ISSN 1817-7077

FAO JECFA Monographs 18

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Joint FAO/WHO Expert Committee on Food Additives

81st meeting 2015

Food and Agriculture Organization of the United Nations

World Health Organization

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
WORLD HEALTH ORGANIZATION
Rome, 2016

ISBN 978-92-5-109210-1

SPECIAL NOTE

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CONTENTS

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Joint FAO/WHO Expert Committee on Food Additives

Rome, 17–26 November 2015

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[1] Attended session on dietary exposure assessment only.

Abbreviations

ADI	Acceptable daily intake
ADME	Absorption, distribution, metabolism and excretion
APCI	atmospheric pressure chemical ionization (in LC/MS and LC-MS/MS)
ARfD	acute reference dose
AUC	area under the curve
bw	bodyweight
CAC	Codex Alimentarius Commission
CAS	Chemical Abstracts Service
CCβ	detection capability
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Foods
Cl_T	total body clearance
C_{max}	maximum concentration (plasma)
C_R	clearance (renal)
CRD	Conference Room Document (CCRVDF)
CV	coefficient of variation
CVMP	Committee for Medicinal Products for Veterinary Use, European Medicines Agency
d	day
DAD	diode array detector (in HPLC)
EC	electrochemical detection (liquid chromatography)
EDI	estimated daily intake
EINECS	European Inventory of Existing Commercial chemical Substances
EMA	European Medicines Agency (formerly EMEA)
EMEA	European Agency for the Evaluation of Medicinal Products
EPA	Environmental Protection Agency (United States of America)
eq	equivalent
ESI	electrospray interface (in LC/MS and LC-MS/MS)
FAO	Food and Agriculture Organization of the United Nations
GC	gas chromatography
GC/MS	gas chromatography/mass spectrometry

GEADE	global estimate of acute dietary exposure
GECDE	global estimate of chronic dietary exposure
GLP	good laboratory practice
GVP	good veterinary practice
h	hour
HPLC/FL	high performance liquid chromatography with fluorescence detection
i.m.	intramuscular [injection]
INN	International Non-proprietary Name
IPCS	International Programme on Chemical Safety
IR	Infrared
IT	ion trap (mass spectrometer)
IUPAC	International Union of Pure and Applied Chemistry
i.v.	intravenous
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
kg	kilogram (10^3 g)
L	litre
LC	liquid chromatography
LC/MS	high performance liquid chromatography/mass spectrometry
LC-MS/MS	high performance liquid chromatography/tandem mass spectrometry
LOAEL	lowest observed adverse effect level
LOD	limit of detection
LOQ	limit of quantification
LSC	liquid scintillation counter
kBq	kilobecquerel
mCi	millicurie
µg	microgram (10^{-6} g)
mg	milligram (10^{-3} g)
min	minimum or minute
mL	millilitre
mM	milliMolar

mmol	millimole
MR	marker residue
MRL	maximum residue limit
MRT	mean residence time
MS	mass spectrometry
MSPD	matrix solid phase dispersion
MW	molecular weight
NA	not analyzed
ND	not detectable
NPD	nitrogen-phosphorus detector (gas chromatography)
ng	nanogram (10^{-9} g)
NOAEL	no observed adverse effect level
NQ	non-quantifiable
ODS	Octadecylsilane
PBPK	physiologically based pharmacokinetic models
PCA	*p*-chloroaniline
PLE	pressurized liquid extraction
QA	quality assurance
QC	quality control
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
RP	reverse phase
rsd	repeatability standard deviation
R_t	retention time
$R_{t/p}$	ratio of tissue radioactivity concentration to that of plasma
s.c.	subcutaneous [injection]
SD	standard deviation
SPE	solid phase extraction
SRM	selected reaction monitoring (in mass spectrometry)
$t_{½}$	half life
$t_{½}\alpha$	plasma distribution half-life
$T_{½}\beta$	plasma elimination half-life

T_{max}	time to peak plasma concentration
TLC	thin layer chromatography
TMDI	theoretical maximum daily intake
TPAR	total pharmacologically active residue
TR	total residue
TRR	total radiolabelled residues
TTC	threshold of toxicological concern
USDA	United States Department of Agriculture
UV	ultraviolet
VD	volume of distribution
$Vd_{(ss)}$	volume of distribution at steady state
VICH	International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products
WHO	World Health Organization

1. Introduction

This volume of FAO JECFA Monographs contains residue evaluation of certain veterinary drugs prepared at the 81st Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held in Rome, Italy, 17–26 November 2015. This was the twenty-fourth meeting of JECFA convened specifically to consider residues of veterinary drugs in food-producing animal species. The tasks for the Committee were to further elaborate principles for evaluating the safety of residues of veterinary drugs in food and for establishing acceptable daily intakes (ADIs) and/or acute reference doses (ARfDs), and to recommend maximum residue limits (MRLs) for substances on the agenda when they are administered to food-producing animals in accordance with good veterinary practice in the use of veterinary drugs. The enclosed monographs provided the scientific basis for the recommendations of MRLs.

Background

In response to the growing use of veterinary medicines in food animal production systems internationally and the potential implications for human health and fair trading practices, a Joint FAO/WHO Expert Consultation on Residues of Veterinary Drugs was convened in Rome in November 1984 (FAO/WHO, 1985). One of the major recommendations of this consultation was the establishment of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) and the periodic convening of an appropriate expert body to provide independent scientific advice to this Committee and to member countries of FAO and WHO. At its first session, in Washington, DC, in November 1986, the CCRVDF reaffirmed the need for such a scientific body and made a number of recommendations and suggestions to be considered by JECFA (CCRVDF, 1986). In response to these recommendations, the 32nd JECFA meeting was devoted entirely to the evaluation of residues of veterinary drugs in food - a new responsibility for the Joint FAO/WHO Expert Committee on Food Additives.

81st Meeting of JECFA

The present volume contains monographs on the residue data of six substances scheduled for evaluation at the 81st Meeting of the Committee. Of the substances on the original agenda, three substances were new evaluations (diflubenzuron, sisapronil, teflubenzuron) and three were re-evaluations (ivermectin, lasalocid sodium, zilpaterol hydrochloride). Lasalocid sodium was re-evaluated in response to issues raised in Concern Forms submitted by members of the Codex Alimentarius (European Union, Canada). No new data were submitted for lasalocid sodium. The Concern Forms submitted requested that JECFA (i) review the approach used to estimate consumer short term exposure to lasalocid residues, (ii) review the proposed MRLs as there was a concern that these MRLs might expose consumers to residues of lasalocid higher than the ADI. The 22nd session of the CCRVDF the Committee had agreed to hold the MRLs recommended for lasalocid sodium at Step 4 for consideration at its next Session based on the recommendations of the 81st JECFA.

The monographs are prepared in a uniform format consistent with the data provided and the specific request for risk assessment by CCRVDF. The format includes identity of substance, residues in food and their evaluation, metabolism studies, tissue residue depletion studies,

methods of residue analysis, a final appraisal of the study results, and if appropriate, recommendations on MRLs. A summary of the recommendations on compounds on the agenda and further information required is included in Annex 1. In addition, a summary of JECFA evaluations of residues of veterinary drugs in foods from the 32nd meeting to the present 81st meeting can be found in Annex 2.

