Critical Reviews in Toxicology

ISSN: 1040-8444 (Print) 1547-6898 (Online) Journal homepage: http://www.tandfonline.com/loi/itxc20

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To cite this article: Alan Boobis, Carl Cerniglia, Alan Chicoine, Vittorio Fattori, Markus Lipp, Rainer Reuss, Philippe Verger & Angelika Tritscher (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, Critical Reviews in Toxicology, DOI: 10.1080/10408444.2017.1340259

To link to this article: http://dx.doi.org/10.1080/10408444.2017.1340259

Published online: 10 Jul 2017.

CRITICAL REVIEWS IN TOXICOLOGY, 2017
https://doi.org/10.1080/10408444.2017.1340259

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REVIEW

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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

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ABSTRACT

The risk assessment of residues of veterinary drugs in food is a field that continues to evolve. The toxicological end-points to be considered are becoming more nuanced and in light of growing concern about the development of antimicrobial resistance, detailed analysis of the antimicrobial activity of the residues of veterinary drugs in food is increasingly incorporated in the assessment. In recent years, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has refined its approaches to provide a more comprehensive and fit-for-purpose risk assessment. This publication describes in detail the consideration of acute and chronic effects, the estimation of acute and chronic dietary exposure, current approaches for including microbiological endpoints in the risk assessment, and JECFA's considerations for the potential effects of food processing on residues from veterinary drugs. JECFA now applies these approaches in the development of health-based guidance values (i.e. safe exposure levels) for residues of veterinary drugs. JECFA, thus, comprehensively addresses acute and chronic risks by using corresponding estimates for acute and chronic exposure and suitable correction for the limited bioavailability of bound residues by the Gallo-Torres model. On a case-by-case basis, JECFA also considers degradation products that occur from normal food processing of food containing veterinary drug residues. These approaches will continue to be refined to ensure the most scientifically sound basis for the establishment of health-based guidance values for veterinary drug residues.

ARTICLE HISTORY
Received 27 March 2017
Revised 22 May 2017
Accepted 6 June 2017

KEYWORDS
Veterinary drug residues; food safety; risk assessment; exposure assessment; JECFA; microbiological effects; toxicological effects

Table of contents

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Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) is an international scientific expert committee that is administered jointly by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO). It has been meeting since 1956, initially to evaluate the safety of food additives. Its work now also includes the evaluation of contaminants, naturally occurring toxicants, and residues of veterinary drugs in food.

JECFA performs risk assessments that serve as the basis for national and international food safety standards and regulations. With respect to residues of veterinary drugs in food, the mandate of JECFA is to

1. Elaborate principles for evaluating their safety and for quantifying their risks.
2. Establish Acceptable Daily Intakes (ADIs) and other guidance values for acute exposure.
3. Recommend Maximum Residue Limits (MRLs) for target tissues.
4. Determine appropriate criteria for, and evaluate methods of, analysis for detecting and/or quantifying residues in food.

It is important to note that risk mitigation and management strategies, including establishing withdrawal periods for veterinary drugs, are outside the scope of JECFA and rather are the purview of national regulatory authorities. However, the withdrawal periods are based on residue depletion studies with radiolabeled as well as unlabeled compound, which are evaluated in the JECFA assessments. The methods and principles underlying the work of JECFA have been laid out in the Environmental Health Criteria (EHC) 240 (FAO/WHO 2009a), and the update and improvement of these risk assessment methods and principles is a core mandate of JECFA.

The safety of veterinary drug residues in human food is typically assessed based on results from studies in laboratory animals. Human data, when available, and results from *in vitro* and in silico studies are also considered in this safety assessment. Because humans could be exposed daily, throughout their lives, to veterinary drug residues through regular consumption of the same food (e.g. meat or fish), and because chronic exposures often have a lower threshold for toxic response than infrequent or acute exposures, residues of veterinary drugs in food are routinely evaluated for effects following chronic exposures, and a corresponding ADI is established. The ADI provides a human Health-Based Guidance Value (HBGV) for chronic or long-term exposures to residues in food, and is most often established from a Point of Departure (POD, e.g. No Observed Adverse Effect Level (NOAEL)) identified from repeated dose exposure studies in experimental animals. This ADI is then compared with the chronic dietary exposure. Until recently, this exposure was estimated using a simple model, resulting in the so-called "Estimated Daily Intake" (EDI). The model was based on a hypothetical diet, the standard food basket (or so-called model diet), intended to cover high consumers of animal products in order to check that the proposed MRLs would not result in exposures in exceedance of the ADI (FAO/WHO 2009a). The model diet assumes that muscle (300 g), liver (100 g), kidney (50 g), fat (50 g), milk (1500 g), eggs (100 g), and honey (50 g), are all consumed at these amounts daily, throughout life, by an adult person weighting 60 kg (Table 1). These amounts of food were then multiplied by the median residue level, which is derived from controlled residue trials, for the respective food category. However, this model diet had a number of limitations detailed below and mainly due to the fact that it is not based on actual consumptions observed in various regions of the world. JECFA has, therefore, developed an alternative approach to estimate chronic exposure – as described below.

JECFA has developed a "Risk-Based Decision Tree Approach for the Safety Evaluation of Residues of Veterinary Drugs" (FAO/WHO 2009b), which highlighted the need for further work in the area of acute health risks as well as in exposure assessment. Indeed, in some instances, there is a potential for veterinary drug residues to cause adverse effects in humans following only a single meal. A historical example of this was the acute intoxication by clenbuterol shortly following consumption of veal liver or lamb and bovine meat in Europe (Pulce et al. 1991; Salleras et al. 1995; Sporano et al. 1998; Barbosa et al. 2005). For a product that is given by injection to food producing animals, acute manifestations of toxicity resulting from the ingestion of the entire injection site that contains high residues in a single meal is another possibility (Sanquer et al. 2006a, 2006b). A further possibility is that people on a special occasion/event or a specific subpopulation may consume a large portion of food derived from an edible tissue where the veterinary drug residues may be more concentrated. In such cases, the ADI and corresponding chronic dietary exposure assessment are not the most appropriate ways to characterize the risk to consumers. Establishment of a HBGV based on acute effects, the acute reference dose (ARfD), as well as an approach to estimate accurately the dietary exposure after a single meal or during 1 d provide an appropriate approach to address this concern.

There are a number of existing guidelines and publications describing the establishment of an ARfD; however, they are neither specific in addressing veterinary drug residues (e.g. EC 2001; FAO/WHO 2005; Solecki et al. 2005; FAO/WHO 2009a; OECD 2010), nor provide guidance regarding when there is a need to and how to establish an ARfD for veterinary drug residues (e.g. VICH 2015a). Therefore, JECFA

Table 1. Food consumption data for estimating chronic exposure to veterinary drug residues for the general population (including children) and children (as of 2016) compared with values used for EDI.

