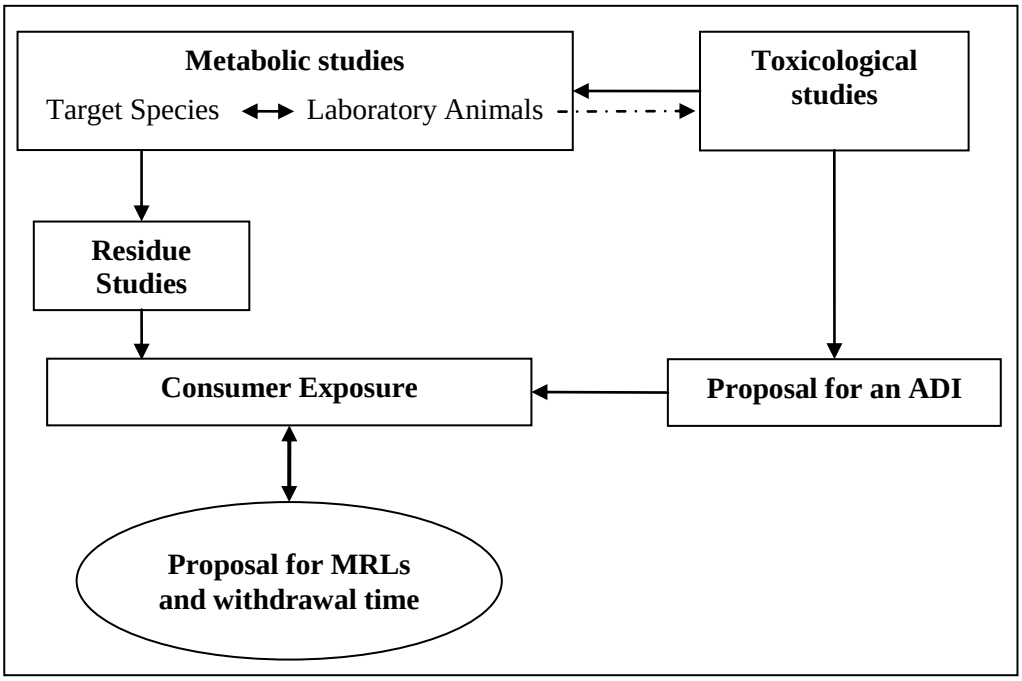

Figure 1: Rationale of the assessment of the safety for the consumer

1. Metabolism and residue studies

The aim of metabolism and residue studies in target species and laboratory animals is:

i) to establish the metabolic pathways of the active substance as a base for its toxicological evaluation,

ii) to identify and quantify residues [unchanged active substance (parent compound) and metabolites] of toxicological relevance in the edible tissues or products derived from animals given the feed or water containing the additive,

iii) to establish the kinetics of total residues and the marker residue in tissues/products (after withdrawal of the additive from the diet of the target animal).

The planning and experimental design of the studies should take into account the anatomical, physiological (age, type, sex) and environmental peculiarities of the target population. When appropriate, the influence of the intestinal or ruminal microflora, enterohepatic circulation or caecotrophy should be considered. The dose regimen tested should be that intended for use, and possibly a multiple of that dose if justified (e.g. in case of very low specific activity of the labelled active substance). The active substance (including the labelled substance) should be incorporated into the feed unless otherwise justified.

1.1. Metabolism studies

The studies required for the assessment of the metabolic fate of the additive in target species are:

1.1.1. a balance study performed on a limited number of animals (e.g., three per sex for chickens for fattening, two dairy cows) administered orally a single dose of the labelled active substance. The dose should correspond to the proposed use (total amount corresponding to the daily

intake) and possibly a multiple of this dose (if justified). A justification of the choice of the label (^{14}C, ^{3}H or other isotope) should be given, based on chemical and biological stability data. The position of the labelling on the molecule should be given, as well as its specific (radio)activity (i.e., Bq/mg), (radio)chemical purity and stability.

The absorption, distribution (plasma/blood, tissues) and excretion [urine, bile, faeces, milk or eggs, expired air (i.e., $^{14}CO_2$) or excretion via gills, where appropriate] should be measured.

