Guidance Document
ADME Evaluation in the context of risk assessment of feed ingredients
January 2024
At Step 4: Public Consultation

ADME EVALUATION IN THE CONTEXT OF RISK ASSESSMENT OF FEED INGREDIENTS

Draft for Public Consultation
January 2024

It is recommended for the companies planning to submit applications/dossiers for pre-market authorization, to contact the jurisdictions of the countries to confirm their acceptance of the current guidance document.

The International Cooperation for Convergence of Technical Requirements for the Assessment of Feed Ingredients (ICCF) was launched in 2017 and aims to develop and establish common guidance documents to provide technical recommendations for the assessment of feed ingredients, including new uses of existing feed ingredients.

This guidance document has been developed by the appropriate ICCF Experts Working Group and was subject to consultation by the Parties, in accordance with the ICCF Process.

The founding members of the ICCF include the Canadian Food Inspection Agency (CFIA), the European Commission (DG SANTE), the U.S. Food and Drug Administration (FDA), as well as the American Feed Industry Association (AFIA), the Animal Nutrition Association of Canada (ANAC), the EU Association of Specialty Feed Ingredients and their Mixtures (FEFANA) and the International Feed Industry Federation (IFIF).

Table of content

ADME EVALUATION IN THE CONTEXT OF RISK ASSESSMENT OF FEED INGREDIENTS

1. INTRODUCTION

1.1 Objective of the guidance document

This guidance document addresses the evaluation of the absorption, distribution, metabolism, and excretion (ADME) of feed ingredients and/or their constituent entity(ies) as a critical component of the risk assessment for the consumer of edible products. The guidance document describes recommended approaches with corresponding endpoints and methods/procedures to support the ADME evaluation of feed ingredients, while minimizing use of animals.

1.2 Definitions

The following definitions apply in the context of this guidance document:

Absorption[1]: The process(es) of uptake of substances into or across tissues after oral uptake of a feed ingredient. Absorption refers to all constituent entity(ies) of the feed ingredient.

Active substance[2]: Any substance in a feed ingredient that contributes to the intended effect[3].

Acceptable Daily Intake (ADI): An estimate of the amount of a substance in food that can be consumed daily over a lifetime without presenting an appreciable risk to human health.

Area under the plasma concentration-time curve[1] (AUC): The area under the curve in a plot of concentration of substance in plasma over time. It represents the total amount of active substance absorbed by the body within a predetermined period. Under linear conditions, the AUC (from time zero to infinity) is proportional to the total amount of active substance absorbed by the body, irrespective of the rate of absorption.

[1] Adapted from the OECD guidelines 417 (Toxicokinetics)
[2] Active substance includes microorganisms that contribute to the intended effect.
[3] The intended effect refers to the conditions of use of the feed ingredient and not to the potential hazardous effect of the substance.

Benchmark dose (BMD): The estimated dose that produces a low but measurable change in the response rate of an adverse effect in the target organ/tissue that is based on all available toxicological data. This predetermined change in response is called the benchmark response.

Bioaccumulation[1]: The increase of the amount of the constituent entity(ies) of the feed ingredient within tissues over time, following repeated exposure.

Bioavailability[1]: The fraction of an administered dose/level of the constituent entity(ies) of a feed ingredient that reaches the systemic circulation or is made available at the site of physiological activity, after oral ingestion of the feed ingredient.

Constituent entity: Any chemical moiety present in the feed ingredient, including active substance(s).

Consumer: The person who ingests edible products, derived from animals that were fed the feed ingredient.

Edible products[4]: The tissues and products of animal origin that can enter the food chain. They include, but are not limited to, muscle, liver, kidney, subcutaneous fat and skin in natural proportion, fat, whole eggs, whole milk, and honey.

Feed Ingredient[5]: A component part or constituent of any combination or mixture making up a feed, whether or not, it has nutritional value in the animal's diet. Ingredients are of plant, animal, microbial or aquatic origin, or other organic or inorganic substances.

***In-silico* models:** Computer models developed to evaluate the ADME properties of constituent entity(ies) in feed ingredients.

***In-vitro* studies:** The studies performed with microorganisms, cells, or biological molecules outside their normal biological context that evaluate the effects of the constituent entities of a feed ingredient.

***In-vivo* studies:** The studies performed with whole living organisms (e.g., animals) that evaluate the effects of the constituent entity(ies) of a feed ingredient.

[4] Adapted from Guidance for Industry #205: Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-producing Animals, Metabolism Study to Determine the Quantity and Identify the Nature of Residues (MRK)

[5] Adapted from Codex Alimentarius, Code of Practice on good animal feeding (CAC/RCP 54-2004)

Ingredient market formulation: The feed ingredient (e.g., active substance(s)) under assessment formulated with carrier(s) and/or other constituent(s). It is the commercial product used to incorporate the feed ingredient under assessment into premixtures, feeds or water.

Laboratory animals[6]: The animals used for testing the feed ingredient or its constituent entity(ies) reared in controlled environmental conditions.

Lowest Observed Adverse Effect Level (LOAEL): The lowest tested level/concentration of a substance that causes an adverse effect in an exposed group compared to a vehicle exposed control group.

Metabolism[1]: The chemical conversion of the constituent entity(ies) of a feed ingredient into (a) different chemical substance (s) within the body. The conversion usually involves endogenous enzymes.

Metabolism pathways: The reaction chains, where chemical products become substrates for the next step in the chain.

Metabolites[1]: The products of metabolism or metabolic processes.

No Observed Adverse Effect Level (NOAEL) [7]: The highest level/concentration of exposure to a substance, at which no adverse effects are observed in an exposed group, when compared to a vehicle exposed control group.

No Observed Effect Level (NOEL)[7]: The highest level/concentration of a substance, found by experiment or observation, that causes no alteration of morphology, functional capacity, growth, development, or lifespan of the target organism distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure.

Point of departure (POD) [8] : The defined point on an experimental dose-response relationship for the adverse effect occurring at the lowest dose level. It may be a NOAEL/LOAEL, but ideally is established from benchmark dose modelling of the experimental data, and generally corresponds to a selected estimated low level of response (e.g., 1 to 10 % response for a quantal effect).