	Adults[a] (g/person/day)	General population[b] (g/person/day)			Children[c] (g/person/day)	
			GECDE		GECDE	
Food type as raw commodity	EDI Model diet[d]	Cluster diets Highest mean[e]	Mean[f]	High-level chronic[g]	Mean[f]	High-level chronic[g]
Mammalian muscle						
All mammalian muscle	*300*	*114*	*100*	*415*	*62*	*478*
Beef and other bovines		47	63	291	37	159
Pork and other porcines		114	58	415	31	162
Sheep and other ovines		21	13	315	13	158
Goat and other caprines		5	1	315	1	67
Horse and other equines		3	1	557	2	478
Rabbit		2	4	309	2	149
Mammalian trimmed fat, skin and added fat excluding butter						
Mammalian fat	*50*	*14*	*4.4*	*125*	*1.7*	*29*
Mammalian offal						
All mammalian offal		*8*	*4*	*269*	*3*	*193*
Mammalian liver	100	–	2	237	3	103
Mammalian kidney	50	–	0.5	166	0.5	150
Fish and seafood						
All fish and *seafood*	*300*	*27*	*75*	*655*	*34*	*189*
Fish		27	26	655	24	226
Crustaceans		4	6	250	3	140
Molluscs		2	*7*	263	3	189
Poultry						
Poultry muscle	300	118	59	352	35	207
Poultry fat and skin	50	1	0.4	23	0.05	3
Poultry offal (all)		5	2	188	0.4	87
Liver	100					
Kidney	50					
Eggs						
Eggs (all)	*100*	*42*	*39*	*169*	*25*	*143*
Milk						
Milk[h]	*1500*	*425*	*1072*	*2917*	*809*	*1736*
Honey						
Honey	*50*	*3*	*5*	*140*	*2*	*84*

[a]JECFA model diet was proposed for an adult person weighing 60 kg and did not including children except those over 15 years old which weigh approximately 60 kg.
[b]Includes children different from those described in the "children" column.
[c]Aged from 36 months up to and including 9 years of age.
[d]Veterinary drugs JECFA model diet consumed daily by an adult person weighing 60 kg.
[e]The highest reported mean chronic consumption is derived from total population across population groups and surveys lasting at least 2 d among countries for which data were made available to FAO/WHO.
[f]per capita estimates are derived from FAO Food Balance Sheet.
[g]The high-level chronic consumption is assessed as the highest 97.5th percentile in consumers only for surveys lasting at least 2 d among countries for which data were made available to FAO/WHO.
[h]Includes whole liquid milk, secondary milk products (e.g. skimmed milk, evaporated milk, milk powders), derived milk products (e.g. cream, butter) and manufactured milk products (yoghurt, cheese, ice cream).

developed a guidance document on establishing ARfDs for veterinary drugs (FAO/WHO 2016a).

The characterization of acute and chronic health risks requires appropriate estimates of acute and chronic exposure. JECFA has been working on the development of suitable methods for this purpose and also to harmonize exposure assessment methodology with work in other areas, in particular the risk assessment of pesticide residues. This paper describes the most recent developments in exposure assessment and hazard characterization of residues of veterinary drugs, as important elements in the overall risk assessment process.

Improving the exposure assessment for residues of veterinary drugs

JECFA has recently refined its approach to assessing dietary exposure to veterinary drug residues to provide estimates that are more accurate. Dietary exposure assessment is a critical step in assessing the public health risk posed by these residues. As data available at international level do not allow for a robust probabilistic approach, a deterministic exposure assessment is necessary, refined as required (FAO/WHO 2009a). Two basic inputs are needed: the concentration of drug residues (derived from residue-depletion studies and suitable withdrawal times) in each animal-derived tissue that may contain the residue (concentration) and the consumption amount of every food consumed (consumption), on a per kg body weight basis. In generic terms, calculation of dietary exposure for N foodstuffs can be expressed as follows:

$$\textit{Dietary exposure} = \sum_{n=1}^{N} \frac{\textit{Concentration of chemical in food} \times \textit{Food consumption}\ (g)}{\textit{kg Body weight}}$$

More accurate and realistic deterministic models to assess chronic and acute dietary exposure

Exposure assessment should be fit for purpose, i.e. the risk to be estimated. Four major shortcomings were identified in the model diet for estimating exposure to veterinary drug residues, used by JECFA as well as many regulatory authorities. First, in some circumstances, the use of the model diet may lead to an overestimation of chronic consumption and result in overly conservative risk assessments, e.g. when residues occur in many food commodities. Second, the use of the model diet underestimated chronic consumption of all individual food commodities that were currently considered in the food basket because it did not take account of habitual high-level consumption of foods that can occur in some populations e.g. lung tissue. Third, the model diet assumes that all foods that are derived from the same tissue type (e.g. muscle) are consumed in similar amounts. For example, the consumption of meat (muscle) and fish (muscle) is assumed to be the same and considered to be mutually exclusive. Finally, the consumption amounts used in the model diet are not suitable for estimating acute exposure as recently defined by JECFA because many individual food consumption surveys are reporting higher values on a single day.

The concentration and exposure data needed for the chronic or the acute risk assessment, respectively, differ in significant and substantial ways. For use in a chronic risk assessment, i.e. comparing exposure levels with the ADI, the relevant concentration data should be represented as the median or the mean concentration of the residue in a food, depending on the expected distribution of concentrations and in particular on the percentage of samples below LOD or LOQ (see FAO/WHO 2009a– Chapter 6, p. 22). The food consumption data should reflect the realistic habitual high-level consumption of all foods that may contain the residue for each population to be protected, e.g. adults, children, pregnant women. For an acute risk assessment, i.e. comparing exposure with the ARfD; however, a high-level concentration of residue should be combined with a high-level consumption of a single food consumed at a single occasion or over 1 d. The body weight should ideally be measured for each individual who participated in the food consumption survey. To address these issues in exposure assessment methodology, JECFA developed and implemented two new models, for estimating chronic and acute dietary exposure, respectively, to residues of veterinary drugs in foods (FAO/WHO 2011):

- The Global Estimate of Chronic Dietary Exposure (GECDE), for chronic exposure assessment.
- The Global Estimate of Acute Dietary Exposure (GEADE), for acute exposure assessment.

Both approaches were piloted by JECFA at its 78th meeting in 2013 (FAO/WHO 2014). The GECDE and GAEDE approaches were subsequently used to estimate chronic and acute dietary exposure at its 81st meeting (FAO/WHO 2016b). The new approaches use food consumption data reported in surveys to provide more realistic and accurate representations of a population's actual consumption patterns.

Table 2. Food consumption data for estimating acute exposure to veterinary drug residues for the general population (including children) and children (as of 2016).

	GEADE	
	97.5th percentile[c]	
Food type as raw commodity	General population[a] (g/person/day)	Children[b] (g/person per day)
Mammalian muscle		
All mammalian muscle	1000	337
Beef and other bovines	514	337
Pork and other porcines	704	312
Sheep and other ovines	1000	311
Goat and other caprines	479	200
Horse and other equines	400	210
Rabbit	780	444
Mammalian trimmed fat, skin, and added fat excluding butter		
Mammalian trimmed fat	258	73
Mammalian offal		
All mammalian offal	1000	300
Mammalian liver	439	165
Mammalian kidney	360	300
Mammalian lung	300	150
Fish and seafood		
All fish and seafood	2000	481
Fish	2000	345
Crustaceans	500	248
Molluscs	832	481
Poultry		
Poultry muscle	1120	467
Poultry fat and skin	50	20
Poultry offal	389	130
Liver		
Kidney		
Eggs		
Eggs (all)	450	196
Milk		
Milk[d]	3235	1551
Honey		
Honey	194	90

[a]General population includes children different from those described in the "children" column.
[b]Children from 2 up to and including 9 years of age.
[c]The high-level acute consumption is assessed as the single highest 97.5th percentile consumption over 1 d among countries for which data were made available to FAO/WHO.
[d]Includes whole liquid milk, secondary milk products (e.g. skimmed milk, evaporated milk, milk powders), derived milk products (e.g. cream, butter) and manufactured milk products (yoghurt, cheese, ice cream).

Furthermore, more recent food consumption data allow more granular exposure assessment of population sub-groups of interest consuming particular animal-derived tissues, e.g. exposure of children to a veterinary drug residue in finfish. It is important to note that food consumption values are reported by national authorities to FAO and WHO. The food consumption data used by the JECFA and displayed in Tables 1 and 2 are updated when necessary to account for new countries submitting data or for countries providing data collected with an improved methodology. Apart from these cases, changes in the natural trend in food consumption appear to be captured adequately through updating the data every 5–10 years (Verger et al. 2002; Kearney 2010).

GECDE: estimating chronic exposure

To reflect chronic dietary exposure, the GECDE utilizes median drug residue concentrations[1] derived from residue depletion studies and suitable withdrawal times, multiplied

Table 3. Hypothetical example of the impact of veterinary drug residue and food consumption estimates on dietary exposure calculations.