The Committee continued to implement some of the more significant recommendations from workshop to update the principles and methods of risk assessment for MRLs for pesticides and veterinary drugs, held jointly by FAO/RIVM/WHO, in Bilthoven, The Netherlands, 7– 11 November 2005. A paper prepared by a working group of JECFA which was intended to elaborate guidance on the establishment of acute reference doses (ARfDs) for veterinary drugs in food by JECFA was discussed. The Committee agreed on principles which will allow the working group to develop guidance on when and how to establish ARfDs for veterinary drugs. A pilot project was continued to evaluate alternate approaches to estimate daily intakes of residues of veterinary drugs in food for chronic and acute exposure intake estimates, based on the recommendations of the Joint FAO/WHO Expert Meeting on Dietary Exposure Assessment Methodologies for Residues of Veterinary Drugs, 7–11 November 2011, Rome, Italy. In the case of zilpaterol hydrochloride (and also ivermectin), the Committee agreed that the Global Estimate of Acute Dietary Exposure (GEADE) was the preferred approach to be used by The Committee in the assessment of potential acute exposure of consumers to residues od these drugs.

The monographs of this volume must be considered in the context of the full report of the meeting, which will be published in the *WHO Technical Report Series*.

On-line editions of *Residues of some veterinary drugs in animals and foods* (from FAO JECFA Monographs and *FAO Food and Nutrition Paper*, No. 41) are available online at http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-vetdrugs/en/. The search interface is available in five languages (Arabic, Chinese, English, French and Spanish) and allows searching for compounds, functional classes, ADI and MRL status.

Contact and feedback

More information on the work of the Committee is available from FAO at http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/en/.

Please send questions and feedback to jecfa@fao.org.

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2. Residue bioavailability and impact on exposure analysis

First draft prepared by

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Background

In the safety evaluation of residues of veterinary drugs, the bioavailability (and thus potential for pharmacological activity) of bound residues has been considered in human exposure when suitable data have been provided to the Committee. Zilpaterol hydrochloride was first assessed at the 78th meeting of JECFA (WHO, 2014). The Committee concluded that it was not possible to recommend MRLs for zilpaterol due to incomplete data. As part of its response to the 78th JECFA decision, the Sponsor questioned why JECFA did not consider the bioavailability of residues in its exposure assessment. Specifically, in a submission to the 81st meeting of JECFA, the Sponsor stated with regard to the procedures used by JECFA for dietary intake assessment:

"The applicant prepared a comprehensive assessment of the pharmacokinetics and pharmacological effects of zilpaterol and other ß-agonist. It is concluded that the pharmacological effect of incurred residues (relay pharmacology) should be quantitatively considered in the dietary intake assessment and the calculation of the maximum residue limits. This would be consistent with previous risk assessments where JECFA has considered poor oral bioavailability of residues in the dietary exposure assessment".

The Sponsor cited as an example the evaluation of triclabendazole by the 70th and 75th meetings of the Committee (WHO, 2009; WHO, 2012) and concluded:

"In summary, pharmacological effects resulting from possible incurred zilpaterol residues (ADI has been set on a pharmacological endpoint) are reduced by a factor of 10 in comparison to an oral bolus administration."

As only bioavailable zilpaterol residues will elicit pharmacological effect (there is no local GI effect of unabsorbed zilpaterol residues), the Committee considered that the request from the Sponsor was that only the *bioavailable* portion of incurred residues be considered in the exposure assessment for zilpaterol hydrochloride. Incurred residues in this context include all (total) zilpaterol residues: parent zilpaterol (free base) + metabolites, and both bound and unbound fractions. The Sponsor had therefore suggested that the evaluation of the pharmacological effect of incurred (i.e. total) zilpaterol residues should be based on an evaluation of the *bioavailable* portion of *total* residues resulting from the use of zilpaterol hydrochloride. The Sponsor referred to this approach as the "relay pharmacology" of residues. The applicant has cited as a precedent for this approach the evaluation of triclabendazole by the 70th and 75th meetings of the Committee (WHO, 2009; WHO, 2012).

Definition of bioavailable residues

The Codex Alimentarius defines *bioavailability* as "The proportion of the ingested nutrient or relate7d substance that is absorbed and utilised through normal metabolic pathways" (FAO/WHO, 2015). The Codex Committee on Residues of Veterinary Drugs in Foods has

further defined the *bioavailable residues* of veterinary drug residues in foods as “Those residues that can be shown, by means of an appropriate method (e.g. Gallo-Torres method) to be absorbed into systemic circulation when fed to laboratory animals” (FAO/WHO, 2003).

Guidance on the assessment of bound residues

The bioavailability of drugs residues in exposure assessment has been considered previously by national and regional regulatory agencies and international organizations, including JECFA.

United States Food & Drug Administration

When the parent compound is not considered a carcinogen, guidance from the United States Food & Drug Administration (FDA) states that the portion of the covalently bound residue that the applicant demonstrates is not bioavailable will be discounted from the residue of toxicological concern, ”provided that a substantial portion (50%) of the covalently bound residue is not bioavailable”(FDA-CVM, 2006). In adjusting “the total residue based on the relative bioavailability of the parent compound and the covalently bound residue”, the FDA guidance notes that the “experimental technique described by Gallo-Torres is an example of an acceptable protocol”.

European Medicines Agency

The issue has been addressed by authorities in the European Economic Community (EEC) in the Reflection Paper on Assessment of Bioavailability of Bound Residues in Food Commodities of Animal Origin in the Context of Council Regulation (EEC) No 2377/90, issued by the Committee for Medicinal Products for Veterinary Use (CVMP) of the European Medicines Agency (EMA, 2008a). The document outlined procedures which were deemed appropriate to address the issue of bioavailability of residues when considering the potential dietary exposure for consumers. In essence, it was considered that when residues are demonstrated to be bound and “non-bioavailable”, such residues may be assumed to be of no consumer concern and can be discounted from the dietary intake dose. It was also noted that the consideration of bound residues in the exposure assessment requires evidence of covalent binding and a subsequent quantification of the bioavailability of such residues.

Important considerations for assessment of bioavailability of incurred residues noted in the Reflection Paper included:

- Quantitative testing of bioavailability and determination of the bioavailable residue fraction is a prerequisite in the exposure assessment of bound residues.
- Bioavailability after oral ingestion will vary depending on the compound involved and on the nature of binding.
- Bioavailability studies typically involve feeding animals (usually rat as default species) tissues which contain radiolabelled incurred residues.
- Experimental parameters must be carefully chosen.
- The Gallo-Torres method is often recommended and has given valuable results.

In comments provided in response to the reflection paper, the International Federation of Animal Health (IFAH – Europe) proposed that the bioavailability of *total* residues should be considered in exposure assessment, instead of just the bioavailability of *bound* residues (EMA, 2008b). In response, the CVMP stated that the focus of the Reflection Paper was the "bioavailability of bound residues". The approach proposed by IFAH – Europe was therefore beyond the scope of the Reflection Paper and the CVMP in consequence rejected the proposal that the bioavailability of total residues should be considered in the dietary intake calculation. In calculating dietary intake, CVMP assumes all non-bound (e.g., all free and extractable residues) have a bioavailability of 100%. The CVMP responded (EMA, 2008b) that their "present approach uses a (worst case) default assumption of 100 % bioavailability of residues (except for residues that have been shown to be bound/non-bioavailable and, in case of antimicrobials, residues that are not bioavailable to the gut flora)".

The final guidance from CVMP was therefore limited in scope to bioavailability of bound residues only.