[6] Adapted from AVMA, 2021; 258 (3)
[7] WHO, EHC 240, 2009
[8] Adapted from the definitions of EFSA, Guidance (2019) and US EPA risk assessment forum (2012)

Quantitative Structure Activity Relationship (QSAR)[9]: A mathematical model, that can be used to predict the physical, chemical, and biological properties, and environmental fate of compounds based on their chemical structure.

Radiolabelled: Labelled with one or more atoms replaced by a radionuclide.

Read-across[7]**:** A method where information about a chemical substance is inferred from a structurally or functionally similar reference compound with known data. This approach assists in predicting properties or behaviours of a target compound when specific data for the target are limited or unavailable.

Target animals: The animal(s) for which the feed ingredient is intended to be used.

Test article: The prototype of the feed ingredient specifically manufactured to test the feed ingredient.

Tissue residue: The constituent entity(ies) of the feed ingredient or its (their) metabolite(s) present in edible products of the target animal species, to which the consumer may be exposed.

Tolerable Upper Intake Level (UL): The maximum level of total chronic intake of a nutrient from all sources to be unlikely to pose a risk of adverse health effects in humans.

Weight of evidence assessment[10]**:** A process in which data from diverse sources is integrated to determine its relative support for possible answers to a scientific question.

1.3 Scope of the guidance document

The scope of this guidance document is the ADME evaluation used in the consumer safety risk assessment of edible products derived from animals fed the feed ingredient under investigation. Beyond this guidance document, the results of the ADME evaluation may also be used in the risk assessment of the feed ingredient for the target animals, the environment (fate of the feed ingredient and its metabolites in the excreta), and/or the workers exposed to the feed ingredient, while handling it.

This guidance document generally applies to risk assessment of new feed ingredients and of already marketed feed ingredients in case of new conditions of use or new target animal.

[9] European Chemical Agency (ECHA), 2020
[10] Adapted from the EFSA Scientific Opinion on the guidance on the use of the weight of evidence approach in scientific assessments, 2017

2. GENERAL PRINCIPLES

Recommendations for the ADME evaluation (when required) of a feed ingredient in this guidance document can be applied to each constituent entity of the feed ingredient or of the ingredient market formulation. The test article should be selected using sound scientific judgement. For example, if the ingredient market formulation influences the absorption of the active substance(s) or the safety of the feed ingredient, it may be recommended to use the ingredient market formulation as a test article (e.g., encapsulated carotenoids). However, if the ingredient market formulation does not influence the absorption of the constituent entity(ies) or the safety of the feed ingredient, the ADME evaluation could be conducted on the individual constituent entity(ies) of the feed ingredient, as needed.

An initial step of the ADME evaluation of feed ingredients to support consumer safety is the review of information available on the ADME properties of feed ingredients or their constituent entity(ies), and the identification of critical gaps in that information using the weight of evidence approach. Gaps in critical information needed for the ADME evaluation of feed ingredients should be assessed on a case-by-case basis. For example, strategies could involve a diversity of approaches (e.g., read-across, new studies). In the case that ADME studies (e.g., *in-vitro*, *in-vivo*) are required to fulfil critical data gaps in information for the ADME evaluation of feed ingredients, the studies should be carefully designed and conducted using scientifically recognized methods (including validated novel technologies and methodologies). The type of study selected should depend on various factors, including but not limited to, the type of feed ingredient and potential metabolites (e.g., substances with bioaccumulation potential).

3. ASPECTS TO CONSIDER IN THE ADME EVALUATION OF FEED INGREDIENTS

Several aspects should be considered when deciding if an ADME evaluation of a feed ingredient is needed for addressing consumer safety. These aspects are summarized in a decision tree included in Annex I. The decision tree also includes recommendations on which types of studies should be conducted for addressing critical data gaps in the ADME evaluation of the feed ingredient, subject to a pre-market authorization or approval.

The initial review of available information to determine whether an ADME evaluation of a feed ingredient or its constituent entity(ies) is needed should consider the following factors, as listed below and in the decision tree in Annex I:

1. Characterization of the feed ingredient. Information on the individual constituent entity(ies) and/or the ingredient market formulation(s) (recommendations on this

topic can be found in the ICCF Guidance Document on 'Identification and Characterization of Feed Ingredient' and other guidance documents),

2. Intended target animal: ADME evaluation of feed ingredient is not required when the feed ingredient is intended to be used only in non-food producing animal species or class,
3. Evaluation of toxicologically relevant information: the toxicity profile of the feed ingredient or its constituent entity(ies) should be evaluated using a weight of evidence approach,
4. Potential safety concerns for the consumer: Potential toxicity of the feed ingredient and/or its constituent entity(ies) and/or ingredient market formulation for the consumer identified from the available information (e.g., from *in-vivo* and *in-vitro* studies) using a weight of evidence approach,
5. The ADME profile of the toxicologically relevant constituent entity(ies) of the feed ingredient: the potential for bioaccumulation and safety for consumers determined using a weight of evidence approach of available literature (e.g., from *in-vivo* studies, *in-vitro* studies, read-across methods, Quantitative Structure Activity Relationship (QSAR) analysis, etc.).

The evaluation of the aspects listed above should allow:

1. Selecting an appropriate point of departure (POD), such as the NOAEL(s) for critical toxicological endpoints. These NOAEL(s) should be used to derive the ADI of the feed ingredient or its constituent entity(ies) for the consumer. If the available information does not allow selecting an appropriate POD (NOEL, NOAEL, BMD, LOAEL), a gap analysis should determine the information needed for selecting a POD.
2. Evaluating the potential consumer exposure to the feed ingredient and/or its constituent entity(ies) based on the conditions of use of the feed ingredient, and/or tissue residue data. In the absence of tissue residue data in edible products and adequate safety data, ADME studies should be considered.
3. Comparing the estimated ADI or UL with the potential consumer exposure allows to identify if there are safety concerns for the consumer due to the exposure of the feed ingredient or its constituent entity(ies) in edible products.

