Assessment of veterinary drug residues in food: Considerations when dealing with sub-optimal data

Alan Chicoine, Holly Erdely, Vittorio Fattori, Anke Finnah, Samuel Fletcher, Markus Lipp, Pascal Sanders, Stefan Scheid

Regulatory Toxicology and Pharmacology 118 (2020) 104806

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Contemporary Review

Assessment of veterinary drug residues in food: Considerations when dealing with sub-optimal data

Alan Chicoine [a,*], Holly Erdely [b], Vittorio Fattori [c], Anke Finnah [d], Samuel Fletcher [e], Markus Lipp [c], Pascal Sanders [f], Stefan Scheid [d]

[a] Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada
[b] U.S. Food and Drug Administration, Center for Veterinary Medicine, Rockville, USA
[c] Food Safety and Quality Unit, Food and Agricultural Organization of the United Nations (FAO), Rome, Italy
[d] German Federal Office of Consumer Protection and Food Safety (BVL), Berlin, Germany
[e] Veterinary Medicines Directorate, United Kingdom
[f] French Agency for Food, Environmental and Occupational Health and Safety, Fougeres, France

ARTICLE INFO

Keywords:
Veterinary drug
Drug residue
Risk assessment
Maximum residue limit (MRL)
Codex alimentarius commission
Joint FAO/WHO Expert committee on food additives (JECFA)
Acceptable daily intake (ADI)
Acute reference dose (ARfD)

ABSTRACT

The use of veterinary drugs in food-producing animals may lead to residues in animal-derived foodstuffs, potentially posing a risk to human safety. While the process of veterinary drug residue risk assessment continues to evolve as new data emerges, a recurring challenge is when sub-optimal or incomplete data are provided with the expectation of supporting a robust risk assessment. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) is comprised of international experts who routinely deal with such data challenges when performing veterinary drug residue evaluations. Recent developments in veterinary drug residue risk assessment are described, including specific consequences of sub-optimal data during the risk assessment process. When feasible, practical solutions to such challenges are also highlighted. Case examples from recent JECFA veterinary drug evaluations are provided to clearly quantify and illustrate the concepts described. The information provided is intended to facilitate the generation of improved quality data, enabling more timely and robust veterinary drug residue risk assessments.

1. Background

The use of veterinary drugs in food-producing animals may result in drug residues in foodstuffs such as meat, milk, eggs, or honey. A robust risk assessment is therefore necessary to ensure that such residues are not present at concentrations posing a risk to human health. Performing such a risk assessment requires substantial toxicological, microbiological, metabolic, pharmacokinetic, and residue depletion data. The objective of this review is to describe the consequences of, and potential solutions for, sub-optimal data when performing international risk assessments of veterinary drug residues, with a specific focus on challenges in residue assessment.

The Codex Alimentarius Commission (CAC) sets maximum residue limits (MRLs) for residues of veterinary drugs in foods. An MRL is the maximum concentration of a specific substance (known as a marker residue, MR) that is allowable in animal-derived foodstuffs. MRLs are a risk management tool designed to ensure consumer safety and facilitate international trade. Consumption of veterinary drug residues at or below MRL concentrations ensures that subsequent human exposure will not exceed specified levels of concern (Health-Based Guidance Values, HBGV). MRLs are also established with consideration of Good Veterinary Practice (GVP), such that foodstuffs derived from treated animals are not harvested before an appropriate time has elapsed since treatment (known as a withdrawal period). Finally, by providing a standardized, recognized limit for veterinary drug residues, MRLs facilitate international trade in animal-derived foodstuffs and minimize potential non-tariff trade barriers.

The CAC establishes MRL values based principally on the risk assessment performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Veterinary drug compounds to be considered for JECFA assessment are nominated by members of the Codex Committee on Veterinary Drug Residues in Food (CCRVDF) and must be registered for veterinary use in at least one member state. Summaries of the risk assessments performed by the JECFA experts are published in joint Technical Report Series, and are available at https://www.who.int

* Corresponding author. Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon, SK, S7N 5B4, Canada.
E-mail address: al.chicoine@usask.ca (A. Chicoine).

https://doi.org/10.1016/j.yrtph.2020.104806
Received 29 July 2020; Received in revised form 11 September 2020; Accepted 9 October 2020
Available online 12 October 2020

Abbreviations:

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
ARfD	acute reference dose
CAC	Codex Alimentarius Commission
CCRVDF	Codex Committee on Veterinary Drug Residues in Food
FAO	United Nations Food and Agricultural Organization
HBGV	health-based guidance balue
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MR	marker residue
MRL	maximum residue limit
M	Tratio of marker residue to total residue
TR	total residues
VICH	International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products
WHO	United Nations World Health Organization
95/95 UTL	upper limit of the one-sided 95% confidence interval over the 95th percentile of marker residue concentrations

/foodsafety/publications/jecfa-reports/en/. Complete residue and toxicological monographs are available from the FAO and WHO, respectively at http://www.fao.org/food-safety/resources/publications/en/and https://www.who.int/foodsafety/publications/monographs/en/.

The JECFA process of risk assessment for veterinary drug residues consists of hazard identification & characterization, exposure assessment, and risk characterization (FAO/WHO, 2009b). An overview of the data requirements and key outputs required to characterize risks of veterinary drug residues are summarized in Fig. 1. For a comprehensive discussion of the JECFA risk assessment process, readers are encouraged to consult Principles and methods for the risk assessment of chemicals in food (EHC 240, 2009).

Step 1 Hazard characterization of the parent compound and its metabolites present in animal-derived foodstuffs is performed, in order to establish a quantitative HBGV representing the maximum safe exposure to veterinary drug residues. The HBGV is based on the most sensitive toxicological or microbiological endpoint, and considers chronic and acute exposure scenarios (Acceptable Daily Intake, ADI; and Acute Reference Dose, ARfD, respectively). HBGVs are derived from the evaluation of multiple studies designed to generate toxicological, microbiological, and/or pharmacokinetic (ADME) data, including in vitro or in vivo (laboratory animals and/or human) studies. Although hazard characterization is integral to a subsequent residue evaluation, a complete review of the study requirements necessary to derive HBGVs is outside the scope of this manuscript. For further information the reader is encouraged to consult relevant guidance documents published by JECFA (Boobis et al., 2017; FAO/WHO, 2009a) and other international agencies. See International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) guidelines for toxicological (22, 23, 28, 31, 32, 33, 37, 54) and microbiological (36r2) studies at https://vichsec.org/en/guidelines/pharmaceuticals/pharma-safety/toxicology.html andhttps://vichsec.org/en/guidelines/pharmaceuticals/pharma-safety/antimicrobial-safety.html.