1.1.2. the identification and quantification of the additive and its metabolite(s) in excreta and tissues following repeated dose administration of the labelled compound (see 1.1.1). The labelled compound should be incorporated into feed (not given by gavage unless properly justified) at the highest dose proposed for use and given to animals until metabolic equilibrium is reached (i.e., when concentration of the labelled compound in plasma reaches a plateau). The identification of metabolites normally should be limited to those representing more than 20% of total (radio)activity in excreta and 10% in edible tissues (liver, kidney, muscle or in the case of fish, muscle + skin in natural proportions, fat or, in the case of pigs and poultry skin + fat in natural proportions) and products (milk or eggs). Depending on structural alerts or toxicological considerations, the identification of minor metabolites (<10%) in tissues and products could be necessary.

Consideration also should be given to the amount and nature of non-extractable residues in edible tissues/products (covalently bound residues or reincorporation of the label into physiological body constituents such as fatty acids, carbohydrates or amino acids/proteins).
The studies should result in metabolic profiles indicating the qualitative and quantitative distribution of the parent compound and identified metabolites in the excreta and edible tissues and products.

Metabolic pathways are assumed to be similar within a species. Therefore, not each category within a species needs to be examined. For example:

- If data for chickens for fattening are provided, no data are necessary for chickens reared for laying, and in case of laying hens only additional data for egg are required. Chicken data cannot be extrapolated to turkeys.
- If data for calves for rearing are provided, no additional data for cattle for fattening is required (and vice-versa), and in case of dairy cows, only additional data for milk are required.
- If data for pigs for fattening are provided, no studies for piglets or sows are required (and vice-versa).

For minor species, see technical guidance on extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition.

A second set of studies of the metabolic fate of the additive should be performed in both sexes of a laboratory animal (i.e., the most sensitive species or strain in the toxicological study data set supporting the lowest NOAEL[4] or the rat by default) to establish a balance study and metabolic profiles of excreta and tissues, according to 1.1.1 and 1.1.2.

1.2. Residue studies

The aim of the studies is to evaluate the total residues and to identify and measure the marker residue of the active substance in edible tissues and products at steady state (metabolic equilibrium) and along the withdrawal period. The marker residue is the residue selected for assay whose concentration is in a known relationship to total residues in tissues and products, ideally constant during depletion. Metabolites of toxicological significance (see 1.1.2) should also be measured.

[4] No observed adverse effect level.

The dose applied should correspond to the highest dose proposed for use and should be incorporated into the feed and administered to animals until steady state is reached.

Two types of studies are in principle required:

1.2.1. a study of total residues, parent compound and identified metabolites in edible tissues and products following achievement of steady-state reached after continuous administration of the labelled active substance via feed. The marker residue should be selected based on this study. The suggested minimum numbers of healthy animals selected at each time point are: four for cattle, pigs and minor species; six for poultry and ten for salmonids and other fish. Sex distribution should be considered.

1.2.2. a kinetic study of the marker residue in edible tissues and products of the target animals administered the additive (not the isolated active substance) according to the proposed conditions of use. The same numbers as listed under 1.2.1 should be applied for tissue samples, while for milk, eight cows should be sampled and for eggs ten laying hens. The minimum duration of the studies should be 28 days. For animals for fattening the studies should be extended until slaughter weight.

The residues should be measured:

- in edible tissues at zero withdrawal time (steady state) and usually three other sampling points thereafter spaced according to the rate of depletion from tissues.
- in milk or eggs (three measurements at steady state made on consecutive days).

Where only residue data are required, 1.2.2 applies.

1.3. Relevance of residues to safety assessment

As a first approach, total residues are considered of toxicological relevance.

In a second step, toxicological relevant residues can be derived from total residues measured in edible tissues and products from target animals administered the (radio) labelled active substance by discounting, on a quantitative basis:

- non-extractable residues consisting of physiological endogenous compounds.
- metabolites which do not retain the full activity of toxicological concern of the parent compound.
- the non-bioavailable fraction of bound residues.

2. Toxicological studies

The safety of the additive is assessed on the basis of the toxicological studies performed *in vitro* and *in vivo* on laboratory animals. They generally include measurements of:

(1) genotoxicity

(2) sub-chronic oral toxicity

(3) chronic oral toxicity/carcinogenicity

(4) reproduction toxicity including developmental toxicity

Further studies providing additional information necessary for the assessment of the safety of the active substance and its residues should be conducted if there is any reason for concern.