4. ADME EVALUATION

In case an ADME evaluation of the feed ingredient or its constituent entity(ies) is considered necessary based on the aspects that should be considered (as listed in Section 3 and in the decision tree of Annex I), a tiered approach is recommended for the ADME evaluation. In this approach, the first tier would be the evaluation of all information available (e.g., from read-across, *in silico* models and/or *in-vitro* or *in-vivo* studies) to conduct the safety risk assessment for the consumer using a weight of evidence approach. A second tier is triggered if critical data

gaps exist in the available information that prevents conducting the ADME evaluation of the feed ingredient or its constituent entity(ies) using the weight of evidence approach. In this case, it is recommended to prioritize studies providing information on the absorption of the feed ingredient or its constituent entity(ies), followed by studies on their metabolism and then, if required, their potential bioaccumulation. There are established models for absorption studies (including *in-vitro* and *in-vivo* studies, and *ex-vivo* absorption and bioavailability models). Demonstration of negligible absorption, either through experimental studies or from theoretical considerations, may provide a scientific justification for not undertaking higher tiered toxicological studies on a feed ingredient or its constituent entity(ies).

4.1 Weight of Evidence Approach

The weight of evidence approach in scientific assessment[11] comprises three (3) basic steps:

- Assembling the evidence,
- Weighing the evidence, and
- Integrating the evidence.

At each step, the reliability, relevance, and consistency of the evidence used should be evaluated. Detailed information on the weight of evidence approach in scientific assessments can be found in the EFSA guidance (13). Although the guidance is not specific for the ADME evaluation of feed ingredient, the principles highlighted in the guidance can be applied to the weight of evidence approach for ADME evaluation of feed ingredient or its constituent entity(ies).

The weight of evidence approach aims at considering all information available on the ADME and toxicologically relevant characteristics of the feed ingredient and/or its constituent entity(ies) from relevant studies/information (e.g., read-across, *in silico*, *in-vitro*, *in-vivo*). These are discussed below.

4.1.1 Read-across Assessment

In the case that no information is available on the specific feed ingredient or its relevant constituent entity(ies), an alternative method that could be used in a case-by-case basis is the read-across assessment (also known as a bridging assessment). This assessment considers all information available on the ADME and toxicologically relevant properties of substances having similar characteristics to the feed ingredient or its constituent entity(ies) from

[11] Adapted from Guidance on the use of the weight of evidence approach in scientific assessments. EFSA Journal 2017;15(8):4971.

studies/information (e.g., *in-silico*, *in-vitro*, *in-vivo* studies). Read-across assessment involves the use of relevant information in the public domain (e.g., in scientific literature from analogous substances (the 'source information') that have similar structure, physical and chemical characteristics to predict the properties and potential behaviour of the feed ingredients of its constituent entity(ies)[12].

The read-across information should provide information on pivotal endpoints required for the ADME evaluation of the feed ingredient or its constituent entity(ies), by extrapolation/interpolation and prediction from available data on the reference substances. These typical endpoints should be selected using scientific judgement considering the feed ingredient under evaluation.

The read-across should cover the evaluation of the analogous substance or group of substances used as reference. The reference substance(s) and the endpoint(s) of interest selected, as well as the approach taken for the literature used for the read-across evaluation should be accurately described, justified, and documented to support the hypothesis considered.

If the read-across provides the information required for the ADME evaluation of the feed ingredient or its constituent entity(ies), the applicant may not be required to provide additional data. Otherwise, further evaluation considering *in-silico* models (if appropriate) is recommended.

4.1.2 *In-Silico Models*

In-silico models such as QSAR(s), and physiologically based pharmacokinetics (PBPK) are computerized models to predict qualitatively and quantitatively the physical, chemical, and biological properties, and environmental fate of substances, based on the information available on their chemical structures. The models are built on all relevant and available information from similar reference substances.

For *in-silico* models, it is important to evaluate the feed ingredient or its constituent entity(ies) against substance(s) within the same applicability domain. Reliability is improved with the use of established models. Example of *in-silico* models available are listed in Annex II.

The selection of an *in-silico* model should be properly justified. Furthermore, the results of the evaluation of the *in-silico* models should be carefully examined to ensure the information obtained from the model can fulfil the requirements for the ADME evaluation of the feed

[12] Adapted from ECHA-17-R-01-EB. Read-across assessment framework (RAAF)

ingredient or its constituent entity(ies). Uncertainties in *in-silico* model results should be addressed and may be compensated by additional information on the feed ingredient.

If the combination of read-across and *in-silico* models provides enough information required for conducting the ADME evaluation, the applicant may not be required to provide additional information. However, if critical data gaps or uncertainties remain after conducting the read-across assessment and *in-silico* models, additional *in-vitro* studies (if appropriate) are recommended.

It is to be noted that, while read-across and *in-silico* models may be used to replace *in-vitro* and/or *in-vivo* studies, they may also be used to provide supplementary information to assess the results from *in-vitro* and/or *in-vivo* studies.

4.1.3 *In-Vitro* Studies

Various *in-vitro* test systems (Annex III) have been published and could be used in the ADME evaluation of feed ingredients and its constituent entity(ies). These test systems may support the evaluation of

- the digestion of the constituent entity(ies) of the feed ingredient, such as the simulated gastric fluid study, simulating the physical and chemical properties of the fluid contained in the stomach of the animals, or the simulated intestinal fluid study, simulating the microbiome, and physical and chemical conditions of the intestinal fluid,
- the absorption of the constituent entity(ies) of the feed ingredient, such as the Caco2 permeability assay, using the Caco2 cells of the intestinal tract of the animals to simulate the absorption of the constituent entity(ies),
- the metabolism of the constituent entities of the feed ingredient after absorption, such as the studies with primary hepatocytes, liver microsomes, S9 sub-cellular fraction, cytosol, liver slices, or whole cell lines.

However, the protocols for the above mentioned *in-vitro* studies have not yet been standardized (e.g., by regulatory bodies). Therefore, when used, it is recommended that good laboratory practices[13] relevant to the test system is used and the reference to the test system selected is properly justified.

The robustness and reliability of *in-vitro* methods can accelerate their use for early screening testing.