International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH)

VICH Guideline 46: Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-Producing Animals: Metabolism Study to Determine the Quantity and Identify the Nature of Residues provides guidance on the assessment of bound residues (VICH, 2011). It notes that the characterization of bound residues "is usually difficult, involving vigorous extraction conditions or enzymic preparations that can lead to residue destruction or artifact formation." In addition, "the biological significance of residues of veterinary drugs in foods usually depends on the degree to which those residues are absorbed when the food is ingested". The guidance further notes that "the determination of the bioavailable residues that result when tissue containing bound residue is fed to test animals can be a useful characterization tool", suggesting also that the method of Gallo-Torres "might be an appropriate procedure for demonstrating bioavailability."

The guideline does not suggest that the bioavailability of total residues should be considered.

Guidance contained in Environmental Health Criteria 240 (EHC 240)

It is stated in EHC240 that "JECFA recognizes that the use of veterinary drugs in food-producing animals can result in residues that cannot be extracted from tissues using mild procedures" (FAO/WHO, 2009). It further notes that there may be cases in studies using radio-labelled drug where "non-extractable residues may be releasable using more specific or vigorous methods, such as the application of procedures for the release of conjugated residue components, without destroying the compounds of interest". In such cases, the "remaining fraction of the bound radioactivity may partly consist of fragments of the drug incorporated into endogenous compounds (endogenous fraction) that would be of no toxicological concern". It is recognized that the bound residues "can frequently not be fully characterized" and notes that "JECFA has developed a procedure to estimate the dietary exposure to residues of a drug that has a bound residue component" (FAO/WHO, 1989). This procedure includes an

accounting for "the toxicological potency and bioavailability of the residues". An equation which describes the calculation of the total residue of (toxicological) concern for a given tissue is provided:

$$Residue = P_0 + \sum_{n=n_1}^{n_x} (Mn * An) + (Bound\ residues * fraction\ bioavailable * A_b)$$

Where

- P_0 is the amount of parent drug per kilogram of tissue,
- $n_1 \ldots n_x$ are the different metabolites of the parent drug,
- Mn is the amount of (unbound) drug metabolite n per kilogram of tissue,
- An is the toxicological potency of n relative to that of parent drug,
- A_b is the estimated relative toxicological potency of the metabolites in the bound residue (when no information is available, use $A_b = 1$).

The following principles are applied:

- Where the endogenous fraction is not known, it should be given a value equal to zero.
- If the bioavailable fraction of the residues is not known, JECFA considers that a bound residue is of no greater concern than the substance for which the ADI was established, and therefore this fraction is taken to be equal to 1.
- In considering the safety of bound residues, JECFA acknowledges that a suitable extractable residue component may be selected as the marker residue used for recommending an MRL if bound residues make up an insignificant portion of the total residue. In these cases, it is not necessary to apply the above calculations.
- Where bound residues become a significant portion of the total residues of concern, then the procedure described may be used to assess their safety.

Past JECFA Decisions

The issue of bioavailability of bound residues was first considered at the 34th meeting of the Committee (FAO/WHO, 1989), which applied a correction for the bioavailability of bound residues in the dietary intake calculation of two substances evaluated at that meeting, albendazole and trenbolone acetate using the Gallo-Torres model (Gallo-Torres, 1977). The Committee also defined "bioavailable residues", providing the definition adopted in CAC/MISC 5-1993 (FAO/WHO, 1993) and provided in Annex 3 of the Meeting Report the procedure to be used in calculating "the daily intake of residues taking into account data on toxicological potency and bioavailability". The procedure is that described in EHC 240 and includes the equation to be used in the calculation that is included in EHC240. With the apparent exception of the decision on triclabendazole (WHO, 2009; WHO 2012) cited by the Sponsor, the Committee has consistently followed the approach described in the Report of the

34th Meeting (WHO, 1989) in assessing the bioavailability of residues in the dietary intake calculations.

Summary of Guidance Documents Reviewed by JECFA

The Committee noted that there is precedent for considering the bioavailability of incurred drug residues when establishing the exposure assessment in humans. Previously published JECFA, VICH, CVM, and EMA documents provide consistent guidance in this matter. All refer back to the Gallo-Torres paper from 1977 as a historical source.

All guidance documents recommend that a correction be applied only for the bioavailability of bound residues in tissue, as clearly illustrated by the trenbolone acetate example from the 34th JECFA report (FAO/WHO, 1989). No guidance document currently recommends the consideration of the bioavailability of total (extractable + bound) residues. Furthermore, consideration of total residue bioavailability was explicitly proposed, and subsequently rejected, in the development of the EMA guidance document.

Conclusion

There is clear direction from regulatory agencies and in EHC 240 regarding the use of bioavailability correction factors for *bound* drug residues in human exposure assessments. Bioavailability of *total* drug residues has not been similarly addressed by regulatory agencies, though the EMA has clearly ruled out this approach at this time. The bioavailability of *total* incurred drug residues appears to have been used in only one drug evaluation previously conducted by the Committee.

Therefore, the Committee considered that the most appropriate approach when assessing human exposure to veterinary drug residues is the Gallo-Torres model as outlined in EHC 240 and in various regulatory guidance documents. This entails applying a bioavailability correction factor for *bound* drug residues, but no correction factor for *free/extractable* residues.

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3. Diflubenzuron

First draft prepared by

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Identity

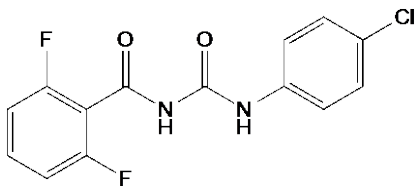
International Non-proprietary Name (INN): Diflubenzuron

Synonyms: Releeze 0.6 g/kg (EWOS AS), EWOS DFB (FAV Recalcine), Dimilin, Micromite, Adept, Du-Dim, Device, DU 112307, PH 60-40, TH 6040, ENT-29054, OMS 1804 (Crompton BV trade names and/or past development codes).

IUPAC Name: 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea

Chemical Abstract Service Number: 35367-38-5

Structural formula:



Molecular formula: $C_{14}H_9ClF_2N_2O_2$

Molecular weight: 310.7 g mol^{-1}

Other information on identity and properties

Pure active ingredient:	Diflubenzuron (purity ≥ 95%)
Appearance:	White crystalline solid
Melting point:	228 °C
Solubility in water:	0.08 mg/L at 25 °C at pH 7
Solubility in acetonitrile:	2.0 g/L
Solubility in acetone:	6.5 g/L
Solubility in dichloromethane:	1.8 g/L
Solubility in n-hexane:	0.063 g/L
Vapor pressure:	≤ 1.2 x 10^{-7} Pa at 25 °C
Log $K_{o/w}$:	3.89 at 22 °C at pH 3
UV_{max}:	257 nm

Background

Diflubenzuron (CAS No. 35367-38-5), besides its use in agriculture, horticulture and forestry against larvae of Lepidoptera, Coleoptera, Diptera, Hymenoptera, and in public health against larvae of mosquitoes, is used as a veterinary drug for the treatment of sea lice (Lepeophtheirus salmonis Krøyer and Caligus rogercresseyi Boxshall and Bravo, 2000) infestations in Atlantic salmon (Salmo salar L.). Diflubenzuron acts by interference with the synthesis of chitin. Demand for chitin synthesis is greatest at the moult between growth stages and hence parasites are killed due to disruption of the moulting process. The fatal effect occurs by the inability of the treated parasites to moult properly due to incomplete development of chitin, with subsequent collapse of the exoskeleton.