			EDI[a] (adults)	GECDE[b] (general population)		GEADE[c] (general population)
Animal-derived tissue	Median drug residue at time × (µg/kg)	95/95 UTL drug residue at time × (µg/kg)	Mean level (µg/person/day)	Mean level (µg/person/day)	High level chronic (µg/person/day)	High level acute (µg/person/day)
Mammalian muscle (sheep and other ovines)	50	200	15 (50 µg/kg × 300 g/1000)	*1.1* (50 µg/kg × 21 g/1000)	15.8 (50 µg/kg × 315 g/1000)	200 (200 µg/kg × 1000 g/1000)
Mammalian liver	200	700	20 (200 µg/kg × 100 g/1000)	0.4 (200 µg/kg × 2 g/1000)	*47.4* (200 µg/kg × 237 g/1000)	307 (700 µg/kg × 439 g/1000)
Mammalian kidney	100	400	5 (100 µg/kg × 50 g/1000)	*0.1* (100 µg/kg × 0.5 g/1000)	16.6 (100 µg/kg × 166 g/1000)	144 (400 µg/kg × 360 g/1000)
Mammalian trimmed fat	20	80	1 (20 µg/kg × 50 g/1000)	*0.3* (20 µg/kg × 14 g/1000)	2.5 (20 µg/kg × 125 g/1000)	21 (80 µg/kg × 258 g/1000)
Milk	2	6	3 (2 µg/kg × 1500 g/1000)	*2.1* (2 µg/kg × 1072 g/1000)	5.8 (2 µg/kg × 2917 g/1000)	19 (6 µg/kg × 3235 g/1000)
Eggs (all)	150	500	15 (150 µg/kg × 100 g/1000)	*6.3* (150 µg/kg × 42 g/1000)	25.4 (150 µg/kg × 169 g/1000)	225 (500 µg/kg × 450 g/1000)
Honey	20	60	1 (20 µg/kg × 50 g/1000)	*0.1* (20 µg/kg × 5 g/1000)	2.8 (20 µg/kg × 140 g/1000	12 (60 µg/kg × 194 g/1000)
Sum			Total mean exposures	Highest exposure high level (liver, 47.4) + total mean exposures (excl. liver, 9.9)		Highest exposure only
Total estimated exposure (µg/person/day)			60	57		307

[a]EDI exposure = sum of (median tissue conc. × tissue consumption) for all tissues for an adult person weighing 60 kg.
[b]GECDE = Highest 97.5th percentile exposure in consumers only from one animal-derived tissue (e.g. liver) + mean exposure in total population of all remaining animal-derived tissue. Numbers in italics are used in the calculation of total estimated exposure.
[c]GEADE = (95/95 UTL residue concentration × the highest 97.5th percentile acute food consumption).

by two different types of consumption values. First, the exposure at the 97.5th percentile of chronic consumption (97.5th percentile consumption multiplied by the median residue concentration) is calculated for all relevant tissues, and the single animal-derived tissue with the highest exposure is selected. Second, the mean dietary exposures (mean food consumption multiplied by the median residue concentration) from all the other relevant animal-derived tissues are then added to the first (high exposure) tissue, in order to estimate total exposure. The highest mean food consumption is derived from the total population; in other words, non-consumers of the food during the survey period are included in the calculation to account for long-term consumption across surveys and population groups. In addition to the general population and children, dietary exposure of infants can also be estimated.

The GECDE assumes that, on average, an individual would be a high-level consumer of only one category of food (hence the 97.5th percentile of chronic consumption for only one tissue), and that his or her consumption of other animal-derived tissues containing the residue would remain at the population average (i.e. mean consumption of all other animal-derived foods tissues). See Table 3 for an example exposure estimate using the GECDE.

The mean and the 97.5th percentile chronic consumption data (Table 1) should be derived from surveys with individual records of two or more days' duration. For the mean consumption, a database on per capita data (FAO 2013) and grouped in 17 cluster diets (Sy et al. 2013) can also be used. For a given food category, the highest value extracted from the cluster diets or reported from an individual food consumption survey is used. The former allows accounting for countries (from the same cluster) for which no individual surveys are available. The rationale for incorporating a 97.5th consumption amount in the GECDE is that it allows the exposure estimate to include chronically high-consuming individuals, while its application for only a single animal-derived foods tissue (as opposed to all foods) ensures that it does not unrealistically inflate chronic food consumption estimates. Furthermore, this percentile is more commonly reported in consumption data submitted to JECFA. A clear indication concerning the minimum number of observations necessary to estimate a given percentile cannot be found in the literature. Different options can be used, none of them being a widely accepted standard. FAO and WHO recommend the number of observations to be at least three, i.e. the 97.5th percentile should be calculated over at least 120 subjects/days. Percentiles calculated over a lower number of subjects/days should be flagged with a warning indicating the need for a cautious interpretation of the results which may not be statistically robust.

For comparison, the chronic consumption values used as inputs in exposure estimates by a variety of regulatory authorities are listed in Table 4.

In summary, the GECDE is calculated as

$$GECDE = Highest\ exposure\ from\ one\ animal\ product + Total\ mean\ exposures\ from\ all\ other\ products$$

Table 4. Daily food consumption for a 60 kg adult used a by various regulatory agencies as inputs into exposure estimates.

	Consumption value (g/person/d)			
Animal-derived tissue	European Medicines Agency (EU)[a]	FDA Center for Veterinary Medicine (USA)[b]	Australia[c]	Veterinary Drugs Directorate (Canada)[d]
Muscle[e]	300	300	Data tables that report mean consumption for Australian NNS	500
Liver[e]	100	100		250
Kidney[e]	50	50		167
Fat[e]	50	50		125
Milk	1500	1500		1670
Eggs	100	100		500
Honey	20	20		167

[a]Consumers are assumed to eat EACH animal-derived tissue listed daily, so the ADI is allocated between all tissue types.
[b]The Center for Veterinary Medicine (CVM) assumes that, when an individual consumes a full portion of edible muscle, liver, kidney, or fat tissue from one species, that individual would not consume a full portion of the same edible tissue from another species on the same day. CVM regulates milk, eggs and honey as independent food commodities; this means that CVM assumes these edible tissues are consumed in addition to the consumption of muscle, fat, kidney, or liver. Note that unlike the EMA approach, the ADI allocated for animal-derived tissue is not split between muscle, fat, kidney, and liver (i.e. a consumer is assumed to eat only one of these animal-derived tissues each day).
[c]FSANZ (2009), p 52f.
[d]Beef-specific tissue consumption factors (values differ for other commodities). As per the USA approach, Canada assumes a consumer will eat an entire portion of only one solid tissue type each day (the entire ADI is allocated to each solid tissue type) (VDD Health Canada 2007).
[e]Mammalian tissues. Consumption amounts may differ for poultry or fish.

In most cases, the tissue with the highest estimate of exposure using the 97.5th percentile consumption value drives the resulting overall dietary exposure estimate. In the rare case where two animal-derived foods tissues have similar 97.5th percentile exposure values, the calculation is undertaken for each one to determine the higher GECDE.

GEADE: estimating acute exposure

Until recently, no appropriate approach to estimate acute exposure to veterinary drug residues was available or indeed needed, as the JECFA was not systematically considering acute risk. However, as JECFA is now considering the need to establish an ARfD for veterinary drugs on a routine basis, suitable methodology for estimating acute exposure was required. JECFA has, therefore, developed the GEADE for this purpose.

GEADE considers high-level exposure from each relevant tissue of animal origin individually. The selected high-residue concentration in each relevant tissue is multiplied by the high daily consumption (97.5th percentile) of that food (e.g. beef and other bovines, poultry muscle, milk) at a single eating occasion or on a single day and calculated for consumers only. The high-residue concentration is usually derived from depletion studies, such as the upper one-sided 95% confidence limit over the 95th percentile residue concentration, or 95/95 upper tolerance limit (UTL).