Additional studies on particular metabolites may be necessary if these metabolites are produced by target species and are not formed to a significant extent in the laboratory test species. If metabolic studies are available in humans, these data should be taken into consideration in deciding the nature of any additional studies.

Toxicological studies should be carried out with the active substance unless the active substance is present in a fermentation product, in which case the fermentation product should be tested. The fermentation product tested must be identical to that to be used in the commercial product.

Where appropriate, validated alternative methods reducing the use of animals can be used.

2.1. Genotoxicity studies including mutagenicity

To identify active substances and, if appropriate, their metabolites and degradation products with mutagenic and genotoxic properties, a selected combination of different genotoxicity tests should be carried out. If appropriate, the tests should be performed with and without mammalian metabolic activation and the compatibility of the test material with the test system should be taken into account.

The following two *in vitro* tests are recommended[5] as the first step:

- a bacterial reverse mutation test (OECD Guideline 471), and
- an *in vitro* mammalian cell micronucleus test (OECD Guideline 487).

This combination of tests fulfils the basic requirements to cover the three genetic endpoints with the minimum number of tests; the bacterial reverse mutation assay covers gene mutations and the *in vitro* micronucleus test covers both structural and numerical chromosome aberrations.

Consideration should be given to whether specific features of the test substance might require substitution of one or more of the recommended *in vitro* tests by other *in vitro* or *in vivo* tests in the basic battery. In the event of positive results from the basic battery, all the available relevant data on the test substance should be reviewed, and where necessary, an appropriate *in vivo* study (or studies) should be conducted to assess whether the genotoxic potential observed *in vitro* is expressed *in vivo*.

The following *in vivo* tests are recommended as follow-up studies:

- a mammalian erythrocyte micronucleus test (OECD Guideline 474),
- a transgenic rodent somatic and germ cell gene mutation assays (OECD Guideline 488), and
- an *in vivo* Comet assay (no OECD guideline at present; internationally agreed protocols available).

The *in vivo* micronucleus test covers the endpoints of structural and numerical chromosomal aberrations and is an appropriate follow-up for *in vitro* clastogens and aneugens. There may be circumstances in which an *in vivo* mammalian bone marrow chromosome aberration test (OECD Guideline 475) may be an alternative follow-up test.

In the *in vivo* genotoxicity studies it is important that evidence of target cell exposure is obtained and, if the test is negative, it may be necessary to consider other relevant tissues (e.g., site of contact tissues for highly reactive substances which are not systemically available).

Transgenic rodent assays can detect point mutations and small deletions and are without tissue restrictions. The *in vivo* Comet assay is considered a useful indicator test in terms of its sensitivity to substances which cause gene mutations and/or structural chromosomal aberrations and can be used with many target tissues.

[5] EFSA Scientific Committee; Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379. [68 pp.] http://www.efsa.europa.eu/en/efsajournal/doc/2379.pdf

2.2. Repeated dose oral toxicity studies

To investigate the sub-chronic toxic potential of the active substance, at least one study on a rodent species must be submitted with a duration of at least 90 days. If the information from a rodent study is not a suitable basis for consumer risk assessment, a further study in a non-rodent species may be required. The test item must be administered orally (preferably incorporated into the diet) with at least three levels in addition to a control group to obtain a dose response. The highest dose used should normally be associated with evidence of adverse effects. The lowest dose level would not be expected to produce any evidence of toxicity.

Protocols for these studies should be in line with the OECD Guidelines 408 (rodents) or 409 (non-rodents).

2.3. Chronic oral toxicity studies (including carcinogenicity studies)

A chronic oral toxicity study must be carried out in at least one species, and should be of at least 12 months' duration. The species chosen should be the most appropriate on the basis of all available scientific data, including the results of the 90-day studies. The default species is the rat. If a second study is necessary, another rodent or a non-rodent mammalian species should be used. The test item must be administered orally (preferably incorporated into the diet) with at least three dose levels in addition to a control group to obtain a dose response.

Carcinogenicity studies should be of at least 18 months for mice and hamsters and 24 months for rats. If the chronic toxicity study is combined with an examination of carcinogenicity, then the duration should be extended to the length of a carcinogenicity study.

Carcinogenicity studies may not be necessary if the active substance and its metabolites:

(1) give consistently negative results in the genotoxicity tests;

(2) are not structurally related to known carcinogens; and

(3) give no effects indicative of potential (pre)neoplasia in chronic toxicity assays.