[13] For further details see the OECD guidelines No. 286

4.2 *In-Vivo* Studies

In the tiered approach recommended in the guidance document, *in-vivo* studies should be considered when critical information required for the ADME evaluation of feed ingredients, or its constituent entity(ies) can not sufficiently be provided by read-across assessment, *in-silico* models, or *in-vitro* studies. The goal of *in-vivo* ADME studies in the risk assessment of a feed ingredient in the context of consumer safety is to generate data on the quantity and nature of residues of toxicological concern in edible products of animals fed the feed ingredient or its constituent entity(ies).

The Veterinary International Commission on Harmonization (VICH) Guideline No. 46 provides a framework for metabolism and residue testing. However, to adequately characterize the residue of concern, it is important that the design of studies remain flexible.

Usually, metabolism studies are accomplished using properly radiolabeled[14] substances, corresponding to the constituent entity(ies) of toxicological concern of the feed ingredient. The radiolabeled constituent entity(ies) are fed to the target animals, after incorporation in feed or water, depending on the conditions of use of the feed ingredient and at the highest concentrations achievable under the proposed conditions of use. As ADME studies are aimed to evaluate the fate of the labeled substances and not their toxicokinetics, the use of single dose is recommended. ADME studies may be envisaged in laboratory animals, if the physiological similarities of the laboratory animals tested with the target animals can be justified, depending on the results obtained in the toxicological studies and *in-vitro* metabolism studies.

Edible products are collected at different time points of the study, to follow the evolution of the concentration of the constituent entity(ies) and/or its metabolites with time, measured as total radioactivity in the relevant edible product, necessary for the evaluation. Enough control and test edible products should be collected to enable the related analytical methods testing (ICCF Guidance Document on Analytical Methods[15]).

[14] Radiolabel should allow the tracking of radioactivity in all relevant metabolites.
[15] Under development at the time of writing this guidance document.

Although the excreta and blood are usually not collected in *in-vivo* ADME studies, the analysis of those samples may provide useful information, such as:

1. Estimating the mass-balance of the radioactivity, supporting the quality of the study,
2. Obtaining information on the metabolites present in excreta and in blood,
3. Using the information for conducting an Environment Risk Assessment (See ICCF Guidance Documents on Environmental Risk Assessment Approach and on Environmental Risk Assessment Phase 2[15]).

Alternative approaches not using radiolabeled materials could be considered, if scientifically justified (e.g., if the substance is not metabolised or the metabolite(s) can be quantified otherwise).

Further to the evaluation of the concentration of metabolites in the edible products, it is recommended to characterize and identify the structure of the major metabolites (i.e., metabolites with concentration higher than 100 µg / kg wet basis or representing more than 10 % of the total residue). An evaluation of the non-extractable residues may warrant discount of some of the residues, as non-extractable residues usually result from incorporation of small fragments of the feed ingredient's constituent entity(ies) in naturally occurring molecules and are not of significance.

The determination of a marker residue (i.e., a metabolite that can be analyzed and has a direct relationship with the total amount of metabolites) is recommended to allow the evaluation of the consumer's exposure to the metabolites from the feed ingredient. This exposure can then be compared with the pre-determined POD.

5. INTERPRETATION OF THE INFORMATION

For each step of the ADME tiered approach, the aim of the information collected should be interpreted to allow:

- For read-across
  - The information gathered for the ADME of the analogous substance(s).
  - The applicability of this information for the feed ingredient.
- For *in-silico* models
  - The information provided by the *in-silico* model(s) regarding the ADME of the feed ingredient, considering the methodology used for the evaluation.
  - The potential presence and concentration of metabolites of toxicological concern when the feed ingredient is fed to target animal species.
- For *in-vitro* studies

- The information provided on the characterization and concentration of metabolites of the feed ingredient in the relevant *in-vitro* studies used.
- The potential for extrapolation of results obtained to laboratory animals and/or to the target animal species.

- For *in-vivo* studies
  - Identification, characterization, and concentration of the metabolites of the feed ingredient, when used under the proposed conditions of use.
  - Evaluation of the toxicological relevance of the metabolites, analysed in the relevant edible products.

And interpreted to achieve the following conclusions:

- For read-across
  - The conclusion with regards to potential presence of metabolites of toxicological concern in the edible products of target animals fed the feed ingredient:
    - If the conclusion allows the consideration of low level of exposure and/or limited toxicological concern, no further evaluation is required.
    - If not, it is recommended to go to the next step of the approach.
- For evaluation of *in-silico* models
  - The conclusion with regards to potential presence of metabolites of toxicological concern in the edible products of target animals fed the feed ingredient:
    - If low level of exposure and/or limited toxicological concern is found, no further evaluation is required.
    - If not, it is recommended to go to the next step of the approach.
- For *in-vitro* studies
  - The conclusion with regards to potential presence of metabolites of toxicological concerns in the edible products of target animals fed the feed ingredient:
    - If the conclusion allows the consideration of low level of exposure and/or limited toxicological concern, no further evaluation is required.
    - If not, it is recommended to go to the next step of the approach.
- For *in-vivo* studies
  - Comparison of the concentration of metabolites of toxicological concern with the relevant POD (reference point), to ensure the safety of the feed ingredient for the consumer:
    - If the concentration of metabolites is lower than the POD, when applying an appropriate uncertainty factor, continue with the risk assessment of the feed ingredient,
    - If the concentration of metabolites is higher than the POD, when applying an appropriate uncertainty factor:

- Modify the conditions of use of the feed ingredient, if possible, to reduce the exposure of the consumer to achieve a safe amount of the toxicologically relevant metabolites (e.g. reduce incorporation rate, propose a withdrawal period),
- Otherwise, evaluate the possibility of stopping the application of the feed ingredient.

6. REPORTING THE INFORMATION

For each step of the ADME approach, data and results should be reported:

- For read-across:
  - Justification of the substances used for the analysis, based on similarity,
  - Approach taken for the extensive literature search,
  - Information gathered and analysed for the evaluation.
- For *in-silico* models:
  - Justification of the methodology used for the evaluation,
  - Information gathered and analysed for the evaluation.
- For *in-vitro* studies:
  - Justification of the studies used for the evaluation,
  - Description and justification of the protocol used for the study,
  - Characterization of the metabolites obtained with the different studies and evaluation of their safety,
  - Analytical methods and their validation
  - Number/amount of metabolites obtained with the different studies.
- For *in-vivo* studies:
  - Description and justification of the protocol used for the study,
  - Characterization and identification of the main metabolites,
  - Analytical methods and their validation
  - Concentration of main metabolites in the edible products,
  - Information on the toxicological properties of the different metabolites.