Step 2 To characterize the residues in food after administration of the veterinary drug, metabolism studies are performed in the target (food) animal species. These studies are typically performed using a radiolabeled drug compound (not the final drug formulation to be marketed).

- The total residues (TR) in edible tissues are determined based on total radioactivity, and individual residue components (parent + metabolites) are quantified (typically via HPLC-MS/MS or radiometric profiling techniques).
- The major residue components (≥10% of total radioactive residues) should be identified (i.e., structure determined). The individual components may also be characterized based on toxicological or microbiological potency, if known (i.e., potency relative to parent compound).
- The drug's metabolic profile is qualitatively compared between laboratory and target food animal species, to ensure that no additional metabolites of concern are present in food animal tissues.
- A marker residue (MR) is determined for regulatory monitoring purposes. The MR should be suitable for monitoring purposes (e.g., stable in the matrix, quantifiable with readily available analytical technology) and be representative of the TR concentration. For most veterinary drugs, the MR will be the parent compound, a metabolite, or some combination thereof.
- Marker:total (M:T) residue ratios are determined over time since final treatment in each edible tissue. In some cases, certain components of the TR do not pose a risk to human safety. This may be due to residue components which are biologically inactive (e.g., inactive drug metabolites), or that are not bioaccessible or bioavailable in the human gastrointestinal tract (e.g., bound tissue residues)(Boobis et al., 2017). In such cases, a "residue of concern" (excluding the inactive or bound components) is defined in lieu of "total residues"(FAO/WHO, 2018b). In many evaluations significant amounts of data are required to adequately characterize the M:T residue ratios, as ratios vary between edible tissues and within the same tissue over time since final treatment.

Step 3 To predict potential real-world human exposure to veterinary drug residues, a non-radiolabeled MR depletion study is performed in a larger number of animals using the final product formulation according to proposed label indications.

- Exponential regression is used to estimate the rate of MR depletion in each tissue. The median MR concentrations are determined over time since last drug administration.
- The median TR (or residue of concern) concentration is then estimated over time, based on the median MR concentration divided by the appropriate M:T ratio (from radiolabeled studies) for that specific tissue/time point.
- The combined exposure to the drug residue is predicted (Global Estimated Acute/Chronic Dietary Exposure). This is based on TR concentrations in edible tissues at various time points from the last drug administration (consistent with the drug product's range of approved withdrawal times in various Member States), multiplied by estimates of acute and chronic human food consumption for each edible tissue (e.g., Global Estimated Acute/Chronic Dietary Intake). [2]
- Predicted human exposures to drug residues at such withdrawal times are compared to the relevant HBGVs (ADI and/or ARfD). If the combined exposure estimates are lower than the relevant HBGVs (i.e., safe for human consumption), potential MRLs for tissues can be calculated.
- The MRL is derived based on the upper limit of the one-sided 95% confidence interval over the 95th percentile of marker residue concentrations (95/95 UTL). Proposed MRLs must be suitably health-protective and considerate of international trade implications. In uncommon cases, exposure estimates derived from the range of approved withdrawal times may be higher than the HBGVs. In this situation, either JECFA will