The toxicity of diflubenzuron was evaluated by the FAO/WHO Joint Meeting on Pesticide Residues (JMPR) in 1981 (JMPR, 1982), 1984 (JMPR, 1985) and 1985 (JMPR, 1986); an ADI of 0 – 0.02 mg/kg bw, based on NOAELs for methaemoglobin formation in the submitted long-term toxicity/carcinogenicity studies in dogs, rat and mice, was established at the latter Meeting. This ADI was maintained by a 1994 WHO Core Assessment Group that prepared Environmental Health Criteria 184. The USA EPA published a Re-registration Decision for diflubenzuron in August 1997 (EPA, 1997). Diflubenzuron has also been reviewed by the European Commission under Directive 91/414/EC and a MRL of 1000 µg/kg, pursuant to Directive 2377/90, based on an ADI of 0.0124 mg/kg bw/day using the mice studies and applying a safety factor of 100, was published in 1999 (EMEA, 1999).

Under the periodic review program, toxicology data for diflubenzuron were re-evaluated by JMPR in 2001 (WHO, 2002) and residues in 2002 (JMPR, 2002) and 2011 (JMPR, 2012). The JMPR has concluded that the long-term intake of residues of diflubenzuron in food resulting from its uses that have been considered by JMPR is unlikely to present a public health concern.

At the 22nd Session of the Codex Committee on Residues of Veterinary Drugs in Food (CCRVDF), concerns were raised about the metabolism of diflubenzuron and formation of the genotoxic metabolite, 4-chloroaniline (p-chloroaniline or PCA). Following discussions, the Committee noted that an ADI of 0-0.02 mg/kg body weight had previously been established by JMPR for diflubenzuron and requested JECFA to recommend MRLs for diflubenzuron in salmon muscle and skin in natural proportion.

Residues in food and their evaluation

Conditions of use

Diflubenzuron is a benzoylurea pesticide used in aquaculture for the treatment of sea lice in Atlantic salmon in the Northern hemisphere and sea lice infestation in salmon in the Southern hemisphere.

Diflubenzuron was first registered as an insecticide in the United States in 1979 (Patterson 2004) and is also used in agriculture, horticulture and forestry against larvae of Lepidoptera, Coleoptera, Diptera, Hymenoptera and in public health against larvae of mosquitoes and other noxious insects.

Dosage

Diflubenzuron is licensed in Norway as a premix (90% pre-concentrate in pelleted diet) at a final concentration of 0.6 g diflubenzuron per kg. The intended oral dose is 3 mg diflubenzuron per kg of fish biomass per day for fourteen consecutive days. The recommended withdrawal period is 105 degree-days. The number of treatment periods per year could be two to three.

In Chile, diflubenzuron is licensed as an oral powder (80% w/w) with an intended oral dose of 6 mg diflubenzuron per kg of fish biomass per day for fourteen consecutive days. The recommended withdrawal period is 300 degree-days.

Pharmacokinetics and metabolism

Pharmacokinetics in laboratory animals

Rats

Single oral doses of 4 to 1000 mg/kg bw, a repeated oral dose of 5 mg/kg bw per day for 14 days, and single dermal doses of 0.05 and 0.5 mg/10 cm^2 of diflubenzuron were administered to rats (EMEA, 1999). Diflubenzuron is absorbed from the gastrointestinal tract and the absorption decreases with increasing dose. Following a dose of 4 mg/kg bw, 42.5% of diflubenzuron was absorbed; however, only 3.7% of a dose of 900 mg/kg bw was absorbed. In rats administered a single oral dose of [^{14}C]-diflubenzuron at 5 mg/kg bw, the highest mean concentrations of radioactivity at 4 h were found in fat (4672 µg eq /kg), ovaries (3737 µg eq /kg), liver (2265 µg eq /kg), heart (1345 µg eq /kg), kidney (1200 µg eq /kg) and brain (984 µg eq /kg). At 48 hours post dose and subsequent times, the highest concentrations were in liver (431 µg eq /kg) and erythrocytes (379 µg eq /kg). No difference was observed in results between males and females. Dermal absorption of diflubenzuron was less than 1%.

The major route of elimination of diflubenzuron is via faeces, urine and bile, as intact diflubenzuron (EMEA, 1999). After administration of a single dose of diflubenzuron, excretion is almost complete within 24 to 48 h, whereas following repeated dosing, the excretion of diflubenzuron and metabolites is slightly slower, being almost complete only after 48 to 96 h. After a single dose of diflubenzuron of 4 mg/kg bw, up to 28%, 30% and 36% of the administered drug could be found in urine, bile and faeces, respectively. Biliary and urinary elimination decreases with increasing dose in a dose dependent manner.

In a study to investigate the intestinal absorption of diflubenzuron in Wistar rats, a mixture labelled with ^{14}C in the amino moiety (31.1 mCi/g) and ^{3}H in the 2,6-difluorobenzoyl moiety (6.3 mCi/g) of diflubenzuron was used (Willems *et al*., 1980). The radiochemical purity, determined by TLC, was >99%. The radiolabelled compound, in suspension (1% tragacanth solution), at doses ranging from 4 mg/kg bw to 1000 mg/kg bw, was administered to female and male rats by gavage. Urine was collected for 6 and 24 h, and also at further 24 h intervals. Faeces were collected at 24 h intervals for 3 days, and then at the conclusion of the experiment. In a second group of female rats, cannulation of the bile duct was performed. Bile was collected at 6, 24, 48 and 72 h, while urine and faeces were collected at 24-h intervals for 72 h. The

cumulative excretion of radioactivity in urine and faeces after oral administration of radiolabelled diflubenzuron is shown in Table 3.1.

Table 3.1. Cumulative excretion (6 days) of radioactivity in urine and faeces after oral administration of [^{3}H, ^{14}C]-diflubenzuron (dose of 5 mg/kg bw) to rats. Results are mean values of 6 animals, with standard deviation in parentheses (Willems *et al*., 1980).

	Percentage of dose	
	[^{3}H]-benzoyl moiety of diflubenzuron	**[^{14}C]-anilino moiety of diflubenzuron**
Urine	24 (3.6)	22 (3.5)
Faeces	69 (3.8)	50 (1.7)
Total	93 (3.6)	72 (3.0

The cumulative excretion of radioactivity in bile and urine over a period of 72 h after oral administration of radiolabelled diflubenzuron to rats with cannulated bile ducts is shown in Table 3.2.

Table 3.2. Cumulative excretion of radioactivity in bile and urine during 72 h after oral administration of [^{3}H, ^{14}C]-diflubenzuron (dose of 5 mg/kg bw) to rats with cannulated bile ducts. Results are given for each of two rats (Willems *et al*., 1980).

	Percentage of dose	
	[^{3}H]-benzoyl moiety of diflubenzuron	**[^{14}C]-anilino moiety of diflubenzuron**
Bile	32 and 23	41 and 27
Urine	19 and 20	22 and 24

In rats with (Table 3.2) and without (Table 3.1) cannulated bile ducts, about 20% of the administered ^{3}H and ^{14}C radiolabelled dose was excreted in the urine. In the bile, an average of 33% of the dose was recovered, with no significant difference between the different labels. The results (sum of the urinary and biliary excretions) indicate that about half of the administered dose was absorbed.

The intestinal absorptions, as a function of dose level, are shown in Tables 3.3 and 3.4. The percentage of the dose excreted in the urine decreased with increasing dosage, while total recoveries remained constant. In bile-cannulated rats, the proportion of biliary to urinary excretion does not change significantly as the dose was increased.

Table 3.3. Excretion of radioactivity in urine and faeces after oral administration of [^{14}C]-diflubenzuron to rats. Duration of the experiment: 120 h. Results are mean values of 6 animals, with standard deviation in parentheses (Willems *et al*., 1980).