The 97.5th percentile food consumption amount is selected as a more statistically robust value than the maximum food consumption amount because it represents an actual distribution of values and this is the high percentile most frequently provided to JECFA. The GEADE is then calculated as follows:

$$GEADE = \frac{97.5th\ percentile\ food\ consumption\ (1\ person-day) \times High\ Residue_{tissue}}{Body\ weight\ (kg)}$$

It should also be noted that the GEADE (like all estimates of acute exposure) is concerned with only a single eating occasion of a single food.[2] See Table 2 for food consumption data used in the GEADE, and Table 3 for an example exposure estimate using the GEADE.

Acute exposure can be estimated for children as well as for the general population, following the widely accepted principle that acute dietary exposure estimates should cover the whole population but should also consider children separately (see Table 2). Children's consumption patterns can differ from those of adults; consequently, the food leading to the highest acute exposure may differ between populations.

Veterinary drug residue concentrations used in JECFA exposure assessment

Concentrations of residues of veterinary drugs and their time courses in different tissues are derived from residue depletion studies with radiolabeled as well as unlabeled compound. Guidance documents on the suitable design and conduct of residue depletion studies have previously been published (VICH 2015a; FDA 2016). The intended target species and class of animal is treated with the veterinary drug according to the proposed dose, route, and duration of use. The residue concentration is determined in the relevant tissues using an appropriate (validated) analytical method. The distribution of tissue residues is then plotted versus time from last treatment (withdrawal time), typically on a log-linear scale. Least squares regression is performed on the logarithmic data set, and used to provide an estimate of the median residue concentrations over the residue depletion study. A more detailed description of this analysis is available in EHC 240 (FAO/WHO 2009a), and statistical software capable of performing residue depletion estimates is freely available from the FAO (FAO 2003). From this analysis the median residue concentration at a given time can be determined and will feed into the chronic dietary exposure estimation (EDI or GECDE). For an acute dietary exposure assessment (GEADE), the high-residue concentration is derived from the residue depletion studies, by determining the upper one-sided 95% confidence limit over the 95th percentile residue concentration. Examples of

applying the new approaches for chronic and acute exposure scenarios from a pilot study can be found in the JECFA Residue Monographs from the 78th meeting of JECFA.

Hazard characterization

The information needed to establish toxicological (including pharmacological)[3] end-points is obtained from studies in experimental animals or (rarely) in humans. However, in those cases where a veterinary drug has also been developed as a human medicine or a candidate human medicine, pharmacological (including pharmacokinetic) studies will have been carried out in human volunteers as part of the drug development program. Where available, this information will be taken into account for the identification of a NOAEL (or similar, such as the BMDL10 (WHO 2016)) and the establishment of an ADI (or ARfD). JECFA will always use human data where appropriate.[4] Unlike the situation with pesticide residues, where many authorities will not accept human data as a basis for establishing health based guidance values, for veterinary drug residues, this is either explicitly (e.g. EC 2005; OECD 2010) or implicitly (e.g. VICH 2009) acceptable. In general, the process for establishing an ADI is the same in different jurisdictions (e.g. VICH, which includes USA, EU and Japan; OECD; EMA; FDA). While for most compounds the same ADI value will be established in each jurisdiction, in rare cases, the ADI may differ between jurisdictions due to differences in assessment of toxicological data (e.g. resulting in use of different uncertainty factors, different interpretation of marginal responses at lower doses).

Assessing chronic effects: ADI

In the risk assessment of chronic effects from exposure to residues of veterinary drugs, the POD for the most sensitive, relevant endpoint (toxicological, pharmacological or microbiological) is used to establish the ADI (FAO/WHO 2009a). This is in line with the underlying concept that the ADI should be established low enough to be protective in situations with regular exposure over a lifetime. Whilst for systemic toxicity, study duration is often chronic, e.g. 2 years in a rat study, for other effects, it could be less than lifetime, for example reproductive effects. Indeed, it may even be the length of only one single exposure, for example to establish a response based on the intended pharmacology of the compound in the target species. In general, regardless of the duration of treatment in the study on which the ADI is based, the ADI is used to characterize the hazards for chronic exposure. In addition, the ADI applies to the general population (including all sub-populations); the differentiation of the often appreciable differences in the food consumption of different sub-populations will be done through characterizing exposure estimates in different sub-populations. The ADI itself, however, as a hazard indicator is independent of exposure.

If the acute effect is the most sensitive, relevant effect observed, it would form the basis of both the ARfD and the ADI (see below). If the ARfD is lower than the ADI, the ADI should be changed to the same numerical value as the ARfD and should be based on this effect. This should be noted in the assessment. Given that in general, the GECDE is less than GEADE, if the GEADE is less than the ARfD, then the GECDE will be less than the ADI. Hence, the ARfD and GEADE together should be protective of all exposure durations.

Consideration for intermitted and less-than-lifetime exposures

However, the situation is getting more complicated than the simple distinction between acute and chronic effects, as in recent years, increasing evidence has shown that, at least for pesticides, the severity of toxicological effects in rodents does not appear to progress after 2–3 months of treatment, so that the POD for lifetime exposure has been observed to be very similar to that in a 90-d study, if and when corrected for the difference in dietary consumption, as the amount of food consumed by experimental animals, such as rats, per kg body weight declines with age (Zarn et al. 2011). As the ADI is based on the most sensitive relevant endpoint, in theory this should make no difference to the risk assessment. However, in practice, it depends on how exposure is estimated. When assessing risks from any excursion of human exposure above the ADI, exposure is averaged over a lifetime, on the assumption that it is the overall exposure over this entire period that determines the outcome. This could lead to an underestimation of risk if exposure over shorter periods appreciably exceeded the ADI, e.g. due to seasonal variation in consumption behavior. As a hypothetical example, assume that the NOAEL in a 2 year study was 1 mg/kg bw per day, after correcting for age-related changes in feed intake, and the NOAEL in a 90 d study was 1.5 mg/kg bw per day. The chemical occurs in only a single food commodity to any appreciable extent. Daily human exposure based on average chronic consumption data is 0.007 mg/kg bw per day. Comparison with an ADI of 10 μg/kg bw (based on the 2-year NOAEL and assuming a 100-fold uncertainty factor) would suggest no appreciable risk. However, consumption of this food commodity is highly seasonal, so that some individuals regularly consume, for 3 months of each year, 4 times more than the overall average. Hence, their exposure will be 0.028 mg/kg bw per day. Comparison with a health-based guidance value of 0.02 mk/kg bw (rounded) (derived from the 90-d study (and an uncertainty factor of 100) would suggest potential concern. Recent analyses for human pharmaceuticals suggest that for this category of chemical, there is no consistent trend between severity of effect and duration of exposure (Roberts et al. 2015). For some compounds, severity progresses with duration from sub-chronic to chronic, whereas for others, it does not. Currently, the authors are not aware of sufficient information to establish how veterinary drugs fit into this pattern. There is also a need to assess the extent to which the current method for estimating chronic exposure could underestimate short-term exceedances. This aspect needs further evaluation before risk assessment methods can take this appropriately into account. Efforts are underway by JECFA and JMPR to address this issue.

Special consideration for microbiologically active compounds

For microbiologically active veterinary drugs, the risk assessment regarding exposure to residues from such a drug needs to consider the potentially harmful effects of the drug residues on the human intestinal microbiota in addition to the toxicological/pharmacological hazard, to account for the critical role of the intestinal microbiome in human health and disease. The VICH has harmonized a process to assess the effects of antimicrobial veterinary drugs used in food-producing animals on the human intestinal microbiota (VICH 2012), which has been incorporated into JECFA procedures (FAO/WHO 2009a) and is currently followed by many national/regional authorities.