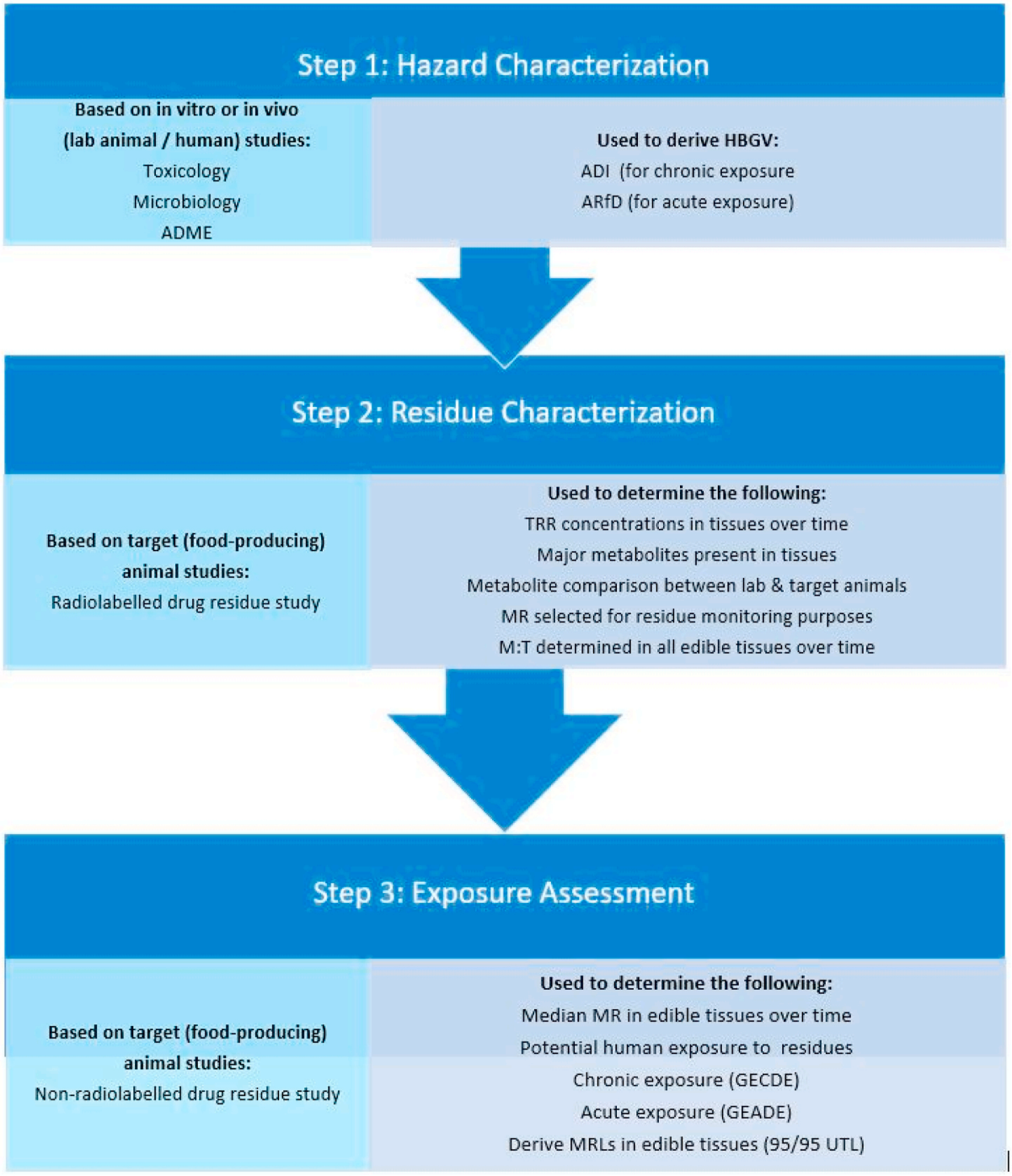

Fig. 1. Summary of steps involved in characterizing risks of veterinary drug residues.

note that Good Veterinary Practices (withdrawal periods) require updating, or MRLs cannot be established.

2. Data requirements and impacts on veterinary drug residue risk assessment

Although each regulatory agency has its own specific requirements and processes for the human safety evaluation of veterinary drug residues in food, a common framework is available for designing the studies that generate the necessary data. In addition to the previously cited JECFA and VICH guidelines for generating toxicological and microbiological data, guidance is also available for conducting studies necessary to perform the residue evaluation (such as metabolism and residue depletion studies). For further information see VICH guidelines #46–49, 56, 57 at https://vichsec.org/en/guidelines/pharmaceuticals/pharma-safety/metabolism-and-residue-kinetics.html (FAO/WHO, 2009a);). Concepts described in international guidance documents help inform risk assessments performed by JECFA and other regulatory agencies.

To evaluate a specific veterinary drug, JECFA requests and expects all relevant data be made available by the sponsor (drug manufacturer and/or Codex member state) but also calls worldwide for any additional relevant data from other sources. Robust risk assessments require data generated from a variety of well-designed in vitro and in vivo studies. However, an "ideal" data set is rarely available in the real world. Sub-

optimal study design, bioanalytical limitations, lack of investment in data generation, and differing regulatory requirements between jurisdictions can result in data "gaps" which impede the risk assessment process.

In some instances, such deficiencies preclude completion of the risk assessment and recommendation of suitable MRLs until suitable data are generated. However, the lack of a completed risk assessment also has significant consequences. Establishment of new Codex MRLs for veterinary drugs is a prolonged process even where there is an 'ideal' data set. There are ramifications when the JECFA risk assessment (and subsequent Codex MRL derivation) are delayed by provision of sub-optimal data. International trade in animal-derived foodstuffs may be impeded due to lack of Codex MRLs. Veterinary drug approval in some countries may also be delayed, as jurisdictions with limited capacities for conducting independent veterinary drug residue risk assessment strongly rely on JECFA's risk assessment and Codex MRLs. This may deprive food-producing animals of newer safe and effective medications in these countries, including analgesics and preventative medicines integral for animal welfare and production efficiency. Veterinarians and producers may continue to rely on older (but approved) products, which may be less safe or effective. If no approved products are available, the use of unapproved formulations of unknown quality (and potentially more significant human safety risks) may occur.

Therefore, JECFA attempts to perform risk assessments for compounds nominated by CCRVDF, even in cases where the assessment is hindered by significant data gaps or flaws. This can raise questions, like: How can JECFA perform an appropriate risk assessment if based on "sub-optimal" data? Can such an assessment fulfill JECFA's critical mandate of protecting consumers from risks of veterinary drug residues in food? And what are potential consequences for a veterinary drug sponsor if comprehensive data are not provided? This review outlines potential challenges faced by JECFA when performing veterinary drug residue risk assessments with sub-optimal datasets, potential strategies considered by JECFA in such cases, and the implications of these approaches on the overall risk assessment. The objective is to provide readers with a better understanding of the consequences of sub-optimal data when performing veterinary drug residue risk assessments, and to discuss practical and feasible solutions.

3. Challenges and approaches for dealing with sub-optimal data

3.1. Hazard characterization challenges

When performing hazard characterization of veterinary drug residues, sub-optimal toxicological or microbiological data can significantly impact derivation of HBGVs. In some cases, a lack of critical toxicological or microbiological data makes completing the hazard characterization impossible, which precludes the remainder of the risk assessment (e.g., if no ADI/ARfD can be established, MRL derivation cannot proceed). Examples of some hazard characterization challenges encountered by JECFA due to data deficiencies are presented in Table 1.

In some situations, hazard characterization challenges can be mitigated through a variety of strategies to facilitate HBGV derivation and continue the residue assessment. Such strategies may include using more conservative uncertainty (i.e., safety) factors, or using surrogate values based on Thresholds of Toxicological Concern (TTCs) or quantitative structure-activity relationship (QSAR) models. However, because JECFA closely integrates the hazard characterization and residue assessment processes, strategies to address hazard characterization deficiencies may impact the residue assessment (such as derivation of more conservative MRLs than necessary had a complete toxicological/microbiological data set been available). A detailed description of the specific impacts of such deficiencies on hazard characterization is outside the scope of this manuscript; the reader is encouraged to consult relevant guidance documents published by JECFA and other international organizations such as VICH(Boobis et al., 2017; FAO/WHO, 2009a).

Table 1
Examples of data deficiencies in recent JECFA hazard characterizations.

Type of data requirement	Deficiency	Impact on hazard characterization	Example JECFA evaluations
Toxicological	Carcinogenic or mutagenic potential of compound	Toxicological ADI cannot be derived	Halquinol (FAO/WHO, 2018a) Diflubenzuron (FAO/WHO, 2016) Xylazine (FAO/WHO, 1998a)
Microbiological	Colonization barrier disruption Resistance development studies	Microbiological ADI cannot be derived	Fosfomycin (FAO/WHO, 2020a)

3.2. Residue assessment challenges

Recent JECFA evaluations of both TR (typically using radiolabeled drug) and MR (non-radiolabeled drug) studies have identified a variety of data gaps or challenges. A brief summary is presented in Table 2 with further explanation in the following text.