Protocols should be in line with OECD Guidelines 452 (chronic toxicity study) or 453 (combined chronic toxicity/carcinogenicity study).

2.4. Reproduction toxicity studies (including prenatal developmental toxicity)

To identify possible impairment of male or female reproductive function or harmful effects on progeny resulting from the administration of the active substance, studies of reproductive function must be carried out by:

(1) a two-generation reproduction toxicity study or an extended one generation reproductive toxicity study; and

(2) prenatal developmental toxicity studies in two species.

The test item must be administered orally (preferably incorporated into the diet) at least at three levels in addition to a control group to obtain a dose response.

2.4.1. Reproduction toxicity study

Studies of reproductive function must be carried out in at least one species, usually a rodent, and may be combined with a developmental toxicity study.

Protocols for the reproduction toxicity studies should be in line with OECD Guidelines 416 (two-generation reproduction toxicity) or 443 (extended one generation reproductive toxicity study).

2.4.2. Prenatal developmental toxicity studies

The objective is to detect any adverse effects on the pregnant female and the development of the embryo and foetus as a result of exposure from implantation through the entire gestation period. Such effects include enhanced toxicity in the pregnant females, embryo-foetal death, altered foetal growth and structural abnormalities and anomalies in the foetus. The preferred species are rat and rabbit.

The second study is unnecessary if the first one proves positive.

Protocols should be in line with OECD Guideline 414 (Prenatal Development Toxicity Study).

2.5. Other specific toxicological and pharmacological studies

Further studies providing additional information useful for the assessment of the safety of the active substance and its residues should be conducted if there are reasons for concern (e.g., if the pharmacodynamic properties of the active substance are such that there is a potential for effects on particular organs or functions of the body). Such studies may include examination of pharmacological effects, effects in juvenile (prepubertal) animals, immunotoxicity or neurotoxicity.

Pharmacological effects in consumers are undesirable, irrespectively of whether or not they have an adverse effect on health.

2.6. Determination of No Observed Adverse Effect Levels (NOAEL)

The NOAEL, expressed as mg per kg body weight per day, is generally based on toxicological effects, but pharmacological effects might occasionally be more appropriate.

The overall NOAEL should be selected from the results of all of the studies conducted. All findings from previous sections together with all other relevant published data (including any relevant information on the effects of the active substance on human) and, where appropriate, information on chemicals having a closely related chemical structure should be taken into consideration in identifying the overall NOAEL.

The benchmark dose (BMD) approach can be used to derive a value which can substitute for a NOAEL. It makes extended use of dose-response data and it provides a quantification of the uncertainty and variability in the dose-response data. However, it is likely that there will continue to be endpoints that are not amenable to modelling and for which a NOAEL/LOAEL (Lowest Observed Adverse Effect Level) approach should be retained. The BMD approach is particularly recommended in cases where no NOAEL but only a LOAEL could be identified. The validity of the BDM approach should be carefully considered (particularly the ratio BMD/BMDL). For details on how to apply the BMD approach see the EFSA guidance on Use of the benchmark dose approach in risk assessment.

3. Proposal of the Acceptable Daily Intake (ADI)

The ADI (expressed as mg of additive or additive related material per person per day) is derived by dividing the overall NOAEL (mg/kg body weight) by an appropriate uncertainty factor and multiplying by a mean human body weight of 60 kg. The BMDL could be used accordingly.

An ADI should, where appropriate, be proposed. An ADI can also be ‘not specified’ because of low toxicity in animal tests. An ADI should not be proposed if the substance shows genotoxic or carcinogenic properties relevant to humans.

The setting of an ADI normally requires the similarity of metabolic fate of the active substance in the target animal and laboratory animal (see 1.1) which ensures that the consumer is exposed to the same residues as the laboratory animals used in toxicological studies. If not, additional studies in a second laboratory animal species or with the metabolites specific to the target species may still allow an ADI

to be set. Under certain conditions the demonstration that the metabolite(s) specific to the target species is (are) not genotoxic could be considered sufficient.

The uncertainty factor used to determine the ADI for a particular additive should take into consideration the nature of the biological effects and the quality of the data used to identify the NOAEL, the relevance of these effects to man and their reversibility and any knowledge of the direct effect(s) of the residues in human.