Dose (mg/kg)	Sex	Cumulative excretion as % of dose	
		Urine	Urine and faeces
4	female	27.6 (1.4)	88.3 (1.2)
16	female	13.0 (0.7)	86.7 (3.6)
48	male	6.2 (0.9)	92.4 (3.3)
128	female	2.7 (0.3)	91.1 (1.6)
128	male	3.4 (0.5)	91.2 (4.2)
1000	male	1.0 (0.1)	84.5 (9.1)

Table 3.4. Urinary and biliary excretion of radioactivity in female rats with cannulated bile ducts after oral administration of [^{14}C]-diflubenzuron. Duration of the experiment: 72 h. Results are mean values, with standard deviation in parentheses (Willems *et al*., 1980).

Dose (mg/kg)	Number of rats	Cumulative excretion as % of dose		
		Urine	Bile	Total
4	3	12.0 (1.0)	30.4 (5.2)	78.1 (1.3)
16	4	7.7 (1.1)	16.4 (1.6)	78.1 (9.2)
128	4	2.9 (0.4)	6.4 (1.8)	84.0 (3.4)
900	4	2.2 (1.1)	1.5 (0.4)	78.8 (8.7)

The data show that the intestinal absorption, measured as the sum of urinary and biliary excretion, diminished with increasing dose, from about 50% at 4 mg/kg to about 4% at 900 mg/kg.

Bluegill sunfish

Diflubenzuron is accumulated from water into fish tissue at levels up to 80-fold. When bluegill sunfish (*Lepomis macrochirus,* 7 cm length) were exposed to water containing 10 µg/L of diflubenzuron for 24 h, 48 h and 72 h, tissue residues were 158, 306 and 266 µg/kg, respectively (Schaefer *et al*., 1979). After 24 to 48 h exposure, fish degrade and eliminated diflubenzuron and the excretory products were neither the parent compound nor *p*-chlorophenylurea.

The bioconcentration of [^{14}C]-diflubenzuron by bluegill sunfish was also evaluated in a dynamic 42-day study (28 days of treatment with diflubenzuron followed by 14 days

depuration) (IPCS, 1996). Radioanalyses of fillet, whole fish and visceral portions were performed throughout the exposure period. Daily bioconcentration factors ranged from 34 to 200, 78 to 360, and 100 to 550 for fillet, whole fish and viscera, respectively. Tissue concentrations of [^{14}C]-diflubenzuron ranged from 0.25 to 1.7 mg/kg for fillet, 0.58 to 3.3 mg/kg for whole fish, and 0.75 to 4.7 mg/kg for viscera. Radioanalysis throughout the depuration period (test fish were placed in clean water for 14 days) indicated 99% depuration each for fillet, whole fish and viscera. The mean concentrations of [^{14}C]-diflubenzuron in fillet decreased from 1.6 mg/kg on day 28 of exposure to 0.012 mg/kg by day 14 of the depuration period. Residue concentrations in whole fish decreased from 3.3 mg/kg on day 28 of exposure to 0.038 mg/kg by day 14 of the depuration period. Concentrations in viscera depleted from 4.4 mg/kg on day 28 of exposure to 0.056 mg/kg by day 14 of depuration. The maximum bioaccumulation factor (550) found in the bluegill sunfish is much lower than that expected based on lipophilicity (7800), indicating rapid degradation and depuration. In addition to the parent compound (80%), 2,6-difluorobenzamide (10-13%) and three other minor metabolites were identified. 4-Chloroaniline was not detected (limit of detection 0.01 mg/kg).

Pharmacokinetic in food producing animals

Chicken

Pharmacokinetic parameters of [^{14}C]-diflubenzuron in White Leghorn (WL) egg-production chickens and Rhode Island Red/Barred Plymouth Rock (RIR/BPR) meat-production chickens were evaluated (Opdycke and Menzer, 1984). Three chickens of each type were given a single bolus intravenous dose of 1 mg/kg of [^{14}C]-diflubenzuron and 3 chickens of each type were given gelatine capsules containing radiolabelled diflubenzuron at a single dose of 5 mg/kg of [^{14}C]-diflubenzuron. Sequential blood samples were taken by heart puncture at 0.15, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, 10, 12 and 22 or 24 hours after injection. Following oral administration, blood was sampled at 2.0, 4.0, 6.0, 8.0, 12, 18, 24, 30 and 36 h and assayed for radioactivity. In addition, excreta were collected continuously during the periods of frequent heart punctures. A two-compartment open model was assumed from the study, following intravenous administration of radiolabelled diflubenzuron. Absorption parameters were estimated using constants determined from the intravenous dose experiment. The half-life of elimination from the central compartment was 14.70 h for WL chickens and 8.45 h for RIR/BPR chickens. Absorption of radiolabelled diflubenzuron after a single oral dose of 5 m/kg bw was both faster and more complete in RIR/BPR chickens. The absorption rate constants were 0.046 h^{-1} and 0.192 h^{-1} for WL and RIR/BPR chickens, respectively. Comparison of the absorption patterns in WL and RIR/BPR chickens indicates both a much faster and greater absorption of diflubenzuron in the RIR/BPR than in the WL chickens. The concentrations of [^{14}C]-diflubenzuron in excreta are shown in Table 3.5.

Table 3.5. Percentage (mean ±SD) of [^{14}C]-diflubenzuron equivalents eliminated following oral and intravenous administration of the radiolabelled compound to chickens {WL: White Leghorn; and RIR/BPR: Rhode Island Red/Barred Plymouth Rock} (Opdycke and Menzer, 1984).

Time post-dose (h)	Percentage of [^{14}C]-diflubenzuron equivalents			
	Intravenous, 1 mg/kg		Oral, 5 mg/kg	
	WL	RIR/BPR	WL	RIR/BPR
0-12	4.2 ± 5	20 ± 4	35 ± 12	33 ± 13
12-24	7.5 ± 5	9 ± 4	10 ± 3[a]	18 ± 5
Total	11.7 ± 5	29 ± 4	45 ± 15	51 ± 18

[a] for 12-36 h; SD = Standard Deviation.

Excretion after a single intravenous dose showed rapid elimination, 11.7% and 29% of the administered dose in 22-24 h for WL and RIR/BPR chickens, respectively.

Salmon

The pharmacokinetic parameters of diflubenzuron in Atlantic salmon smolts (approx. 60 g, 22 fish) were studied after a single dose via gavage of 75 mg /kg bw of [^{14}C]-radiolabelled diflubenzuron at 8 °C (Horsberg and Hoy, 1991). The [^{14}C]-diflubenzuron (18.38 mg) was mixed with non-radiolabelled diflubenzuron (81.67 mg) and suspended in 7 mL peanut oil. After a 21-day acclimatization period, a stomach tube was inserted and 0.3 mL of the suspension was administered to each fish. After 2 h, 12 h, 2 d, 6 d, 10 d, 13 d, 20 d and 27 d, fish were slaughtered and 1 to 2 fish were sampled for autoradiography. Samples were taken from blood, brain, muscle, abdominal fat, kidney, liver, bile cartilage and cutaneous mucus. An estimate of the percentage of the administered dose present in liver, kidney, blood and muscle at different sampling times was calculated using the total content of radioactivity in the organ, the weight of the fish and the total dose of radioactivity administered to each fish. Whole-body autoradiography, liquid scintillation counting and TLC were used to evaluate the kinetic properties. The concentration of radioactivity in brain and cartilage was highest 12 h after administration, with concentrations of 13.8 µg/g and 10.9 µg/g, respectively. In bile, the concentration of radioactivity varied between 275 and 1066 µg/g the first 10 days after administration, then dropped to less than 4 µg/g for the rest of the period.