3.2.1. ***Residue assessment challenge #1:*** *Issues arising from radiolabeled drug studies*

Performing veterinary drug residue assessments requires sufficient knowledge of the drug's metabolic profile in both the target and laboratory animal species. Such data may be missing or incomplete, often because the studies required to generate metabolite data (typically a radiolabeled drug study) are costly, technically challenging, and require a long preparation time. Production of radiolabeled drug requires careful determination of the exact position of sometimes multiple radiolabels. Enriching the labelled drug purity for sufficient radioactivity levels required to quantify and qualify minor metabolites is a technically challenging process, with inherent limitations of purification. Animals for these studies must be housed in a fully contained environment, and drug administration and animal care requires additional occupational exposure considerations. Finally, all waste and biological materials are radioactive and require appropriate disposal. Alternative approaches (i.e., not using radiolabeled drug) to characterize the components of residues in food derived from treated animals might be suitable, but use of such methods is technically and analytically challenging and has not been validated.

3.2.1.1. ***Scenario 1A)*** *Inability to estimate total residue concentrations in tissues.* In cases where only a limited component of the TR (typically the putative MR or parent drug) has been quantified in edible tissues, but TR concentrations in the same tissues are unknown, no M:T can be derived. This can occur when the only data available for the assessment are non-radiolabeled residue depletion data, and the sponsor has not provided any TR (radiolabeled) studies. The resulting assessment cannot adequately quantify the total exposure, as only concentrations of the putative marker residue or parent compound (but not all metabolites) are known. Furthermore, the nature of the hazard cannot be adequately determined due to the potential presence of metabolites with different toxicological profiles than the parent compound (see 1C). Without adequate knowledge of the metabolite profile in the target animal, the toxicological risk of the TR cannot be characterized.

- Example: In the JECFA assessment of the organophosphate insecticide ethion for use in cattle, the submitted residue depletion studies only measured parent ethion but not the active monoxon metabolite. "The Committee noted that the lack of qualitative or quantitative metabolite data is a major omission, and must be addressed before

Table 2
Examples of data deficiencies in recent JECFA residue assessments.

Study	Scenario	Deficiency	Impact on residue assessment	Example JECFA evaluations
Total residue[a]	1A	No radiolabeled study performed	Cannot quantify TR concentrations in tissues	Ethion (FAO/WHO, 2018a)
	1B	No radiolabeled study performed Insufficient radiolabel dose/activity Insufficient duration of depletion study	Cannot derive M:T in tissues	Halquinol (FAO/WHO, 2018a; FAO/WHO, 2020b) Flumethrin (FAO/WHO, 2020a; FAO/WHO, 2020b)
	1C	Difference in metabolites between lab and target animal species Insufficient radiolabel dose/activity Structure/identity of residues not determined	Metabolite comparison and characterization incomplete	Xylazine (FAO/WHO, 1998a) Flumethrin (FAO/WHO, 2020a) Halquinol (FAO/WHO, 2018a; FAO/WHO, 2020b)
	1D	No radiolabeled data in both species Low M:T in one/both species	Extrapolation of M:T data between species	Fig. 2
Marker residue[b]	2	Lack of residue studies with relevant drug dose regimens, routes of administration, or usage conditions	Residue study not representative of full range of drug uses	Ampicillin (FAO/WHO, 2018a) Lufenuron (FAO/WHO, 2018a) Ethion (FAO/WHO, 2018a)
Any	3	UTL derivation Food consumption estimates	Errors in data presentation or different methods of data analysis	(Boobis et al., 2017; FAO/WHO, 2009a)

[a] Typically performed with radiolabeled drug compound.
[b] Typically performed with non-radiolabeled drug (final drug formulation).

any MRLs can be determined for this substance. The toxicological assessment revealed that at least one metabolite (ethion monoxon) retains significant anticholinesterase activity, and therefore must be accounted for in the residue assessment. In addition, the available data did not identify all the metabolites of concern that lead to the identified reproductive toxicity … To estimate the toxicological activity of the total ethion residues (including metabolites), knowledge of the M:T over time will be required. As such data are not currently available, an accurate assessment of the total toxicological activity of ethion residues (and subsequent residue exposure assessment) cannot be performed." (FAO/WHO, 2018a) Without the relevant TR data, residue depletion studies detecting only the proposed MR (parent ethion) are insufficient for assessing overall exposure to residues of toxicological concern. Therefore no MRLs can be derived based on the proposed MR until evidence is generated demonstrating that commensurate exposure to TR remains below the HBGV.

3.2.1.2. ***Scenario 1B)*** *Inability to estimate M:T residue concentrations in tissues.* Even if a TR (radiolabeled) study has been performed in the target species, subsequent estimation of M:T in tissues is not always possible. Poor quality radiolabeled studies exacerbate uncertainty of the quantitative data, diminish confidence in the derived M:T estimates, and result in insufficiently robust risk characterizations. The study provided may not utilize a sufficient dose or specific activity of radiolabeled compound, may not be conducted for a sufficient duration, or use slaughter time points which are not appropriate for determining relevant M:T.

- Example: The initial JECFA assessment of halquinol (FAO/WHO, 2018a) noted that the radiolabeled data were insufficient to derive a robust M:T in muscle and skin/fat of pigs. Due to the limited extractability of radioactive residues, and very low overall tissue radioactivity, only one sample at one time point (first slaughter point at 3 h post dose) had quantifiable residues. Subsequently provided radiolabeled halquinol studies utilized considerably higher radiolabel specific activity, larger doses, and more rigorous extraction techniques (FAO/WHO, 2020b). The resulting total radioactive residues were quantifiable in tissue samples from more pigs, and for a longer duration, than the previous radiolabeled study. The substantial increase in total halquinol residue data allowed for derivation of more robust M:T estimates in these tissues, ultimately facilitating the derivation of MRLs.
- Example: When JECFA evaluated flumethrin for use in cattle, the provided radiolabeled data were designed primarily to elucidate metabolic pathways (i.e., not designed as a residue depletion study). The M:T ratios were therefore determined at a very short duration (<24 h) after final drug administration, and M:T ratios could not be determined at later timepoints relevant for assessing potential human exposure (i.e., at label withdrawal periods up to 21 days) (FAO/WHO, 2020a; FAO/WHO, 2020b).