The microbiological endpoints of human health concern identified in the guideline that are considered when establishing a microbiological ADI are disruption of the colonization barrier and an increase in the selection and emergence of antimicrobial resistant bacteria. A microbiological ADI may be established for one or both effects of human health concern and is derived experimentally from *in vitro* or *in vivo* studies, published scientific literature and other appropriate data sources. Where the residues of a drug reach the colon and remain microbiologically active against the human intestinal microbiota, a microbiological ADI for an antimicrobial drug can be established according to VICH GL36(R) (VICH 2012) and EHC 240 (FAO/WHO 2009a). However, for antimicrobial drugs where the microbiologically active residues might not reach and/or remain microbiologically active in the colon, it may not be necessary to establish a microbiological ADI. In addition, establishing a microbiological ADI may not be necessary if it can be demonstrated that the concentrations of the active residues in the colon are sufficiently low that they would be very unlikely to affect the ecology of the intestinal microbiota. In cases where the drug has effects on more than one microbiological endpoint of human health concern, the more sensitive is used to establish the microbiological ADI.

Derivation of a microbiological ADI from in vitro data

$$\text{ADI} = \frac{\text{MIC}_{\text{calc}}\ (\text{NOAEC}) \times \text{Mass of colon}\ (220\ \text{g/day})}{\text{Fraction of oral dose available to microorganisms} \times 60\ \text{kg person}}$$

The MIC_{calc} is derived from the lower 90% confidence limit for the mean MIC_{50} of the relevant genera for which the drug is active. For the No Observable Adverse Effect Concentration (NOAEC), it is recommended that the NOAEC (based on *in vitro* data other than MIC data) derived from the lower 90% confidence limit for the mean NOAEC from *in vitro* systems be used to account for the variability of the data. Therefore, in this formula, uncertainty factors are not generally needed to determine the microbiological ADI. The fraction of an oral dose available for colonic microorganisms is based on in vivo measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction (of an oral dose) excreted in urine.

Derivation of a microbiological ADI from in vivo data

In vivo test systems using human flora-associated and conventional laboratory animals may be suitable for the assessment of disruption of the colonization barrier and potential for resistance emergence. The microbiological ADI is calculated from the No Observable Adverse Effect Level (NOAEL) divided by the uncertainty factor.

The "microbiological ADI" is then compared with the "toxicological ADI" and the lower of the two ADIs for the drug is used to establish the overall ADI ("the ADI") to ensure safety to the consumer. An update on the safety evaluation of veterinary antimicrobial agents in food to determine effects on the human intestinal microbiota has recently been published (Cerniglia et al. 2016).

Assessing acute effects: ARfD

In addition to routinely assessing the potential effects of chronic exposure, JECFA has recently agreed that the need to establish an ARfD should be considered for all veterinary drugs and that where appropriate an ARfD should be established (FAO/WHO 2016b). JECFA has prepared draft guidance for this (FAO/WHO 2016a), building on the Joint Meeting on Pesticide Residues (JMPR) guidance where relevant (Solecki et al. 2005). This covers many of the endpoints that will be of concern to JECFA, but additional consideration is needed of microbiological effects and transient pharmacological effects. In establishing an ARfD, the same general principles apply, as when establishing an ADI, i.e. the POD (NOAEL or benchmark dose) for the most sensitive, relevant endpoint should be used. However, as the concept of an ARfD for residues of veterinary drugs is relatively recent, specific guidance by some authorities is at varying stages of development. As with the example of pesticide residues, it is likely that some years of practical experience will lead to greater harmonization of the approach to establish ARfDs for residues of veterinary drugs in food.

The POD used as the basis of an ARfD may be from studies in experimental animals, but particularly for the acute effects of veterinary drugs, may be from studies in humans, as some veterinary drugs are also used as human medicines and will, therefore, have been ethically tested in humans (see above). The most likely effect observed in these latter studies is a reflection of the desired pharmacology in the target veterinary species, for example beta-adrenoceptor stimulation. This would be considered an undesirable effect of a veterinary residue in humans and hence could serve as the basis for establishing an ARfD. However, to protect against all possible adverse health effects, the totality of the database from humans and experimental animals needs to be taken into account. This can be particularly relevant for endpoints such as developmental toxicity, which cannot be assessed from the types of studies possible in humans. As an example, based on a NOAEL for acute beta-adrenoceptor stimulation in a well-conducted, ethical clinical study of a veterinary drug (being investigated as a candidate human medicine) in volunteers of 1 μg/kg bw, one might propose an ARfD of 0.1 μg/kg bw, by applying the default uncertainty factor of 10 for

interindividual variability. However, if the drug also produced developmental toxicity in rabbits with a NOAEL of 3 μg/kg bw per day, the proposed ARfD would not be sufficiently protective for this effect, assuming the default 10-fold inter-species difference and 10-fold interindividual variability. Hence, in this case, it would be necessary to establish an ARfD of 0.03 μg/kg bw.

Information may be available on the *in vitro* or *ex vivo* pharmacological effects of veterinary drugs on the target system from a range of species, including humans. This information should be used on a case-by-case basis for assessing the relevance of effects, relative sensitivity of humans and possibly in the choice of uncertainty factor for toxicodynamics (inter- and/or intra-species).

The effect on which the ARfD is based should be acute. However, the toxicological information available to JECFA rarely covers all relevant endpoints after acute exposure. Hence, it can become necessary to consider effects observed in repeated dose studies for the possibility that they could occur after a single exposure. Guidance on the endpoints where this might be anticipated has been proposed by JECFA (based on the JMPR guidance and practical experience from previous evaluations) and on how to assess studies for such effects, including biological plausibility (FAO/WHO 2016a). For example, there may be interim determinations of a range of parameters in repeated dose studies, such as body weight, food and water consumption, clinical chemistry, hematology, urinalysis, behavior, and other clinical signs. Changes in such parameters observed after only a few days may plausibly occur after only a single exposure. This should be assessed on a case-by-case basis, referring to the guidance. Knowledge of mode of action will be of assistance in the choice of the appropriate dose metric for inter-species comparisons, e.g. parent or biologically active metabolite; the maximal observed concentration in relevant tissue/body fluid, C_{max} (for transient, reversible effects) or total area-under-the-curve, AUC (for less rapidly reversing effects).

Some acute effects may be biologically relevant to only some sub-populations. The most obvious such effect is developmental toxicity, which is relevant only to pregnant women. Developmental toxicity is considered an acute effect in the absence of evidence to the contrary, due to the existence of windows of sensitivity during the developmental process. Hence, when an ARfD is established based on such an effect, in strictly scientific terms, this would be applicable only to pregnant women. In the case of pesticide residues, this is of practical significance due to differences in dietary exposure between adults and children, which affects the risk characterization.[5] One could, therefore, consider the establishment of different ARfDs for pregnant women and the general population (which would include children) when the most sensitive effect is developmental toxicity (FAO/WHO 2009a), to be compared with specific dietary exposure estimates for the different population groups.

It makes little sense to spend the time and effort to establish an ARfD when the acute toxicity is so low (i.e. the threshold or POD of the acute toxicological endpoint is so high) that it would not give rise to any concern even at the upper limit of human consumption. Estimates of extreme exposure, i.e. based on the highest MRLs established by Codex, EU, and USA and the 97.5th percentile highest consumption (consumer only, on 1 d) value for each edible tissue from FAO and WHO consumption data, suggested a cutoff for exposure of 1 mg/kg bw, above which an ARfD would not be necessary. This value was based on an upper bound exposure estimate of 0.3 mg/kg bw and multiplied by a factor of 3 (with rounding up to 1 mg/kg bw) to allow for uncertainty in the exposure estimate (FAO/WHO 2016a). JMPR had undertaken a similar exercise for pesticide residues and concluded that an appropriate cutoff would be 5 mg/kg bw (FAO/WHO 2009a). The higher value is, in part, due to inter-unit/lot variability in residue levels of pesticides. As a number of substances have dual use as pesticides and veterinary drugs, JECFA concluded that to ensure consistency in ARfDs for such substances, a cutoff of 5 mg/kg bw should also be used for veterinary drug residues, as this is the more conservative value (FAO/WHO 2016a). Should JMPR revise the cutoff used for pesticide residues, it would be appropriate to reconsider the cutoff for veterinary drug residues. A cutoff of 5 mg/kg bw equates to an NOAEL of 500 mg/kg bw in studies in experimental animals with the application of a default uncertainty factor of 100.