The calculated percentages of the administered dose, which were present in muscle, liver, blood and kidney, are shown in Figure 3.1. The highest amount of radioactivity was detected 12 h after administration of [^{14}C]-diflubenzuron. It was concluded that diflubenzuron is poorly absorbed from the intestine, because only 3.7% of the administered dose was detected in blood, muscle, liver and kidney 12 h after administration. The radioactivity in bile was very high, indicating that the major excretion pathway for the drug is the biliary route.

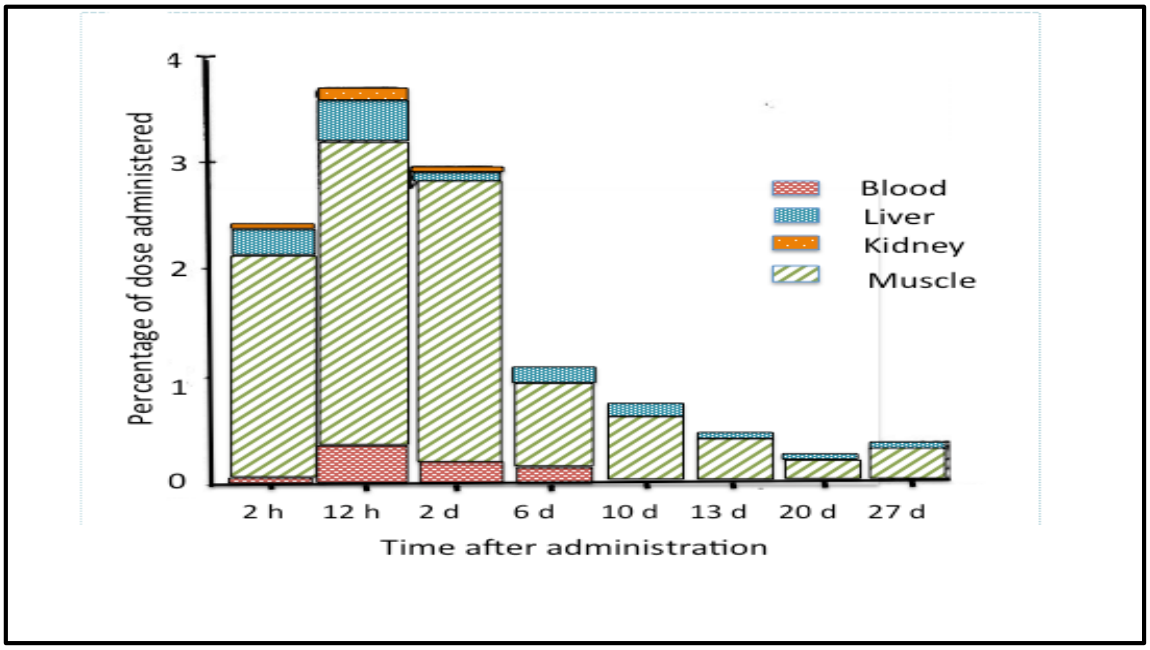


Figure 3.1. Percentage of the administered dose present in muscle, liver, kidney and blood at different intervals after oral administration of 4.3 mg/kg bw [^{14}C]-diflubenzuron to Atlantic salmon (Adapted from Horsberg and& Hoy, 1991).

In a field trial, conducted at a commercial fish farm in Norway, with full scale stocking densities of Atlantic salmon, the clearance time of diflubenzuron was established for liver, skin and muscle (Wallace *et al.*, 1997). The study was conducted at a water temperature of 14.6 to 15.6 °C. Diflubenzuron medicated pellets (0.63 g/kg) were administered to the fish by way of automatic feeding machines, for 14 consecutive days. The daily dose of diflubenzuron ranged from 2.66 to 3.2 mg/kg bw. The clearance times (days after treatment) were calculated using a first order kinetic model and were: 15 days for liver, 18 days for muscle and 14 days for skin.

Metabolism in laboratory animals

Rats

In rats, the major route of metabolism for diflubenzuron is via hydroxylation of the phenyl moieties of diflubenzuron (approximately 80%) and, to a lesser extent, cleavage of the benzoyl-ureido bridge (20%) (EMEA, 1999). The main metabolites identified by HPLC or TLC in urine and bile were 2,6-difluoro-3-hydroxy-diflubenzuron, 2,6-difluorobenzoic acid, 2-hydroxy-diflubenzuron and 4-chloro-2-hydroxydiflubenzuron, 4-chloro-3-hydroxydiflubenzuron, 2,6-difluorohippuric acid and 2,6-difluorobenzamide. The cleavage product 4-chlorophenyl urea also was identified in a concentration of approximately 3 to 5%. The metabolite 4-chloroaniline was detected at very low concentrations (less than 0.01% of the absorbed dose)in urine of rats given a very high dose of diflubenzuron (100 g/kg feed, equal to 7.8 g/kg bw/day) for 4 days.

In an ADME study, diflubenzuron was administered by gavage as [^{14}C]-diflubenzuron to male and female Wistar rats either at single dose of 5 or 100 mg/kg bw or at a dose level of 5 mg/kg bw following 14 days of non-radiolabelled diflubenzuron in the diet at a dose level of 5 mg/kg bw/day (EPA, 1997). An additional group of rats, with cannulated bile ducts, was also treated

with a single oral dose of 5 mg/kg bw of [^{14}C]-diflubenzuron. The rats only partially absorbed diflubenzuron from the gastrointestinal tract. In the bile duct cannulated rats, about 33% of the administered dose was absorbed and about 50% of the 33% (17% of the administered dose) was excreted in the bile. By the seventh day, 19-21% of the administered dose had been recovered from the urine and 77-80% from the faeces of rats receiving the lower doses of 5 mg/kg bw. Also by the seventh day, 3% of the administered dose had been recovered from the urine and 96% from the faeces of rats receiving the higher dose of 100 mg/kg bw. The half-life of radioactivity in blood was about 14 hours. Over 98% of the administered radioactivity had been excreted by the seventh day. Very little bioaccumulation in tissues was observed. The highest concentrations of radioactivity were observed in the erythrocytes and liver at 48 hours. Ten urinary metabolites were identified, including 4-chloroaniline and *p*-chlorophenylurea, which together accounted for about 2% of the administered dose (at 5 mg/kg). In the faeces, only unchanged parent compound was detected.

In another study, the metabolic fate of radiolabelled [^{14}C]- and [^{3}H]-diflubenzuron in Wistar rats was investigated (Willems *et al*., 1980). A mixture labelled with ^{14}C in the amino moiety (31.1 mCi/g) and ^{3}H in the 2,6-difluorobenzoyl moiety (6.3 mCi/g) of diflubenzuron was used. The radiochemical purity, determined by TLC, was greater than 99%. The radiolabelled compound, in suspension (1% tragacanth solution), at a dose of 5 mg/kg bw, was administered by gavage after a 16 h fast period. Urine was collected for 6 and 24 h and faeces at 24 h intervals for 72 h. Excretion was almost complete at 72 h after dosing and about 80% of the metabolites appeared to have the basic diflubenzuron structure. Two major routes of degradation were discernible, hydroxylation of the aromatic rings and scission of the benzoyl-ureido bridge. About 20% underwent cleavage of the ureido bridge but neither 4-chlorophenyl urea nor 4-chloroaniline was not present in urine or bile in appreciable quantities.