3.2.1.3. ***Scenario 1C:*** *Radiolabeled study design not suitable for characterization of metabolites.* Performance of a TR (radiolabeled) study in the target animal species does not guarantee data of sufficient quality to adequately characterize the metabolites produced, especially in cases where the ADI is based on toxicological endpoints derived from laboratory animal species. For the lab-animal derived ADI to be applicable to residues present in tissues of food-producing animals, the metabolic profile of the drug in the target (food) animal species must be demonstrated as comparable to the laboratory species (see VICH guidelines 46 and 47, respectively). For example, if a target species produces a certain drug metabolite not produced in laboratory animals, and this metabolite is significantly more toxic than the parent compound or other metabolites produced in laboratory animals, the toxicological ADI derived from lab animal studies may underestimate the residue hazard. Furthermore, if the metabolite produced in the target species elicits a different toxicological effect or mode of action (e.g. the metabolite is a genotoxic carcinogen) than other metabolites, the endpoint selected based on laboratory animal studies may not be appropriate. Therefore the presence of particularly toxic metabolites in edible tissues must be ruled out (or confirmed to occur at levels below a safe threshold), or else the ADI derived from laboratory animal studies may not be sufficiently conservative.

- Example: Administration of the sedative xylazine in cattle produces the genotoxic and carcinogenic metabolite 2,6-xylidine (2,6-dimethylaniline). However, data available from limited xylazine metabolism studies in rats did not indicate substantial production of 2,6-xylidine. The metabolic characterization of xylazine was considered inadequate, and contributed to an inability to derive MRLs in cattle (FAO/WHO, 1998a).

A robust comparison of drug metabolite profiles between laboratory and food animals requires adequate metabolite characterization in both species. In other words, simply determining the total radioactive residues (e.g., scintillation counting) in tissues is insufficient. The residue components must be identified (typically via HPLC) and the structure(s) of major metabolites characterized (via MS) so that potential

toxicological and/or microbiological natures of the metabolites can be assessed.

- Example: In the aforementioned flumethrin evaluation [6], although radiolabeled data were provided indicating initial metabolite formation, critical moieties of the molecule lacked labeling. Therefore complete elucidation of the metabolite pathways was not possible as any metabolites comprised of the non-radiolabeled moiety would not be quantified or characterized. A suitable marker residue could not be confirmed until all relevant flumethrin metabolites are assessed.
- Example: In JECFA's initial halquinol assessment (FAO/WHO, 2018a), the limited quantity and characterization of extractable metabolites in liver and kidney precluded a robust risk characterization and validation of the proposed marker residue (a mixture of parent halquinol and specific metabolites). Subsequent radiolabel studies, using more vigorous extraction techniques and more detailed chromatographic separation techniques, identified previously-unreported glucose conjugates of halquinol components. The more rigorous metabolite characterization helped lead to recommendations for both a marker residue and MRLs for halquinol (FAO/WHO, 2020b).

3.2.1.4. ***Scenario 1D)*** *Extrapolation of M:T data between species.* Extrapolation of known M:T data from one food-producing species to another may not be appropriate if differences in metabolic pathways exist. This may be encountered during extension of MRLs to other species, or when considering MRLs in pre-ruminating (veal) calves based on metabolism studies in older, ruminating cattle. In practice this is not typically a significant limitation as drug metabolism pathways are often similar between related mammalian species, and comparable M:T ratios are expected (VICH 46). From a quantitative perspective, if the M:T ratio is high (i.e., close to 1.0), small differences in metabolite profiles between species minimally impact the M:T. The risk due to such M:T differences is mitigated by the otherwise conservative nature of drug residue risk assessment (e.g., ADI derivation safety factors, excessive food consumption estimates, etc.) However, if the known M:T is much