Considerations for injection sites

This cutoff would be adequately protective of the toxicological effects of residues in any typically consumed tissue from animals treated with a veterinary drug, as maximum concentrations of residue would be limited by good practice in the use of veterinary drugs (GPVD) which is an important consideration when JECFA undertakes its assessment and recommends MRLs. However, injection sites for veterinary drugs could potentially lead to exceptions to this. In general, there are three different routes of administration for injectable drugs. Intra-venous injection, where the drug quickly enters the bloodstream and unless a vein is punctured as a consequence of poor technique, no residues will remain at the injection site. However, injectable products for subcutaneous administration (s.c.) and intramuscular (i.m.) administration are often formulated for slow release, depositing the drug at high concentration either under the skin or in the muscle, respectively, and frequently result in considerable amounts of residue at the injection site for long periods. While it is possible to cutout the injection sites during the slaughtering process and divert them from the food chain, this may not always be done reliably and special considerations need to be applied when assessing the safety of the residues found at s.c. and i.m. injection sites, particularly the latter as these are more likely to be consumed. Hence, consumers of the injection site tissue are potentially exposed to appreciably higher residues than consumers of other muscle tissue. In addition to the potentially high residues in the injection site tissue, there is potential for short-term (acute) ingestion of a significant quantity of injection site muscle (whereas injection site muscle is unlikely to be consumed chronically). Therefore, the likelihood that an ARfD will be needed increases when the veterinary drug is administered to the food animal by intramuscular or subcutaneous injection. In these circumstances, coordination between the residue and toxicological

experts is key to determine the possible need for an ARfD. Other unique conditions of exposure might exist that could result in high acute exposure, necessitating a similar interaction. However, the guidance document for the establishment of ARfD for veterinary drug residues in food (FAO/WHO 2016a) lays out conditions under which it is not necessary to establish an ARfD because it is considered extremely unlikely that exposure to residues of such a drug would lead to public health concerns. A key presumption here is that the drug is used in accordance with GPVD, respecting the recommended/authorized dosage and withdrawal periods that, as noted above, are established by relevant national regulatory authorities to ensure that residues in all edible tissues (including injection site tissues), do not pose risks to consumers.

Special consideration for microbiologically active compounds

For drugs with antimicrobial activity, the possibility of acute effects of residues on the human intestinal microbiota needs to be considered. While several organizations have recently developed approaches to address the acute toxicological and pharmacological effects of veterinary drugs (VICH 2015b; OECD 2010), based on previous work on pesticides, to date little work has been undertaken to address acute antimicrobial effects. For example, the VICH guidance on ARfD states that the microbiological ARfD should be same as the microbiological ADI (VICH 2015b). However, this does not take into account several important differences between the acute and the chronic exposure scenario of the intestinal microbiota. To address this, JECFA developed supplemental guidance to inform the establishment of a microbiological ARfD for veterinary drugs (FAO/WHO 2016a).

There are differences in exposure of the intestinal microbiota in the colon following a single, acute exposure, and chronic daily veterinary drug exposure. When establishing a microbiological ADI for a veterinary drug, it is assumed that there could be daily ingestion of veterinary drug residues in food (i.e. chronic exposure) at the upper level of the microbiological ADI. In other words, on any 1 d, the ingested dose is loaded into the gastrointestinal tract that already is exposed to the drug on a daily basis, over a life-time (steady state). However, in the case of acute drug exposure, there would be only a single exposure wherein the dose is ingested as a one-mealtime event and transits down the gastrointestinal tract into the colon that does not have any residue present from prior daily meals. Thus, exposure levels of the intestinal microbiota would be lower than those occurring on chronic ingestion at the ADI.

The same scientific approach used to determine a microbiological ADI for chronic exposure, using the VICH GL36(R) (VICH 2012) approach, as currently used by JECFA (FAO/WHO 2009a), would be applicable in determining a microbiological ARfD, using either *in vitro* or in vivo methods. However, the microbiological ADI determined using VICH GL36(R) (VICH 2012) should be evaluated first and independently of acute exposure. When establishing a microbiological ARfD, the overall database needs to be evaluated, as is the case when establishing a microbiological ADI.

While both disruption of the colonization barrier and antimicrobial resistance are theoretically possible following acute exposure of the intestinal microbiota to an antimicrobial drug, JECFA concluded that a single exposure to residues of a veterinary drug is unlikely to provide the selective pressure necessary to change the susceptibility of the bacterial population within the microbiome (i.e. antimicrobial resistance) (FAO/WHO 2016a). Hence, the most relevant microbiological end-point for acute exposure would be disruption of the colonization barrier and the emergence of resistance would not normally be evaluated, unless there was some evidence for such a concern following a single exposure.

There is built-in conservatism in a number of the values (i.e. MIC_{calc}, bioavailability of drug residue, colon content, volume) used in deriving the microbiological ADI in the formula described in the VICH GL36(R) (VICH 2012) and EHC240 (FAO/WHO 2009a) guideline for chronic exposure. The conservatism in a number of these values is likely to be even greater when the formula is used to derive a microbiological ARfD and this has yet to be addressed by VICH. JECFA reviewed available information on physical and temporal dilution of the gastrointestinal contents as a consequence of regular meal consumption and intestinal transit (FAO/WHO 2016a). It was concluded on such considerations that inclusion of a dilution factor of 3 (i.e. three meals per day) in the numerator of the formula used in deriving the ARfD for microbiological effects would be appropriate.

The value for the colon volume in the VICH GL36(R) equation (VICH 2012) used for deriving a microbiological ADI is 220 ml (mass of colon content of 220 g). As this is an anatomical parameter, the same value should be applicable when deriving a microbiological ARfD. However, in developing its guidance for establishing a microbiological ARfD, JECFA (FAO/WHO 2016a) noted that a number of recent studies (Khashab et al. 2009; Pritchard et al. 2014; Nilsson et al. 2015) using state-of-the-art imaging techniques, had indicated that the true volume of the hydrated colon of healthy individuals is greater than the estimate used in VICH GL36(R) (VICH 2012) and in EHC 240 (FAO/WHO 2009a). JECFA concluded that a more realistic, conservative estimate of this parameter, applicable to the derivation of both a microbiological ADI and a microbiological ARfD, would be 500 ml, but that this should be subject to further review before a final decision is taken on the value for future use.

Derivation of a microbiological ARfD from in vitro data

$$\text{microbiological ARfD} = \frac{(MIC_{calc} \text{ or NOAEC}) \times \text{Correction Factors} \times \text{colon volume}}{\text{Fraction of oral dose available to microorganisms} \times 60\text{ kg person}}$$

MIC_{calc} represents the lower 90% confidence limit for the mean MIC_{50} of the relevant genera for which the drug is active; the NOAEC is determined based on a single acute dosing in an *in vitro* system (e.g. continuous or semi-continuous culture of fecal contents); the colon volume is 500 ml. The fraction of an oral dose available to colonic microorganisms is ideally based on *in vivo* measurements for the drug administered orally. Alternatively, if sufficient data are available,

it can be calculated as 1 minus the fraction of an oral dose excreted in urine.