Metabolism in food producing animals

The metabolic fate of diflubenzuron has been evaluated in various species, including cattle, sheep, swine, chickens and salmon.

Cattle

In a non-GLP compliant study, a single oral dose of 10 mg/kg bw of [^{14}C]-diflubenzuron (equally labelled in both phenyl moieties, specific activity 17.4 µCi/mol, radiochemical purity > 99.0%) was administered, as a slurry in water, by stomach tube to a catheterized 360 kg lactating Jersey cow (Ivie, 1978). The [^{14}C]-diflubenzuron formulation in water was diluted with non-radiolabelled diflubenzuron such that the final treatment mixture contained 3.6 g of diflubenzuron active ingredient and a total of 0.65 µCi of radiolabelled diflubenzuron. After treatment, urine and faeces were collected at 24-hours intervals, and the cow was milked every 12 hours. Seven days after treatment, the animal was slaughtered and tissues collected for analysis of total radiocarbon residues. Radioactive residues in liquid phases were quantified by direct liquid scintillation counting. Metabolites in milk, urine, bile and faeces were resolved by 2-dimensional TLC. The metabolites determined in urine and faeces of samples are presented in Table 3.6.

Table 3.6. Metabolites in urine and faeces from a lactating cow after oral treatment with [^{14}C]-diflubenzuron (10 mg/kg bw) {Adapted from Ivie, 1978}.

	Percentage of TRR	
Metabolite	**Urine[a]**	**Faeces[b]**
2,6-Difluoro-3-hydroxydiflubenzuron	45.0	17.6
4-Chloro-2-hydroxydiflubenzuron	1.6	0.4
4-Chloro-3-hydroxydiflubenzuron	3.7	0.8
4-Chlorophenylurea	0.6	--
2,6-Difluorobenzoic acid	6.0	--
2,6 Difluorohippuric acid	6.9	--

[a] samples collected after 1 day treatment; [b] samples collected 2 days after treatment; -- = not detected.

It was verified that about 85% of the administered dose was eliminated in the faeces and 15% in the urine during the 7-day post treatment period. Only 0.2% was secreted into the milk. Analysis of tissue samples (brain, liver, kidney, muscle, fat and skin) collected 7 days after treatment revealed that only the liver contained appreciable radiocarbon residues, ranging from 2.3 to 3.6 mg eq/kg. Residues of 0.8 mg eq/kg found in skin were attributed to surface contamination through the faeces. In all other tissues collected, residues lower than 0.1 mg eq/kg were determined. In urine, 4 compounds remained unknown and in faeces another 4 compounds also remained unidentified.

In another metabolism study, dairy cows were dosed orally via capsule for up to 28 days with double ring-labelled [^{14}C]-diflubenzuron at rates equivalent to 0.05, 0.5, and 5 mg/kg in the diet (EPA, 1997). At the 0.05 and 0.5 mg/kg dose levels, no radioactive residues, expressed in diflubenzuron equivalents, were detectable in milk. At the 5 mg/kg dose level, radioactive residues in milk plateaued after 4 days between 6.3 and 13.4 µg/kg. After 28 days of dosing, radioactive residues in muscle, fat, and kidney were non-detectable at the 0.05 mg/kg, 0.5 mg/kg and 5 mg/kg dose levels. Radioactive residues in liver were 7.1 µg/kg at the 0.05 mg/kg level, 70.8 µg/kg at the 0.5 mg/kg level, and 540 µg/kg at the 5 mg/kg level.

Swine

[^{14}C]-Radiolabelled diflubenzuron was administered orally at a dose of 5 mg/kg bw (405 µCi) to a female Duroc-Poland China pig (46 kg) (Opdycke *et al*., 1982a). Urine and faeces were collected at 12-h intervals. After 11 days, the pig was slaughtered and samples of brain, heart, lung, liver, gallbladder, kidney, blood, lymph, fat, ovary and oviduct, stomach wall, pancreas, skin and bone were collected for diflubenzuron quantification. More than 88% of the administered dose was accounted for, with over 82% in the faeces and 5% in the urine. The highest concentrations of [^{14}C]-diflubenzuron equivalents were determined in the gallbladder

(0.43 mg/kg), fat (0.30 mg/kg) and liver (0.23 mg/kg). Metabolites identified by TLC and HPLC coupled to a UV detector (HPLC-UV) in the urine included 4-chlorophenyl urea (0.82% of dose), 2,6-difluorobenzoic acid (0.83% of dose), 4-chloroaniline (1.03% of dose) and 2,6-difluorobenzamide (0.29% of dose).

Sheep

In a similar non GLP-compliant study as reported for cattle (Ivie, 1978), the fate of diflubenzuron was evaluated in four mixed breed castrated male sheep (28-42 kg). For measurement of the elimination of radiocarbon in the bile, the bile ducts of two sheep were cannulated 7 days before treatment. One cannulated and one uncannulated sheep were treated orally with [^{14}C]-diflubenzuron by the same procedure described for the cattle (Ivie 1978). The other two sheep (one cannulated and one uncannulated) were treated orally with [^{14}C]-diflubenzuron at 500 mg/kg bw, in order to allow isolation of larger quantities of metabolites. Total urine, bile and faeces were collected at 24-h intervals after treatment. After 4 days, the two sheep treated at 10 mg/kg bw were slaughtered, and tissue samples were collected for combustion analysis. Analysis of tissue samples (brain, liver, kidney, muscle and fat) collected 4 days after treatment revealed that only the liver contained appreciable radiocarbon residues (3.6 mg eq/kg in the cannulated sheep and 2.30 mg eq/kg in the uncannulated sheep). Kidney samples from the bile-duct cannulated sheep contained low levels of radiocarbon, whereas the uncannulated sheep did not have detectable residues. In all other collected tissues, residues lower than 0.2 mg eq/kg were determined.

In the 4-day post-treatment period, the uncannulated sheep treated with 10 mg/kg bw eliminated 43% of the administered dose in the faeces and 41% in the urine. The cannulated sheep at the same dose eliminated 36% in the bile, 32% in the faeces and 24% in the urine. In the same period the uncannulated sheep treated with 500 mg/kg bw of radiolabelled diflubenzuron eliminated 79% in the faeces and 10% in the urine. The cannulated sheep at this high dose eliminated 5% in the bile, 74% in the faeces and 7% in the urine. The major radioactive component in all faeces extracts was identified as unmetabolized diflubenzuron (97.7% in the bile-duct cannulated sheep and 40.0% in the uncannulated sheep).

Although sheep had qualitatively similar metabolic profiles to cow, there were quantitative differences in the relative amounts of metabolites. The major metabolite in the cow urine resulted from hydroxylation of the 2,6-difluorobenzoyl ring and comprised almost half of the radiocarbon in the first day's urine sample. In contrast, this metabolite was a minor product in sheep urine, in which the major metabolites resulted from cleavage of the amide group at the benzoyl carbon forming 2,6-difluorbenzoic acid that was subsequently conjugated with glycine to the hippuric acid.

The metabolites determined in urine and faeces samples from sheep by 2D-TLC and identified by comparison with reference compounds, followed by mass spectrometry or NMR, are presented in Table 3.7.