It is customary to employ an uncertainty factor of at least 100 (i.e., a factor of ten to allow for potential interspecies variation and a further factor of ten to allow for possible differences in response between individual humans) in calculating the ADI (if a full toxicological package has been provided). When data on the active substance are available for human beings a lower uncertainty factor may be acceptable. Higher uncertainty factors might be applied in order to account for additional sources of uncertainty in the data or when the NOAEL is set on the basis of a particularly critical endpoint, such as prenatal development toxicity.

3.1. Tolerable upper intake level (UL)

For some additives (e.g. nutritional additives, substances which add colour to food of animal origin, zootechnical additives favourably affecting the characteristics of animal products) it may be more appropriate to base the safety assessment on the UL. This is defined as the maximum level of total chronic daily intake of a nutrient (from all sources) judged (by national or international scientific bodies) to be unlikely to pose a risk of adverse health effects to consumers or specific groups of consumers.

For example, the nutritional additives vitamins and trace elements are essential dietary components to support vital functions of the animal. They are incorporated/stored in the body in different quantities or used for synthesis of other body constituents. In this respect, they cannot be considered as residues in the usual acceptance of the term. They contribute to the nutritional supply of the consumer. However, depending on the quantity consumed they could cause adverse effects when exceeding the UL.

Published ULs can be found in "Tolerable upper intake levels for vitamins and minerals", by the Scientific Committee for Food of the European Commission and the Scientific Panel on Dietetic Products, Nutrition and Allergies (NDA) from EFSA. In case an UL is not provided in the above reference, other national or international comparable threshold values could be used.

4. Consumer exposure

The dossier should contain all relevant data which allow the assessor to calculate the potential exposure of the consumer which would result from the use of the additive and to enable comparisons with the exposure to the same active substance from other sources, where relevant.

Calculation of the daily intake is based on:

1. the concentrations of total relevant residues (as the arithmetic mean ± 2 standard deviations or the highest single value in case of less than six animals) as described in section 1.2, and
2. default values for daily food consumption by adults shown in Table 1.

Table 1: Default daily adult human consumption figures (g wet tissue/products)[6]

	Mammals	Birds	Fish
Muscle	300	300	300*
Liver	100	100	-
Kidney	50	10	-
Fat	50**	90***	-
+ Milk	1500	-	-
+ Eggs	-	100	-

*: Muscle and skin in natural proportions
**: For pigs 50 g of fat and skin in natural proportions
***: Fat and skin in natural proportions

The scenario (Table 1) should be followed for all additives for which residue data is required.

For additives intended for multi-species use, the daily exposure resulting from the consumption of tissues should be independently calculated for all target species for which data is available. The highest value for each tissue is taken as representative of human exposure from edible tissues. Where appropriate, exposure from milk and eggs should be added to this figure. If bees are identified as the target species, honey (20 g, 8 samples per time point) should be considered.

The model above is included in EU legislation and is widely used by other European and international risk assessment bodies. However, it has limitations. It reflects only chronic intake, only addresses adults, fixes a consumption pattern and assumes that all adults are consumers of each food item. As an alternative, the default values shown in Table 2 could be used. These values are derived from the EFSA Comprehensive European Food Consumption Database[7] and represent the high intake (95th percentile) of consumers only for each food item listed in the table and differentiates between chronic and acute intake.

Table 2: Default values of EU food consumption for high consuming adults and toddlers (g/day)

	Chronic intake[1]		Acute intake[2]	
	Toddlers[3]	Adults[4]	Toddlers	Adults
Meat[5]	90	290	135	390
Liver	-	60	-	170
Kidney	-	15	-	100
Animal fat	-	30	-	40
Milk[6]	1050	1500	1500	2000
Eggs	35	70	50	130
Honey	-	30	-	50
Fish	65	125	130	280
Seafood	-	75	-	200
Fish + seafood	-	165	-	360

[1] Chronic intake is the 95th percentile of the distribution of average individual consumption levels (over the survey period) for consumers only from all available EU national surveys.
[2] Acute intake is the 95th percentile of the distribution of daily consumption levels (all days considered as independent) for consuming days only from all available EU national surveys.
[3] Toddlers: 1-3 years of age, 12 kg body weight.
[4] Adults: 18-65 years of age, 60 kg body weight (presently under consideration by EFSA).
[5] Meat including processed meat products.
[6] Milk including dairy products.