7. ACRONYMS

ADME	Absorption, Distribution, Metabolism, and Excretion
ADI	Acceptable Daily Intake
AUC	Area Under the plasma concentration time Curve
BMD	Benchmark Dose

LOAEL	Lowest Observed Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
PBPK	Physiologically Based PharmacoKinetic
PEC	Predicted Environmental Concentration
POD	Point of Departure
QSAR	Quantitative Structure Activity Relationship
UL	Tolerable Upper Intake Level

8. BIBLIOGRAPHY

8.1 OECD Guidelines

1. Organisation for Economic Co-operation and Development (OECD) (2007) Environment Health and Safety Publications Series on Testing and Assessment No. 69: Guidance document on the validation of (quantitative structure activity relationship [(Q)SAR] models, 154 pages.
2. Organisation for Economic Co-operation and Development (OECD) (2009) Environment, Health, and Safety Publications Series on Testing and Assessment No. 102: Guidance document for using the OECD (Q)SAR application toolbox to develop chemical categories according to the OECD guidance on grouping of chemicals, 118 pages.
3. Organisation for Economic Co-operation and Development (OECD) (2010) Test Guideline No. 417: Toxicokinetics, OECD Guidelines for the Testing of Chemicals, 20 pages.
4. Organisation for Economic Co-operation and Development (OECD) (2014) Environment, Health, and Safety Publications Series on Testing and Assessment No. 194: Guidance on grouping of chemicals, Second Edition, 141 pages.
5. Organisation for Economic Co-operation and Development (OECD) (2018) OECD Series on testing and assessment No. 286: Guidance Document on good *in-vitro* Methods Practices, 206 pages.
6. Organisation for Economic Co-operation and Development (OECD) QSAR Toolbox software available online - The OECD QSAR Toolbox - OECD.

8.2 VICH Guidelines

7. VICH Guideline No. 46 (MRK) – Metabolism and Residue Kinetics (2011) Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food producing animals: metabolism study to determine quantity and identify the nature of residues, 13 pages.

8. VICH Guideline No. 47 (MRK) – Metabolism and Residue Kinetics (2011) Studies to evaluate the metabolism and residue kinetics of veterinary drug in food producing animals: laboratory animal comparative metabolism studies, 10 pages.
9. VICH Guideline No 48(R) (MRK) – Metabolism and Residue Kinetics (2015) Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: marker residue depletion studies to establish product withdrawal periods, 16 pages.

8.3 European Union

10. Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation od feed additives – Official Journal of the European Union L133/1 http://data.europa.eu/eli/reg/2008/429/oj
11. European Chemicals Agency (2008) Guidance on information requirements and chemical safety assessment. Chapter R.6: QSARs and grouping of chemicals, 134 pages.
12. European Chemicals Agency (ECHA) (2017) Read-Across Assessment Framework (RAAF), 60 pages.
13. European Food Safety Authority Panel on Food Additives and Nutrient Sources added to food (ANS) (2012) Guidance for submission for food additive evaluations, EFSA Journal; 10(7):2760, 53 pages. Doi: 10.2903/j.efsa.2012.2076
14. EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V, Solecki R, Turck D, Benfenati E, Chaudhry QM, Craig P, Frampton G, Greiner M, Hart A, Hogstrand C, Lambre C, Luttik R, Makowski D, Siani A, Wahlstroem H, Aguilera J, Dorne J-L, Fernandez Dumont A, Hempen M, Valtuena Martinez S, Martino L, Smeraldi C, Terron A, Georgiadis N and Younes M (2017). Scientific Opinion on the guidance on the use of the weight of evidence approach in scientific assessments. EFSA Journal 2017;15(8):4971, 69 pages. https://doi.org/10.2903/j.efsa.2017.4971
15. EFSA FEEDAP Panel (EFSA Panel on Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Dujardin B, Galobart J and Innocenti ML (2017). Guidance on the assessment of the safety of feed additives for the consumer. EFSA Journal 2017;15(10):5022, 17 pages. https://doi.org/10.2903/j.efsa.2017.5022
16. EFSA Scientific Committee, More SJ, Bampidis V, Benford D, Bennekou SH, Bragard C, Halldorsson TU, Hernández-Jerez AF, Kousoumanis K, Naegeli H, Schlatter JR, Silano V, Nielsen SS, Schrenk D, Turck D, Ylunes M, Benfenati E, Castle L, Cedergreen N, Hardy A,

Laskowski R, Leblanc JC, Kortenkamp A, Ragas A, Posthuma L, Svendsen C, Solecki R, Testai E, Dujardin B, Kass GEN, Manini P, Jeddi MZ, Dorne J-LCM and Hogstrand C (2019). Guidance on harmonised methodologies for human health, animal health and ecological exposure to multiple chemicals. EFSA Journal 2019;17(3):5634, 77 pages. https://doi.org/10.2903/j.efsa.2019.5634

17. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, Official Journal of the European Union L276/33.