Correction factors (where appropriate) take into account considerations not applicable for the microbiological ADI, but may be appropriate to the microbiological ARfD. This includes a factor of 3 to allow for temporal dilution during gastrointestinal transit and for dilution by consumption of additional meals. Additional factors may be considered, to take into account the inoculum effect on MIC determinations, pH effects on MIC, and possibly other physico-chemical-specific factors of the growth conditions used in testing (e.g. incubation atmosphere, growth substrates/factors that affect growth and metabolism of the tested organisms or continuous or semi-continuous culture and batch fed culture used in deriving a NOAEC, data from studies of the effects of an acute dose (one-time exposure) of the drug on the intestinal microbiota should be evaluated; however, if this information is not available, then studies of repeated doses or continuous exposure to drug (i.e. after one or a few days of drug added to the test systems) may yield a NOAEC for acute exposure, or may provide sufficient information to derive a correction factor.

Derivation of a microbiological ARfD from in vivo data

The microbiological ARfD is calculated from the No Observable Adverse Effect Level (NOAEL) divided by the suitable uncertainty factor.

The "microbiological ARfD" is compared with the "toxicological ARfD" (if one has been established) and the lower of the two ARfDs for the drug is used to establish the overall ARfD ("the ARfD") to ensure safety to the consumer.

Other considerations

Systemic exposure to residues of veterinary drugs in food: bioaccessibility versus bioavailability

Exposure to residues of veterinary drugs occurs after consumption of food from an animal that has been treated with the drug, i.e. so-called incurred residues (residue incorporated into the food matrix after drug administration to the animal). Hence, the systemic exposure to such residues will depend on the amount of drug that desorbs from the food in a form that is available for absorption (bioaccessibility) and the extent to which these residues enter the systemic circulation (bioavailability) (Sensoy 2014).

Bioaccessibility

Not all of the incurred residues in food as consumed may be bioaccessible; some may be non-extractable (e.g. covalently bound) and thus not available for mucosal absorption over the range of physiological conditions (ECETOC 2013). Although the consumer may not be systemically exposed to such non-extractable residues, local exposure in the GI tract to microbiologically active residues will need to be considered on a case-by-case basis. If it can be demonstrated that such non-extractable residues are truly not bioaccessible, and do not otherwise constitute a concern to human safety, they can be excluded from the exposure assessment by applying a "correction factor" for the reduced accessibility.

Although this concept has been routinely applied by various concerned bodies evaluating veterinary drug residues (EMA, CVM, JECFA), all have proposed that correction for limited oral availability of drug residues should be considered only for bound (non-extractable) drug residues. This follows the Gallo–Torres model, in which the combined absolute bioaccessibility and bioavailability of incurred residues (availability) is determined experimentally in rats (Gallo-Torres 1977). However, to the knowledge of the authors, no regulatory agency yet routinely considers correcting for any potentially limited oral exposure to total veterinary drug residues (the total of bound, non-extractable, residues; plus non-bound, free or extractable residues). The only known exception to this approach was the triclabendazole assessment performed by the 70th JECFA (FAO/WHO 2009c), which incorporated an availability correction factor for total incurred residues, an approach that was subsequently explicitly rejected at the 81st JECFA (FAO/WHO 2016b). This is consistent with the use of conventional uncertainty factors to address possible inter-species and inter-individual differences in oral bioavailability (see below).

Bioavailability

Studies used to assess the toxicity of residues of veterinary drugs in experimental animals utilize the oral route of exposure, as this is the route by which humans will be exposed. The POD (e.g. NOAEL or benchmark dose) for toxicological effects is based on the administered dose (measured or estimated), expressed in mg/kg body weight. No explicit consideration is given to oral bioavailability, which may be less than 100%, because of low absorption or high pre-systemic metabolism. The POD for the critical effect is used as the basis to establish the toxicological ADI or ARfD by incorporation of a suitable uncertainty factor, typically 100 (see under hazard characterization). This in part reflects the uncertainty about inter-species differences and inter-individual variability in kinetics. Hence, any species differences in bioavailability due to pre-systemic metabolism are assumed to be covered by this uncertainty factor. If low absorption is due to the physicochemical characteristics of the compound, this is less likely to show marked inter or intra-species differences and conventional (default) uncertainty factors are assumed to be adequately protective of human health. If low absorption is due to efflux transport, again this is assumed to be covered by the uncertainty factors. Nevertheless, advances in understanding in the biological determinants of bioavailability do provide opportunities for refinement of the exposure assessment and/or risk characterization. Hence, it might be possible in the risk assessment to use appropriate *in vitro*/vivo information regarding potential bioavailability differences, therefore, accounting for differences in the actual drug exposure between and within species (i.e. chemical specific, or data informed adjustment factors (WHO 2001; FAO/WHO 2009a).

Table 5. Impact of variance in veterinary drug residue availability, and extremely low availability, on hypothetical correction factors for use in exposure assessments.

Compound	Mean availability of incurred residues	Availability range of incurred residues	Correction factor for mean availability	Correction factors for range of availability
A1	0.75	0.70–0.80	1.33	1.25–1.43
A2	0.75	0.60–0.90	1.33	1.11–1.67
B	0.15	0.10–0.20	6.67	5.0–10

Relay pharmacology

At its 81st meeting, JECFA further noted that the related topic of relay pharmacology – study of the pharmacodynamic/toxic effect of incurred drug residues – is different from (although related to) the consideration of oral availability of incurred drug residues. This is because the former addresses only biologically active forms of the drug (parent and/or metabolites), whereas the latter addresses total residues absorbed. A study used to determine the relay pharmacology of incurred residues (i.e. investigation of effects after administration of drug via incurred residues in food) need not be identical to one used to determine oral availability of incurred residues (measurement of plasma concentrations after administration of drug via incurred residues in food), although the same study could conceivably achieve both objectives.

Future directions regarding bioaccessibility/bioavailability/ of veterinary drug residues

Although limited oral bioavailability of non-bound residues is currently not considered by JECFA when performing human exposure assessments, this may be considered in the future if additional data necessary to adequately quantify such exposure can be provided. As recently discussed at the 81st meeting of JECFA, such data may include (but is not limited to) differences in oral bioavailability of non-bound drug residues between humans and animal species, or the effect of food processing on oral bioavailability of residues (FAO/WHO 2016b).

Finally, care must be taken when incorporating any potential correction factors for limited availability, as shown in Table 5. Although incurred drug residues of compounds A1 and A2 have the same mean oral availability (0.75), and thus the same mean correction factor (1.33), the larger range of estimated uncertainty for compound A2 results in a correspondingly larger range of correction factors. A conservative risk assessment would utilize the lowest correction factor (i.e. highest bioavailability) in the range. For compounds with low bioavailability of incurred residues (e.g. compound B), the magnitude of the correction factor (5–10-fold) becomes increasingly consequential to the risk assessment. The feasibility of applying such correction factors must, therefore, be carefully considered, as this may unduly affect the final risk characterization.

Metabolites and processing of residues of veterinary drugs

Consumers of food from animals treated with veterinary drugs will be exposed (or potentially exposed) not only to the parent compound but also to any metabolites produced in the target species and possibly also to degradation products formed on processing of the food (e.g. during heating). If metabolites present in food have toxicological or microbiological activity, the possibility that they should be included in the exposure estimate used in risk characterization needs to be considered, i.e. residue definition for risk assessment. JMPR has recently developed some detailed guidance on this, for the case of pesticide residues (WHO 2015). Less comprehensive guidance is available for residues of veterinary drugs, but JECFA has provided some information on this in its recently published guidance for monographers (WHO 2016).

Evaluation of a veterinary drug typically includes detailed qualitative and quantitative information on metabolism in (usually) one of the species used for toxicity testing (typically in rats) and in the target, food-producing species. Where metabolites produced in food-producing species are also formed in the toxicity-testing species at more than a few percent, the toxicity of these metabolites is considered to have been covered by testing of the parent compound and no additional toxicological information would be required. However, in the case of an antimicrobial, as testing for effects on the intestinal microbiota is often *in vitro*, separate consideration would need to be given to the possibility that such metabolites themselves have antimicrobial effects.