[6] Regulation (EC) No 429/2008.
[7] European Food Safety Authority; Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097. [34 pp.] doi:10.2903/j.efsa.2011.2097. Available online: www.efsa.europa.eu/efsajournal.htm

An analysis showed that there is a very low likelihood that the same high consumer will be found in more than two food groups at the same time. For risk assessment, the intake of both consumer groups (adults and toddlers) should be calculated for all food items listed in the table. The sum of the two highest values is then taken as total intake.

If the ADI is based on a pharmacological effect, the acute intake data should be taken for the calculation following the procedure above.

5. Proposal for Maximum Residue Limits (MRLs)

Maximum residue limit means the maximum concentration of residue (expressed as mg marker residue per kg of edible wet tissue or product) which may be accepted by the European Union to be legally permitted or recognized as acceptable in food. It is based on the type and amount of residue considered to be without any toxicological risk for human health as expressed by the ADI. In principle, an MRL cannot be set in the absence of an ADI.

When establishing MRLs for feed additives, EFSA will follow the calculations detailed in the Appendix. Consideration will be given to consumer exposure to residues of the same substance coming from other sources (e.g., food of plant origin, veterinary use).

For certain additives, residues could arise below the MRL values in milk, eggs or meat which could nonetheless interfere with food quality in particular food processing procedures. For such additives, it may be appropriate to consider a "maximum (food product) processing compatible residue" (MPCR) in addition to establishing MRL values.

APPENDIX

Calculation of Maximum Residue Limits (MRL)

Individual MRLs will ideally be set for the different tissues or products of the target animal species. The individual MRLs in different tissues/products will reflect the depletion kinetics and variability of the residue levels within those tissues/products in the animal species. Variability is normally reflected by using the 95% confidence limit of the mean. If the confidence limit cannot be calculated due to low number of samples, the highest individual value will be taken instead.

Table A1: Definitions used in deriving an MRL

$_{i\text{-}j}$	Individual tissues/products (liver, kidney, muscle, skin + fat, milk, eggs) at different times
$MRL_{i\text{-}j}$	Maximum residue limit in tissues/products (mg marker substance/kg)
$Qt_{i\text{-}j}$	Daily human consumption of individual tissues/products (kg)
$TRC_{i\text{-}j}$	Total residue concentration in individual tissues/products (mg/kg)
$MRC_{i\text{-}j}$	Marker residue concentration in individual tissues/products (mg/kg)
$RMTR_{i\text{-}j}$	Ratio $MRC_{i\text{-}j}$ to $TRC_{i\text{-}j}$ for individual tissues/products
$DITR_{i\text{-}j}$	Dietary intake for individual tissues/products calculated from total residues (mg) $DITR_{i\text{-}j} = Qt_{i\text{-}j} \times TRC_{i\text{-}j}$
$DITR_{MRLi\text{-}j}$	Dietary intake calculated from MRLs (mg) of individual tissues/products $DITR_{MRLi\text{-}j} = Qt_{i\text{-}j} \times MRL_{i\text{-}j} / RMTR_{i\text{-}j}$

Deriving a MRL is an iterative process which is summarised in Figure A1.