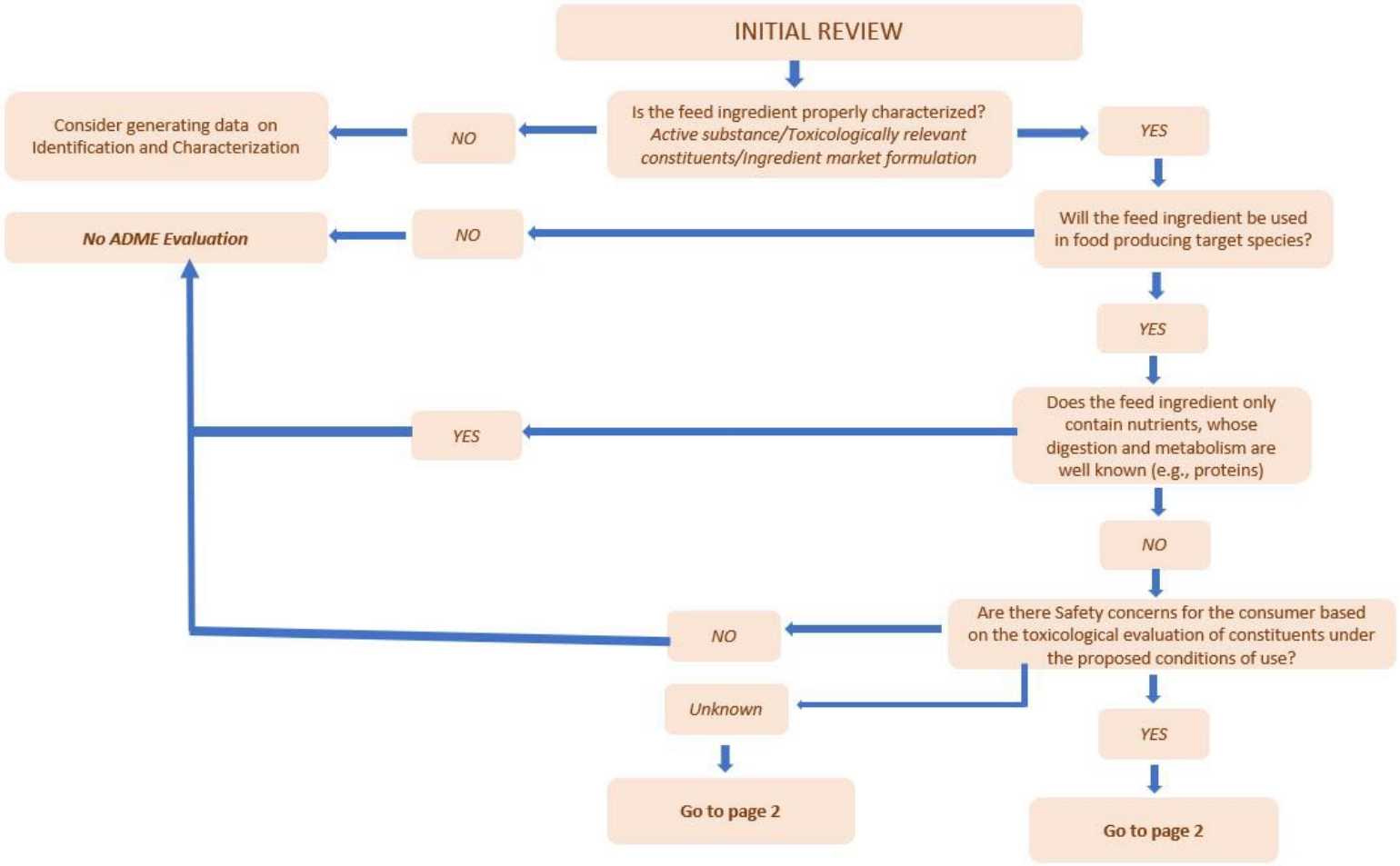
8.4 United States of America

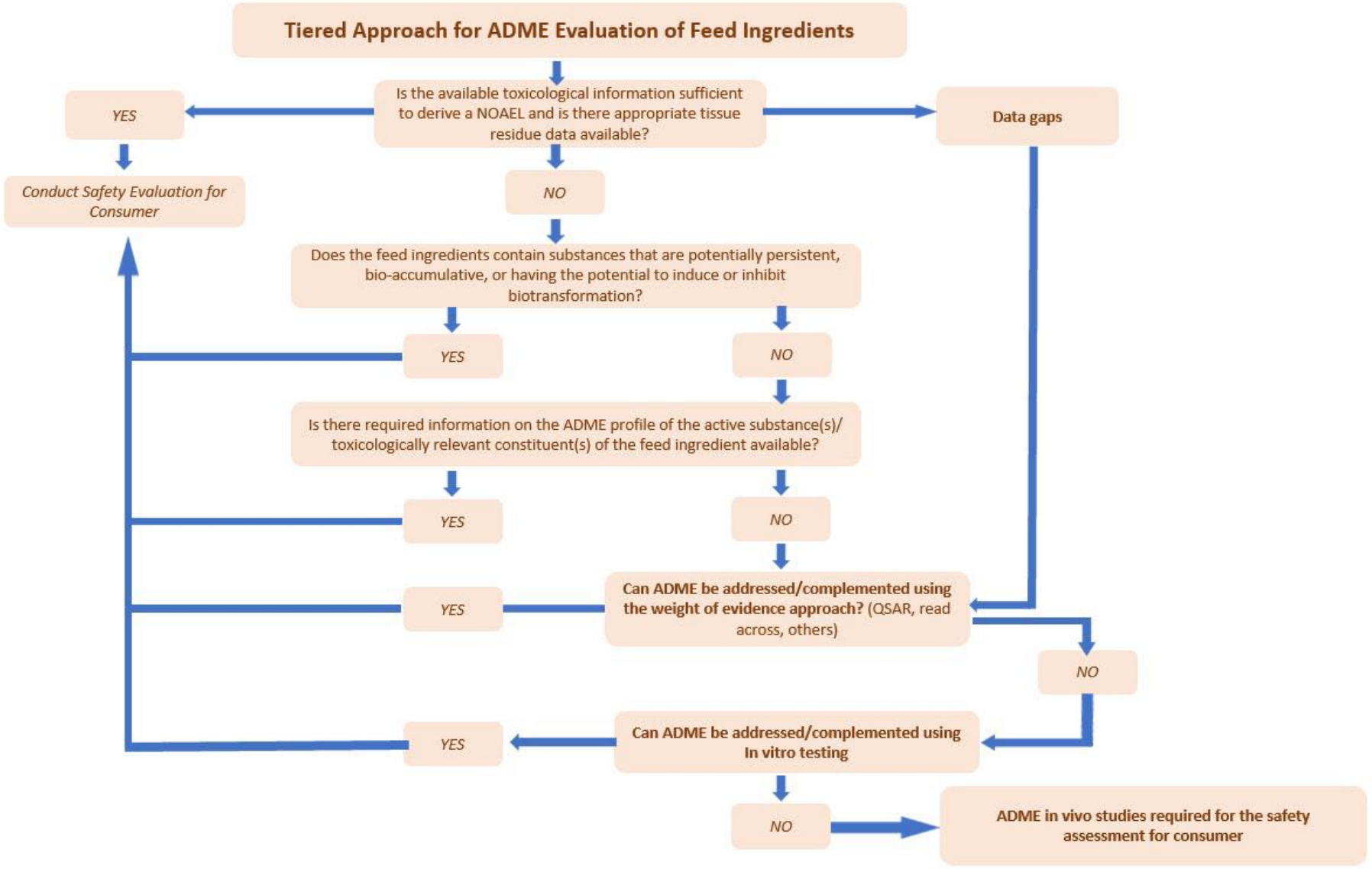
18. Guidance for Industry #205 (US FDA): Studies to Evaluate the Metabolism and Residue Kinetics of veterinary Drugs in Food-producing Animals: Metabolism Studies to Determine the Quantity and Identify the Nature of Residues (MRK).
19. Benchmark Dose Technical Guidance (US EPA); Risk Assessment Forum June 2012, 99 pages.