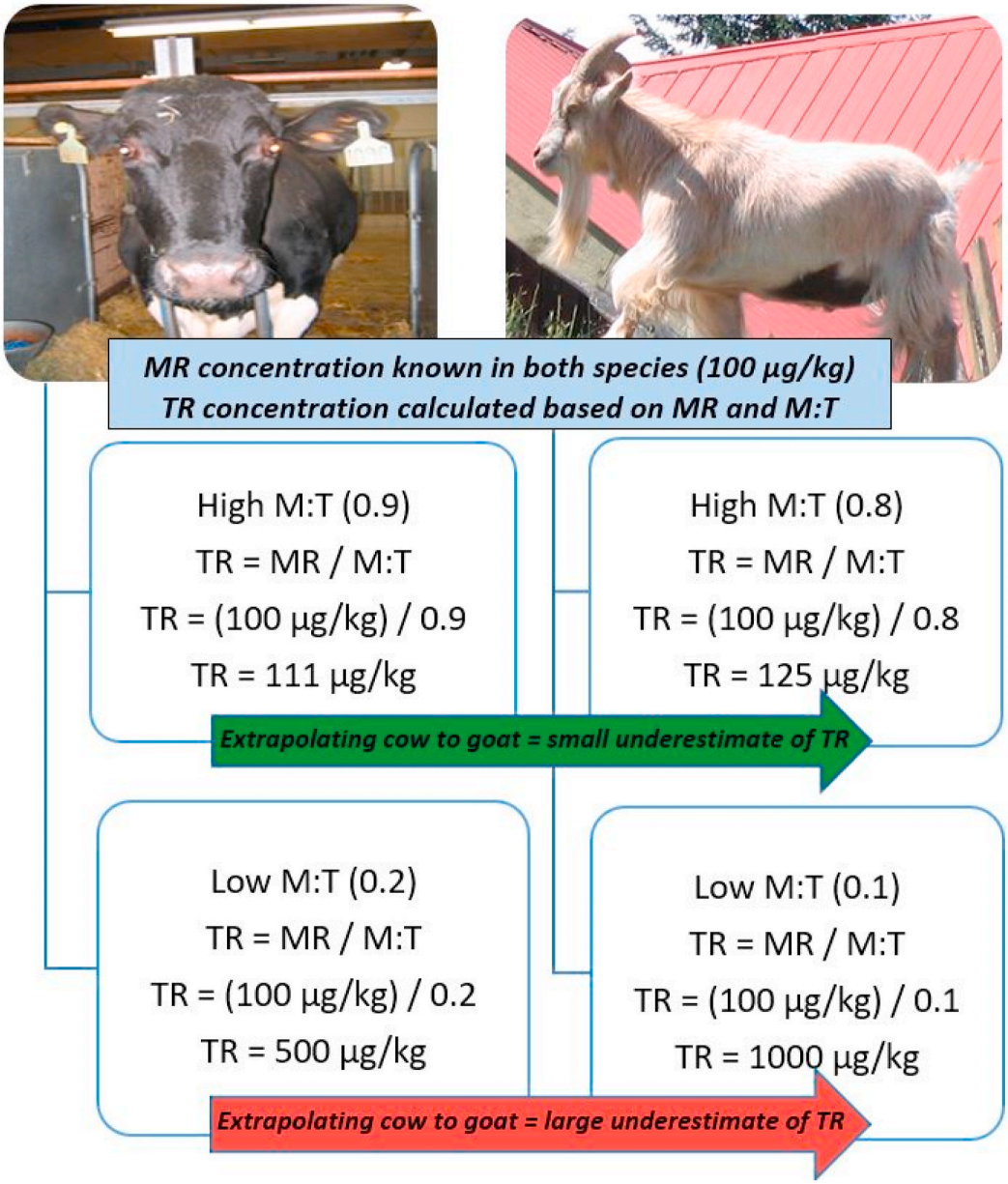

Fig. 2. Impact of differences in M:T residue ratios between species on veterinary drug residue risk assessment.

lower, variance in M:T between species has a proportionately greater effect on the overall risk assessment (Fig. 2). In such cases, extrapolation of M:T to another species could significantly underestimate the actual TR concentrations.

- Example: Consider two species in Fig. 2, with the same MR concentrations in tissue (100 μg/kg), but where the M:T is known for one species (cattle) and not the other (goats). If the known M:T is high, any likely differences in M:T are unlikely to have a significant impact on predicted TR concentrations. For example, if goats had a slightly lower M:T (due to more extensive metabolism) than cows, but the cow M:T is applied for goats, the TR estimate in goat tissues will be only slightly underestimated. Even without knowing the precise goat M:T in goats, extrapolation of MRLs from cattle to goat tissues would pose minimal risk in this case. But if the known M:T is low (e.g., extensive metabolism and thus the MR comprises only a small proportion TR), estimated total residues in tissue will be much greater than MR concentrations. If the cattle M:T is extrapolated to goats, even a small difference in actual M:T may lead to a significant underestimation of the actual TR present in goat tissues. Extrapolation of MRLs from cattle to goat tissues may not be appropriate in this case unless the actual M:T in goat tissues are verified.

Because radiolabeled studies are typically performed with small sample sizes (as few as 3 animals per slaughter time (as per VICH 46)), it can be difficult to adequately assess M:T variance within and between species.

3.3. Potential solutions for challenges related to total residue or metabolite data

Depending on the nature of the deficiency, the assessment may still be performed should one of the following conditions be met:

- If the parent compound (MR) is excreted mostly unchanged (i.e., little or no metabolism occurring), the MR will comprise most or all of the TR (e.g., M:T is ≥ 0.9). Determination of the MR concentration in tissues will be sufficient (even without a TR study), as differences between MR and TR concentrations will be insignificant. Examples of such drugs include oxytetracycline and chlortetracycline (FAO/WHO, 1990).
- If metabolites are known to have limited toxicological or microbiological significance, and the parent compound is the MR, then the "total residue" is not relevant for the risk characterization. Only the "total residue of concern" (i.e., MR) need be quantified, and the metabolite portion of the TR can be discounted from the exposure assessment. For example, because metabolites of amoxicillin are microbiologically inactive, and the ADI for amoxicillin is based on microbiological endpoints, the metabolic characterization of amoxicillin in finfish was not necessary to derive MRLs in these species (FAO/WHO, 2020a).
- If the HBGV derived from lab animal studies is sufficiently high (i.e., the drug is considered of low toxicity), or the MR concentrations in edible tissues are very low, an accurate estimation of M:T may not be necessary to perform the risk assessment. If even a very conservative (i.e., low) M:T corresponds with TR exposure significantly below the ADI, refinement of the M:T will not significantly alter the risk characterization. However, this requires sufficient confidence in the data provided (i.e., minimal uncertainty) to ensure that such conclusions are valid. In reality, this may require full MR and TR data, so that it can be validated that changing initial M:T estimates has minimal impact on the exposure assessment. The approach described here is not applicable if only substandard data are available, such as insufficient information available on metabolite formation or quantities. The M:T must still be derived with a degree of confidence, and the metabolites comprising the TR be properly characterized.
- If the bioanalytical method converts all biologically-active moieties into a common form, the precise composition of the TR is not relevant (i.e., the assay estimates "total residue of concern" by converting all residues into one form). For example, many ceftiofur assays convert all microbiologically-active residues with a functional beta-lactam ring (such as parent ceftiofur and primary metabolite desfuroylceftiofur) into a single component, the marker residue desfuroylceftiofur (FAO/WHO, 1998b).

3.3.1. ***Residue assessment challenge #2***: *Issues concerning marker residue depletion studies*

Issues can arise when JECFA evaluates marker residue depletion data originally generated to support approval under limited conditions of use in only one jurisdiction.