When metabolites are formed in food-producing species, but not or to very minor extent in toxicological test species, the potential toxicological (including pharmacological) and microbiological relevance of such metabolites should be considered. Potential toxicity might be addressed by specific studies conducted for this purpose, either *in vitro* or *in vivo* or by applying expert judgment, based for example on read across from structurally similar compounds, or the known effects of specific metabolic transformations on biological activity. For example, if the toxicological concern for the parent were interaction with a specific receptor, for which the pharmacophore was known, metabolic destruction of the pharmacophore would provide reassurance that there was little or no concern for the metabolite. The application of the threshold of toxicological concern (TTC) decision tree (EFSA/WHO 2016) can also be applied in the evaluation of such metabolites or degradation products.

As an example, such approaches were used in the recent evaluation of the β2-adrenoceptor agonist zilpaterol by JECFA (FAO/WHO 2014, 2016b). The critical toxicological (pharmacological) effect, and the basis of both the ADI and the ARfD, was activation of β2-adrenoceptors. The potency of deisopropyl zilpaterol, the main metabolite, was assessed by a combination of *in vitro* and in vivo studies. The effect of the minor metabolite N-acetylated deisopropyl zilpaterol was

assessed to be negligible, based on reasoned argument of the structural requirements of the relevant pharmacophore.

The effect of food processing (freeze/thaw/cook, etc.) on residues of veterinary drugs has not been routinely evaluated. However, changes in hazard due to food processing could be of significance when considering the risk. For example, at the 81st JECFA meeting in 2015 (FAO/WHO 2016b), concerns were raised during the assessment of diflubenzuron about 4-chloroaniline (*p*-chloroaniline, PCA), a possible metabolite but also a degradation product formed on heating at temperatures above 100 °C. 4-Chloroaniline is considered by many authorities as genotoxic and carcinogenic and hence raised more concern than the parent compound. As the temperature of its formation is readily achieved during cooking, the issue of processing-formed degradates of residues of veterinary drugs was raised more generally. Should the possibility of the formation of such compounds be assessed routinely? Such information is not currently required from sponsors by any major regulatory authority. JECFA noted that due to a number of factors, the task of routinely assessing the effects of processing of foods on residues of veterinary drug would be very complex and onerous, much more so than when assessing pesticide residues, for example, where this is routinely undertaken. It was concluded that routine assessment of the effects of processing foods on residues of veterinary drugs was not recommended. However, where there is reason to suspect that processing of foods containing residues of veterinary drugs could have toxicological implications, the effect of processing should be addressed in the assessment of the compound (FAO/WHO 2016b). In addition, if the limited availability of incurred (but non-bound) drug residues were to be taken into account in the exposure assessment, any changes in residue availability due to food processing would also need to be considered.

Conclusions

In order to ensure the protection of consumers' health, the safety of veterinary drug residues in food is routinely assessed by estimating chronic exposure, and verifying that this does not result in an exceedance of the corresponding ADI. However, some veterinary drugs can also present an acute hazard, and while this is considered routinely in the evaluation of pesticide residues resulting in the establishment of an ARfD where necessary, this has so far not been routinely considered for veterinary drugs. Building on the experience with pesticides and taking special considerations for veterinary drugs into account, JECFA developed a guidance on when and how to establish an ARfD for veterinary drugs. One specific consideration is for compounds with antimicrobial activity. JECFA routinely considers for chronic exposures the need to establish microbiological health-based guidance values for potentially harmful effects of the drug residues on the human intestinal microbiota, in addition to the toxicological/pharmacological hazard. The microbiological endpoints of human health concern when establishing a microbiological ADI are disruption of the colonization barrier and an increase in the selection and emergence of antimicrobial resistant bacteria. JECFA now also considers acute exposure scenarios for these effects and has concluded that the latter of these microbiological endpoints will rarely be of relevance in an acute scenario as a single exposure is unlikely to elicit the necessary selective pressure to contribute the emergence of antimicrobial resistant bacteria.

Considerations beyond the acute and chronic exposure scenarios are required to consider the impact of intermittent and less-then-lifetime exposures to residues of veterinary drugs. While for pesticide residues a recent study indicates that the severity of toxicological effects in rodents does not progress after 2–3 months of treatment, the situation is less consistent for human pharmaceuticals, and hence a detailed analysis for veterinary drugs is required before considering any implications for risk assessment.

The differentiation of acute and chronic health risks requires appropriate methodology to correspondingly estimate acute and chronic exposure. Traditionally a simple model diet approach has been used, which is clearly limited with respect to types of animal-derived foods to be considered, as well as to appropriately differentiate between chronic and acute exposure scenarios. Therefore, JECFA developed a more suitable exposure assessment approach, based on high or median concentration levels combined with individual food consumption data from appropriate surveys, the GAEDE to estimate acute exposure and the GECDE to estimate chronic exposure.

Bioaccessibility and bioavailability of veterinary drug residues can potentially impact the exposure assessment and ultimately the risk assessment. To date, JECFA has corrected for the limited bioaccessibility of non-extractable (bound) residues of veterinary drugs according to the Gallo–Torres model, when appropriate. However, a similar correction for bioavailability of the total incurred residues is not presently applied, and additional data to that normally available would be necessary to further refine and quantify the human exposure assessment in such a way. For compounds with very low or variable bioaccessibility/bioavailability, the impact of correction factors on the estimates of exposure may be disproportionately confounded by the uncertainty and thus should be applied with caution.

Recent developments and refinements of dietary risk assessment of residues of veterinary drugs in food undertaken by JECFA highlight the importance of updating risk assessment methods and principles in the regulatory setting, to reflect and integrate the latest scientific knowledge in the process. One remaining challenge in this context is acceptance by regulatory authorities, and international harmonization of updated risk assessment methods and principles.

JECFA will continue in its efforts to update its risk assessment approaches with the aim of improving the soundness and accuracy of the assessments – taking into consideration relevant new scientific developments. In this respect, JECFA is continuing to address the elements laid out in its "decision-tree" and will also address new challenges such as dietary exposure estimation for compounds that are used both as veterinary drugs and as pesticides.

The authors believe that the improvements described in this paper will result in a more differentiated and robust risk assessment for residues of veterinary drugs, enabling sound

evidence-based risk management decisions for the protection of consumers' health.

Acknowledgements

The authors wish to thank Dr Jean Charles LeBlanc, Dr Lynn G. Friedlander, and Dr Holly Erdely for their valuable review of the manuscript; the kind support provided by Ms Isadora Pontalti in organizing the references is also acknowledged. The authors gratefully acknowledge the usefulness of four sets of comments provided by reviewers selected by the Editor and anonymous to the authors.

Declaration of interest

The authors report no conflict of interest. None of the authors has received compensation for their contribution to this manuscript. None of the authors have appeared in regulatory or legal proceedings during the past 5 years with regard to matters discussed in the paper.

Disclaimer

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

Notes

1. Note that median drug residue concentrations have been used by JECFA in chronic exposure assessment since the adoption of the EDI approach in 2006.
2. Or over 1 d, when it is not possible to distinguish a single eating occasion from the data provided.
3. Many authorities make a distinction between the toxicological and the pharmacological effects of veterinary drugs. In practice, they are assessed in the same way when establishing health-based guidance values, and in any event such distinctions are sometimes difficult if not impossible.
4. In assessing the suitability of human data, considerations include the ethics of the study, its scientific quality and suitability for establishing health based guidance values, e.g. number of subjects, representation of the population (e.g. males and/or females), sensitivity of endpoints in experimental studies not covered in humans.
5. Exposure in pregnant women may be sufficiently low that there is no concern for acute effects (i.e. it is less than the ARfD) but exposure in (young) children may exceed the ARfD. This would raise unnecessary concern, as the ARfD is not toxicological relevant to this sub-population.

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