8.5 Literature

20. Knights K.M., Stresser D.M., Miners J.O., Crespi C.L., 2016 - In Vitro drug metabolism using liver microsomes. Current protocols in pharmacology 74:7.8.1-7.8.24
21. Madden J.C., Enoch S.J., Paini A., Cronin M. T.D., 2020. A review of in silico tools as alternatives to animal testing: principles resources and applications. Alternatives to Laboratory Animals 48(4) 146-172
22. Open Systems Pharmacology Suite Manual available online: Introduction - Open Systems Pharmacology (open-systems-pharmacology.org)
23. TIMES (Tissue metabolism simulator) software available online with a licence fee - Software (oasis-lmc.org)

ANNEX I – DECISION TREE




Tiered Approach for ADME Evaluation of Feed Ingredients
Is the available toxicological information sufficient to derive a NOAEL and is there appropriate tissue residue data available?
YES
Conduct Safety Evaluation for Consumer
Data gaps
NO
Does the feed ingredients contain substances that are potentially persistent, bio-accumulative, or having the potential to induce or inhibit biotransformation?
YES
NO
Is there required information on the ADME profile of the active substance(s)/ toxicologically relevant constituent(s) of the feed ingredient available?
YES
NO
Can ADME be addressed/complemented using the weight of evidence approach? (QSAR, read across, others)
YES
NO
Can ADME be addressed/complemented using In vitro testing
YES
NO
ADME in vivo studies required for the safety assessment for consumer

ANNEX II – *IN-SILICO* MODELS

Table 1 provides summary information available at the time of the publication of this guidance document. Further models may be developed and used at the time of the preparation of the submission package.

Table 1 – Freely available databases for toxicological, physical, and chemical, and other relevant information for risk assessment

Database	Website details and further information
AMBIT	http://cefic-lri.org/ambit/ Developed by the European Chemical Industry Council's Long-Range Initiative (cefic-lri). It contains information on > 450.000 chemicals including the European Chemical Agency's (ECHA's) REACH data
Chemspider	http://www.chemspider.com/ Developed by the Royal Society of Chemistry, it provides information on over 83 million chemicals, using 275 data sources: includes direct links to other relevant sources
ChemIDplus	https://chem.nlm.nih.gov/chemidplus/ Developed by the US National Library of Medicine: contains information relating to > 300.000 chemical structures including physico-chemical property and toxicity data
Computational Toxicology Dashboard	https://comptox.epa.gov/dashboard Hosted by the US Environmental Protection Agency (US EPA): a repository of data currently for 875.000 chemicals: links out to additional data sources: integrates data e.g. from ToxCast/Tox21 high-throughput screening initiatives.
eChemPortal	http://www.echemportal.org Developed in collaboration with the Organisation for Economic Cooperation and Development (Organisation for Economic Co-operation and Development (OECD)), provides links to information prepared for governmental chemical reviews at national and international levels, including submissions to the European Chemicals Agency (ECHA): provides exposure and use information
EMBL-EBI/ChEMBL	https://www.ebi.ac.uk/ https://www.ebi.ac.uk/chemb/ European Molecular Biology Laboratory's European Bioinformatics Institute (EMBL-EBI): source of biological and biomolecular data incorporating the ChEMBL

	database of bioactive molecules with druglike properties (>15 million values from >1.8 million chemicals)
OCHEM	https://lochem.eu/home/show.da Online chemical database with modelling environment: 2.9 million records for over 600 properties, based on the wiki principle
QSAR Toolbox	https://www.qsartoolbox.org/ Developed to help fill data gaps in (eco)toxicity data: version 4.4 contains 57 databases with 2.6 million data points for 92.134 chemicals
PubChem	https://pubchem.ncbi.nlm.nih.gov/ Open chemistry database from US National Institutes of Health (NIH) with data on over 102 million chemicals.
OpenFoodTox 2.0	https://www.efsa.europa.eu/en/data-report/chemical-hazards-database-openfoodtox Openfoodtox is a compilation of chemical and toxicological information on chemicals assessed by EFSA since its creation and included in already published scientific opinions. The database represents the data that was available to EFSA at the time of assessment and does not provide any reassurance on whether any of the chemicals are suitable or not for food applications in Europe. EFSA owns this database and its content

Source: adapted from Madden et al. 2020

PK-SIM: PK-SIM is a publicly available tool. https://www.open-systems-pharmacology.org/. This tool is using mathematical models for studying systems. The model used aggregates and integrates existing knowledge with an aim to systematically analyse systems behaviour, test, generate hypothesis and plan experimental next steps, as appropriate. The approach taken by PK-SIM is selected and biased, as it focused on organisms and topics of broader relevance in pharmaceutical research and development, i.e., systems pharmacology. However, facets of the tool can be used beyond systems pharmacology.

TIMES (tissue metabolism simulator) is a heuristic algorithm used to generate plausible metabolic maps from a comprehensive library of biotransformation and abiotic reactions. The ability of TIMES to predict in the same interface the metabolism of chemicals and toxicity resulting from their metabolic activation is an important advantage of the method. The software is available online and requires a licence fee (http://oasis-lmc.org/products/software.aspx).