- If the drug is approved in multiple Member States, but the dose, route, or frequency of administration differs dramatically between jurisdictions, the submitted marker residue depletion data may not encompass the entirety of approved use. In such cases, data from multiple residue depletion studies (i.e., residue data used to facilitate approval in the other jurisdictions), may be required for a robust residue exposure assessment.
- In some cases, marker residue depletion data are not universally applicable due to differences in animal husbandry, production practices, and animal genetics between jurisdictions. Examples include differences in milk residue depletion between dairy cattle with differing quantity and quality of milk (Han et al., 2017), or breed differences which can impact residue depletion of some formulations (Chang et al., 2010). For some externally-applied formulations, volatilization of the product and skin permeability of the treated animals may differ between warmer and colder climates, affecting the rate and extent of drug absorption(FDA, 2017). Water temperature can impact residue depletion in fish, with higher absorption and faster elimination in fish when kept in warmer water (see VICH GL57). Multiple studies utilizing different water temperatures may facilitate JECFA's evaluation by encompassing a range of conditions encountered in member states (see JECFA's ampicillin and lufenuron evaluations (FAO/WHO, 2018a)).

3.3.1.1. Possible solutions for incomplete non-radiolabeled residue depletion data. Data gaps or deficiencies in non-radiolabeled residue depletion data (including the bioanalytical method used to quantify marker residues in tissues) are less common than encountered with radiolabeled residue data. This may be due to:

- Non-radiolabeled residue depletion studies in the target species are generally less costly and simpler to perform than radiolabeled studies;
- Technical requirements of marker residue depletion studies (including sample sizes, slaughter times, and tissue sample requirements) and bioanalytical method validation are generally well understood and harmonized internationally (see VICH GL 48 and 49, respectively);
- Although the JECFA MRL derivation approach is distinct from approaches used to establish withdrawal periods, both processes incentivize the generation of robust residue depletion data sets. More robust residue depletion data can result in shorter withdrawal periods in some jurisdictions. For example, North American regulatory agencies derive withdrawal periods for veterinary drugs based on the time at which the 99/95 UTL reaches a safe level (i.e., the WP is determined when the 99th percentile, or slowest-depleting animals, reach the MRL/tolerance). Studies with larger sample sizes generally have narrower 95% confidence intervals, thus a "lower" 99th

percentile and a correspondingly shorter WP. Similarly, for MRLs based upon comparison of acute dietary exposure with the ARfD, JECFA derives the MRL from worst-case, high-concentration residues in tissues (e.g., 95/95 UTL, as opposed to median residue concentrations for estimates of chronic dietary exposure). Larger data sets with narrower confidence intervals lead to relatively lower acute exposure estimates, facilitating MRL derivation.

- In some cases, extrapolation of tissue residues based on differing dose regimens can be attempted. Should residue depletion studies using the intended dose regimen be lacking, other available residue depletion data may be useful in the exposure assessment. For compounds exhibiting non-linear plasma kinetics (e.g., saturation of transporters involved in ADME) within the intended dosage range, separate residue depletion studies are typically required.
- For sponsors with incomplete non-radiolabeled residue data for a compound, such as residue data for only one specific dosage regimen or type of animal, relevant additional data may be available from other sources. This may include data from the published scientific literature, or from pharmaceutical companies with other products containing the same active ingredient but utilizing different formulations or dose regimens. JECFA routinely uses such data (if available) when performing veterinary drug risk assessments (e.g., ethion (FAO/WHO, 2018a),). Note that data obtained from the scientific literature, while often helpful supporting information, may have inherent limitations if used as a primary source for JECFA risk assessments (e.g., lack of raw data necessary for 95/95 UTL modelling, limited method validation). For further discussion, see "General considerations about the use of scientific literature in risk assessment" (FAO/WHO, 2020a).

3.3.2. ***Residue assessment challenge #3:*** *Errors in data presentation or analysis*

3.3.2.1. ***Scenario 3A)*** *Errors or gaps in data presentation.* Obvious typographical or calculation errors have been noted in data submitted, such as transposing digits when reproducing tissue concentrations from raw data to the study summary. Some basic calculation errors are relatively minor, such as using an inappropriately large number of significant figures (ascribing greater precision to the results than is warranted). However, major errors can also occur, such as using the wrong decimal place or incorrect unit conversion, leading to errors of a factor of 10, 100, or 1000.

An isolated error in data presentation is understandable, and is typically easy to resolve. But if otherwise random errors are noted consistently, confidence in the overall data package is reduced and scrutiny is increased. Such errors are particularly problematic if compounding other, more structural problems or deficiencies in the data presentation. For example, JECFA has received data packages comprised primarily of studies performed by 3rd-parties and published in the scientific literature. Such studies do not always adequately describe the drug dosing information, such as injection technique or location. Drug administration in feed requires particular attention, as substantial data are required to accurately determine the dose administered per body weight:

- A thorough characterization of the feed admixture (including an analysis of the medicated feed using a validated assay);
- A thorough description of methods used to determine feed intake by individuals or groups of animals, in order to confirm actual dose consumed;
- The body weight of individuals or groups of animals over the course of the study.

If such detailed dosing information is not present in published literature, evaluators cannot be certain the residue concentrations determined in such studies are applicable to the approved dosage regimens.

3.3.2.2. ***Scenario 3B)*** *Differences in approaches to analyze data.* Sponsors should be aware that some approaches to residue evaluation may differ between JECFA and other regulatory authorities. The same data submitted to a national authority may be analyzed differently when submitted for JECFA evaluation. Differences in data analysis between jurisdictions is generally simple to address, provided the data submitted are organized, detailed, and robust. However, if only a summary of the residue evaluation is presented (e.g., only UTL curves without underlying residue data), JECFA cannot adequately perform its own evaluation. Examples of differences in residue assessment methods between JECFA and some jurisdictions include:

- JECFA utilizes 95/95 UTLs when deriving MRLs, as opposed to 99/95 UTLs used to calculate withdrawal periods in North America. Therefore sponsors should carefully note the percentiles used in UTL calculations. Although different statistical programs can be used to derive 95/95 or 99/95 UTLs, the authors are not aware of substantial differences in outcomes between programs. The JECFA MRL derivation tool is freely available at http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/guidelines0/residue-depletion/en/
- JECFA bases dietary residue exposure estimates on Global Estimated Chronic Dietary Intake values (Arcella et al., 2019; Boobis et al., 2017), rather than the "model food basket" or other food consumption values used by other jurisdictions (FAO/WHO, 2009a).