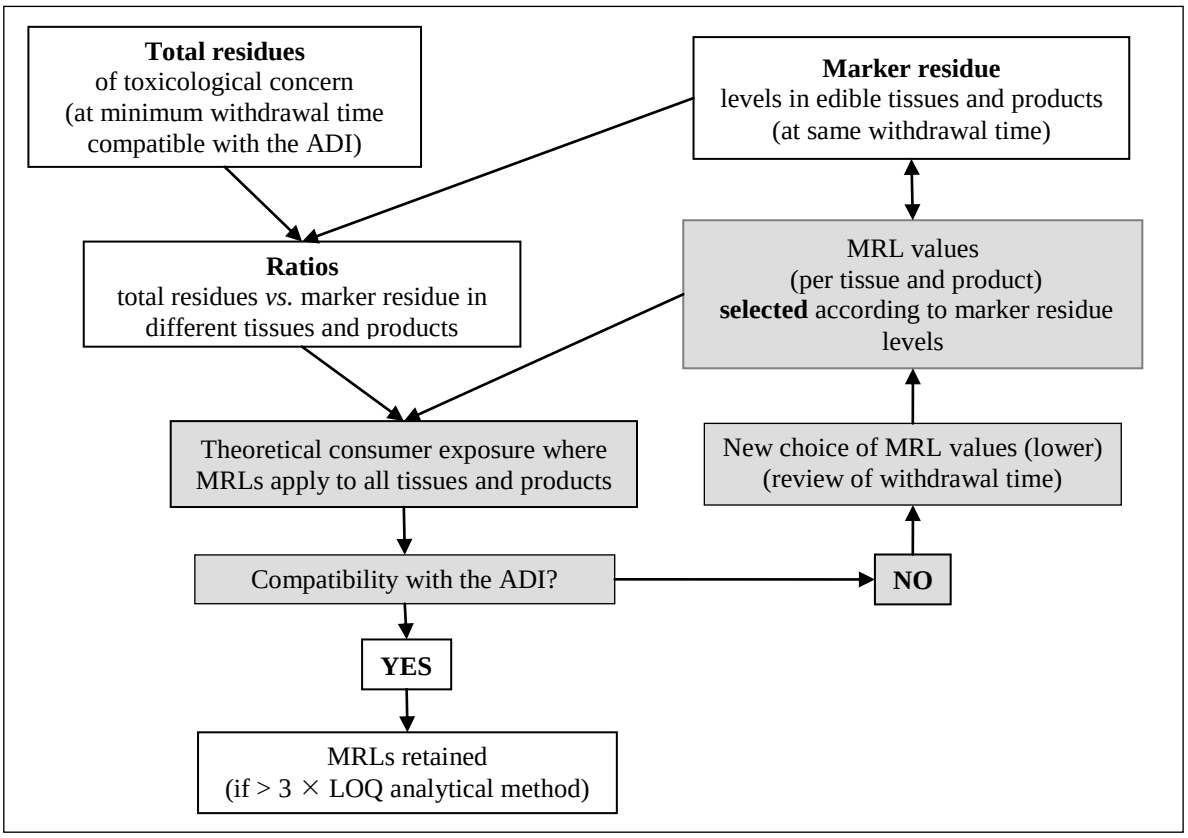

Figure A1: Rationale for setting MRLs

The different steps are the following:

i) select the minimum withdrawal time for which the sum of the tissue/product specific DITRs is below the ADI. If the ADI is exceeded whatever the withdrawal time for which data are available, a new data set will be generated using a longer withdrawal time or lower dosages.

ii) for all tissues/products, calculate the ratios marker *vs.* total toxicologically relevant metabolites corresponding to the withdrawal selected in step i). The measured values for TRC and MRC will be inserted as appropriate in the template shown in Table A2 and the other values calculated. Ideally, the ratio values will be in the same range (similar) for all withdrawal times. In cases where a full data set is not available because values fall below the LOD,[8] an extrapolation of RMTR may be acceptable.

Where the marker residue represents a major fraction of total residues and as TRCs and MRCs are determined in separate experiments, TRCs may appear to be lower than MRCs which leads to ratios >1. In that case MRCs will be considered as the reference and TRCs back calculated using the RMTR.

iii) considering the MRCs measured in the different tissues/products (including 2SD or the highest values where a reduced number of animals is available) as a guide, and taking into consideration the LOQ[9] of the analytical method of the marker residue in the different tissues/products, select a first set of MRL values.

iv) check that the sum of the $DITR_{MRL}$ obtained from the proposed MRLs is below the ADI and close to the sum of the individual DITRs. If the ADI is exceeded, then a set of lower MRLs will be selected and the comparison repeated.

Table A2: Template for deriving a MRL proposal

	Liver	**Kidney[4]**	**Muscle**	**Fat or skin + fat[4]**	**Milk**	**Eggs**	**Sum**
TRC[1] (mg/kg)							-
MRC[2] (mg/kg)							-
RMTR[2]							-
DITR[3] (mg)							
MRL proposed (mg/kg)							-
$DITR_{MRL}$(mg)							

[1] Considering the proposed withdrawal time.
[2] Ideally established at the same time as TRC.
[3] Calculated from TRC values.
[4] See Table 1.

In all cases, an analytical method of sufficient sensitivity must be available before MRLs can be set. The LOQ of the method should be at least three times lower than the MRL.

For minor species, see technical guidance on extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition.

Withdrawal period

The withdrawal time comprises the period after cessation of the administration of the additive which is necessary to enable the residue levels to fall below the MRLs.

[8] Limit of detection.
[9] Limit of quantification.