OECD toolbox: The OECD toolbox is a software application intended to be used in filling gaps in (eco)toxicity data needed for assessing the hazards of chemicals. The seminal features of the toolbox are:

- Identification of relevant structural characteristics and potential mechanism or mode of action of a target chemical,
- Identification of other chemicals that have the same structural characteristics and/or mechanisms or mode of action,
- Use of existing experimental data to fill the data gap(s). The toolbox is publicly available. (https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm).

ANNEX III – *IN-VITRO* STUDIES

This Annex provides summary information for well-established *in-vitro* ADME studies at the time of the publication of this guidance document. Further studies may be developed and used at the time of the preparation of the submission package.

Absorption studies

In-vitro digestion models simulate the conditions of the gastrointestinal tract in livestock (ruminants, non-ruminants) by adjusting the ionic strength and pH, as well as addition of enzymes, bile salts, mechanical stresses, and even fermentation reactions to simulate colon (hindgut) conditions. It includes simulated gastric fluid and simulated intestinal fluid studies.

Caco-2 permeability assays for membrane transfer: *In-vitro* Caco-2 permeability assays provide a measure of the permeability of a substance across the intestinal barrier and its potential for interactions with transporters:

- The Caco-2 cell line is derived from a human colon carcinoma. The cells have characteristics that resemble intestinal epithelial cells such as the formation of a polarised monolayer, well defined brush border on the apical surface and intercellular junctions.
- These cells differentiate spontaneously after 14-21 days of incubation in a culture medium. The cell monolayer divides the apical and basolateral sides of absorption. With Hank's balanced salt solution (HBSS) as the carrier fluid, the analyte is introduced onto the apical side, and the absorbed moiety is collected on the basolateral side at the desired time intervals. Percent of analyte that is absorbed through the Caco-2 cells is determined by analytical methods.

Distribution studies

Blood/plasma stability: Blood/plasma stability assay measures the stability of molecules in mouse, rat, and human (and/or other species) blood/plasma. The molecule to be evaluated and controls (positive and negative) are incubated with blood/plasma for a defined period and the percent remaining and half-life determined.

Protein binding assays are used to measure distribution in tissue. Binding is evaluated by equilibrium dialysis and ultrafiltration methods, which determine the proportion or percentage of the substance that is bound to proteins and free in solution, as generally only the unbound substance is available for passive diffusion to extravascular or tissue sites. It is therefore an important factor for the efficacy of a substance. Several different binding tests are recognised

and used based on specific requirements, e.g. brain tissue binding, plasma protein binding, whole blood binding, microsomal binding, blood to plasma ratio.

Metabolism studies

Measuring the metabolic profile of a molecule *in-vitro* gives an estimate concerning its stability and thus elimination rate by metabolism in the body. The liver is the most important site of metabolism in the body. Therefore, hepatic clearance is a critical parameter for the assessment of the metabolic stability. In *vitro* metabolism systems include primary hepatocytes, liver microsomes; liver slices, S9 sub cellular fraction, cytosol, whole cell lines, recombinant enzymes, or extrahepatic tissues, as described below.

Microsomes and hepatic assays for comparable metabolism: Microsomes and hepatic assays aims to evaluate the metabolic stability of a chemical substance and aims at predicting the pharmacokinetic parameters underpinning the use of the substance. Microsomes are typically used as the enzyme source for the measurement of metabolic stability.

Primary (fresh or cryopreserved) hepatocytes contain functional biochemical pathways typical of the liver. Hence, the primary cultures of hepatocytes carry enzymes and cofactors at physiological concentrations and closely mimic the moiety metabolism *in-vivo.*

Liver microsomes are subcellular fractions that are useful to model hepatic clearance *in-vitro*. They contain many of the metabolizing enzymes found in the liver.

S9 sub-cellular fraction consist of both microsomal and cytosolic enzymes that help understanding the metabolism of chemical moiety *in-vivo*. The system may be supplemented with co-factors such as Uridine Diphosphate Glucuronic Acid and 3'-PhosphoAdenosine-5'-PhosphoSulfate Phase II metabolic pathways, such as N-acetylation, methylation, cysteine and glucuronidation binding.

Cytosol is used to identify the soluble enzymes involved in metabolic pathways of chemical moiety. The assays complement microsomal studies for assessing chemical moiety metabolism pathways.

Other methods such as the liver slices and the whole cell lines may also be used.

ANNEX IV- *IN-VIVO* STUDIES

The following guidance documents may be used when designing a ADME *in-vivo* study:

- OECD Guidelines for the testing of chemicals. Section 4: Health effects. Test No. 417: Toxicokinetics.
- VICH Guidelines No. 46 Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food producing animals: metabolism study to determine quantity and identify the nature of residues.
  EFSA Guidance Document on the safety of feed additives for the consumers, European Food Safety Authority (EFSA) Journal 2017;15(10):5022 www.efsa.europa.eu/efsajournal
- Guidance For Industry #205. Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-producing Animals: Metabolism Study to Determine the Quantity and Identify the Nature of Residues (MRK).

Parameters	OECD	VICH	EFSA	FDA
Types of animals	Young adult laboratory animals (usually, rats) 6-10 weeks Weight +/- 20%	Target animal (representative) - swine - sheep - poultry (laying hens for evaluation egg concentration) - beef or dairy (dairy necessary for evaluation milk concentration)	Target animal	Target animal (representative) - swine (40-80kg) - sheep (40-60 kg) - poultry (laying hens for evaluation egg concentration) - beef (250-400 kg) or dairy (dairy necessary for evaluating milk concentration)
Housing	Individual housing	Individual housing	Individual housing	Individual housing

Parameters	OECD	VICH	EFSA	FDA
Number of animals	≥ 4 animals per dose (one sex)	≥ 3 animals per euthanasia time (depending on withdrawal period) ≥ 8 cows with different milk production Laying hens to allow for the collection of at least 10 eggs	≥ 3 animals	≥ 3 animals per euthanasia time (depending on withdrawal period) ≥ 8 cows with different milk production Laying hens to allow for the collection of at least 10 eggs
Dose	Highest dose from toxicology and a fraction of this dose (2 treatments)	Intended maximum dose used (steady state)	Highest proposed dose (single dose)	Intended maximum dose used (steady state)