4. Consequences, conclusions, and ways forward

Although JECFA endeavors to complete veterinary drug residue assessments with the data presented, provision of substandard or incomplete data inherently leads to sub-optimal outcomes. This may include delays or failure in completing the residue assessment (and thus no MRLs derived), or deriving more conservative (lower) MRLs than possible had higher quality data been available. As noted in Table 3, the quality of data package available to JECFA reviewers has significant consequences in the overall residue assessment. There are specific components of the residue evaluation process where the quality of data will determine the level of refinement used in the assessment. Robust data typically leads to increased refinement, decreased uncertainty, and derivation of MRLs that may be higher than MRLs derived from poorer quality data.

- Example: If metabolites of a veterinary drug are demonstrated to be less toxicologically or microbiologically active than parent compound (based on likely metabolic pathways and metabolite structures), the metabolites can be discounted from the "residue of concern". However, without complete characterization of such metabolites, it is assumed that metabolites and parent drug have comparable toxicity and the residue of concern remains TR. The subsequent exposure assessment will then compare TR (not MR) exposure with relevant HBGVs. The resulting MRL, derived from MR concentrations, may therefore be unnecessarily low as it will not reflect a concentration justified by the potential residue of concern. Conversely, a more thorough metabolite characterization may allow for refinement of the exposure assessment (e.g., discounting inactive metabolites or bound residues) and will help facilitate subsequent MRL derivation (e.g., zilpaterol (FAO/WHO, 2016),).
- Deficiencies in study design (e.g., small sample sizes, low doses, poor assay sensitivity) may result in limited quantifiable residues, hindering a robust evaluation. With limited quantifiable data to derive exposure estimates, conservative assumptions must be used in the assessment. If quantifiable MR or TR data is limited, the lowest calculated M:T values will be used in the assessment. This leads to

Table 3
Summary of impact of data quality on MRL recommendation.

Data challenge	Assessment Uncertainty	Impact on Residue Assessment	Actions Required	Likely Consequence	Example JECFA evaluations
Relevant data not available (i.e., lack of critical tox, micro, metabolism, or residue depletion study)	Very High	Not possible to complete initial residue assessment	Relevant data must be provided (i.e., data obtained from other source, data generated from new study)	No MRLs derived	Ethion (FAO/WHO, 2018a) Halquinol (FAO/WHO, 2018a) Diflubenzuron (FAO/WHO, 2016)
Relevant data available, but with significant data flaws	High	May be possible to complete initial residue assessment, but not possible to derive MRLs	Sponsor addresses data flaws Re-evaluate at next JECFA meeting	Delay in MRL adoption (typically 2+ years)	Halquinol, (FAO/WHO, 2018a; FAO/WHO, 2020a)
Relevant data available, but less robust or with minor flaws	Moderate	If conservative assumptions used, may be possible to complete initial residue assessment and derive MRLs	None	Derivation of low (er) MRLs than potentially possible with higher quality data	Ivermectin (extension of MRLs to pig and goat tissues) (FAO/WHO, 2020a)
Relevant data available, robust and high quality	Low	Refinement of assumptions used, possible to complete residue assessment and derive MRLs	None	Derivation of appropriate MRLs	Zilpaterol (FAO/WHO, 2016)

higher predicted TR at each time point, potentially resulting in low (er) MRLs.

It is in the interest of all stakeholders (drug sponsors, JECFA evaluators, CCRVDF members, animal production industry, and consumers) that JECFA perform residue assessments based on robust and complete data sets. Familiarity with JECFA evaluation processes may help sponsors identify and rectify data deficiencies prior to formal JECFA evaluation.

Disclaimer

The views expressed in this publication are those of the authors and do not necessarily reflect the views of FAO, WHO, or the authors' respective institutions.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Arcella, D., et al., 2019. Harmonized methodology to assess chronic dietary exposure to residues from compounds used as pesticide and veterinary drug. Crit. Rev. Toxicol. 49, 1–10.

Boobis, A., et al., 2017. Characterizing chronic and acute health risks of residues of veterinary drugs in food: latest methodological developments by the joint FAO/WHO expert committee on food additives. Crit. Rev. Toxicol. 1–15.

Chang, S.K., et al., 2010. Pharmacokinetics and tissue depletion of florfenicol in Leghorn and Taiwan Native chickens. J. Vet. Pharmacol. Therapeut. 33, 471–479.

FAO/WHO, 1990. Evaluation of Certain Veterinary Drug Residues in Food: Thirty-Sixth Report of the Joint FAO/WHO Expert Committee on Food Additives.

FAO/WHO, 1998a. Evaluation of Certain Veterinary Drug Residues in Food. 47th Report of the Joint FAO/WHO Expert Committee of Food Additives. WHO Technical Report Series, No. 876.

FAO/WHO, 1998b. Evaluation of Certain Veterinary Drug Residues in Food. 48th Report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No. 879.

FAO/WHO, 2009a. Maximum Residue Limits for Pesticides and Veterinary Drugs. Principles and Methods for the Risk Assessment of Chemicals in Food. Environmental Health Criteria 240.

FAO/WHO, 2009b. Risk Assessment and its Role in Risk analysis., Principles and Methods for the Risk Assessment of Chemicals in Food. Environmental Health Criteria 240.

FAO/WHO, 2016. Residue Evaluation of Certain Veterinary Drugs. Joint FAO/WHO Expert Committee on Food Additives, 81st Meeting 2015. FAO JECFA Monographs 18.

FAO/WHO, 2018a. Residue Evaluation of Certain Veterinary Drugs. Joint FAO/WHO Expert Committee on Food Additives - 85th Meeting 2017. FAO Monographs 21., Rome, Italy.

FAO/WHO, 2018b. Summary Report of the JECFA/JMPR Working Group on Residue Definition.

FAO/WHO, 2020a. Evaluation of Veterinary Drug Residues in Food. 88th Report of the Joint FAO/WHO Committee on Food Additives. WHO Technical Report Series No. 1023.

FAO/WHO, 2020b. Residue Evaluation of Certain Veterinary Drugs. Joint FAO/WHO Expert Committee of Food Additives, 88th Meeting, 2019. FAO JECFA Monographs 24.

FDA, 2017. Freedom of Information Summary. NADA 141-450. Banamine Transdermal flunixin transdermal solution.

Han, R., et al., 2017. Elimination kinetics of ceftiofur hydrochloride in milk after an 8-day extended intramammary administration in healthy and infected cows. PLoS One 12, e0187261.