Table 3.7. Metabolites in urine and faeces from sheep after oral treatment with [^{14}C]-diflubenzuron at a dose of 10 mg/kg bw (Adapted from Ivie, 1978)**.**

	Percentage of TRR			
	Bile-duct cannulated		Uncannulated	
Metabolite	**Urine[a]**	**Faeces[b]**	**Urine[a]**	**Faeces[b]**
2,6-Difluoro-3-hydroxydiflubenzuron	1.2	ND	1.4	0.4
4-Chloro-2-hydroxydiflubenzuron	0.8	ND	0.2	0.8
4-Chloro-3-hydroxydiflubenzuron	0.4	ND	ND	0.4
4-Chlorophenylurea	ND	ND	ND	
2,6-Difluorobenzoic acid	15.1	ND	26.7	
2,6 Difluorohippuric acid	30.2	ND	22.3	

[a] samples collected after 1 day treatment; [b] samples collected 2 days after treatment; ND = Not Detected (the LOD was not reported).

Goats

In a subsequent metabolism study, four lactating goats were dosed orally via capsule for 3 consecutive days with double ring-labelled [^{14}C]-diflubenzuron (EPA, 1997). Two goats were dosed at a rate of approximately 10 mg/kg in the diet and two at a rate of approximately 250 mg/kg. Radioactive residues in the faeces and urine accounted for approximately 88% of the administered dose for both low- and high-dose goats. After 3 days of dosing, total radiolabelled residues (TRRs) in the low-dose (10 mg/kg) goats were 7 to 9 µg/kg in milk, 217 to 262 µg/kg in liver, 16 to 19 µg/kg in kidney, 1 µg/kg or less in muscle, and at most 4 µg/kg in fat. TRRs in the high-dose (250 mg/kg) goats were 220 µg/kg in milk, 324 to 606 µg/kg in liver, 360 to 1020 µg/kg in kidney, 20 to 50 µg/kg in muscle, and 120 to 300 µg/kg in fat. The radioactive residues were characterized in milk and liver. Extraction of milk released 85% of the TRR. The principle residues identified consisted of *p*-chlorophenylurea (29-55% TRR) and 2,6 difluorobenzamide (6-8% TRR). 4-chloroaniline was non-detectable (less than 1 µg/kg) in milk from either low- or high-dose goats. Extraction of liver recovered 90% of the TRR. The principle residues identified were diflubenzuron (7% TRR), 2-hydroxydiflubenzuron (7% TRR), *p*-chlorophenylurea (16% TRR), and 2,6-difluorobenzamide (1% TRR). 4-Chloroaniline was not detectable in liver from the low dose goats but accounted for approximately 0.4% of the TRR (11 to 28 µg/kg) in the liver of the high-dose goats.

Chicken

The metabolism and fate of [^{14}C]-diflubenzuron in four White Leghorn (WL, 36 weeks old, about 1500 g) egg-production chickens and four Rhode Island Red/Barred Plymouth Rock (RIR/RB, 46 weeks old, about 2600 g) meat-production chickens after single oral dose of 5 mg/kg bw (25 µCi to WL and 5 µCi to RIR/RB chickens) were investigated (Opdycke *et al.*, 1982b). Administration of the radiolabelled diflubenzuron was achieved by dissolving the drug

into 4 mL of acetone and adding 1 mL to each of four gelatine capsules containing feed. Excreta were collected from individual chicken at 4, 8, 12, 24, 36, 48, 60, 72, 84, 96, 120, 192 and 288 or 312 h after treatment. The chickens were sacrificed after 12 and 13 days and samples of fat, liver, kidney, gizzard, ovary with internal eggs, breast, muscle, heart, brain and intestine were collected for analysis. Unextracted residues were combusted for radioassay. Diflubenzuron and metabolites in organic fractions were characterized by TLC co-chromatography and high performance liquid chromatography (HPLC) with the reference compounds. A total of 91% of the administered dose was recovered from the WL and 82% from the RIR/BPR excreta, respectively. Rapid elimination of 65% and 43% of the dose within the first 8 h after administration suggests similar excretion patterns for the WL and RIR/BPR chickens. Residual radioactivity in tissues is shown in Table 3.8.

Table 3.8. Residual radioactivity in tissues following treatment of chickens with a single oral dose of diflubenzuron ; 5 mg/kg oral dose, 25 µCi to WL and 5 µCi to RIR/RB chickens (Opdycke *et al.*, 1982b).

	Concentration of radioactivity (mg eq/kg)	
Tissue	**WL**	**RIR/BPR**
Fat	0.01	0.04
Liver	0.06	0.15
Kidney	0.19	0.14
Gizzard	0.01	0.04
Ovary with internal eggs	0.16	0.09
Breast muscle	0.01	0.03
Egg shells	0.40	ND
Brain	ND	0.25
Heart	0.01	ND
Intestine and contents	0.01	ND

ND = None Detected; Limit of detectability was considered to be the mean of the individual background counts plus twice the standard deviation of the background counts; WL = White Leghorn; RIR/BPR = Rhode Island Red/Barred Plymouth Rock.

Table 3.9 presents the percentage of administered dose for each of the metabolites isolated from the organic phase of the chicken excreta. WL chickens metabolized a greater percentage of the radiolabelled diflubenzuron than RIR/BPR chicken and a larger number of compounds were detected. In WL chickens, 16% of the administered dose was transformed to [^{14}C]-labelled metabolites, while RIR/BPR chickens transformed only 3.4% of the dose. The major residue was unchanged diflubenzuron in the two breeds of chicken. Up to five metabolites were not identified.

Table 3.9. [^{14}C]-Diflubenzuron and metabolites identified in organic fraction of chicken excreta (Adapted from Opdycke *et al.*, 1982b).

	Percentage (%) of dose	
Metabolite	**(WL)**	**(RIR/BPR)**
Diflubenzuron	49.90	63.39
4-Chloroaniline	0.44	0.58
2,6-Difluorobenzamide	1.98	
4-Chlorophenyl(urea)	3.14	0.38
2,6 Difluorobenzoic acid	1.35	0.22

Residual radioactivity in the eggs was entirely from the parent compound; no metabolites were identified.

Salmon

The metabolic profile of diflubenzuron in Atlantic salmon (*Salmo salar*) has been evaluated according to EEC Regulation No 762/92 in two GLP-compliant experiments (Auger, 1997) after single dosing (gavage) of radiolabelled [^{14}C]-diflubenzuron and multiple dosing (13 days of feeding of non-radiolabelled diflubenzuron followed by a single dose of radiolabelled [^{14}C]-diflubenzuron) at the recommended dose of 3 mg/kg bw (water temperature +15 °C). In both experiments, the fish were treated with radiolabelled diflubenzuron at concentrations of 1.0 g/kg and 0.6 g/kg for the single dose and repeated dose, respectively. The higher concentration of the drug used in the single dose study was chosen to reduce gavage to 0.3% of bw in order to minimize risk of stomach rupture. Analysis of the treated feed before and after dosing confirmed a radiopurity higher than 99%.

Liver, fillet (muscle and skin), gall bladder (including bile) and residual carcass were collected from 10 fish each at 1 and 7 days (single dose) and 1, 4 and 7 days (repeated dose) post final dose administration. Samples of tissues were collected for TRR determination using liquid scintillation (counting). The limits of detection were 2 µg eq/kg for liver and 0.6 µg eq/kg for fillet and carcass, respectively. Acetonitrile and ethyl acetate tissue sample extracts were also analysed using reversed-phase HPLC-UV at 254 nm. Finally, fish fillet extracts were analysed by liquid chromatography coupled to mass spectrometry (LC-MS).

Diflubenzuron was found as the main TRR both in fillet and in liver, corresponding to 94.8 and 72.2%, respectively, at day 1 after the repeated dosing regimen. For the single dose regime, diflubenzuron represented 88.6% and 69.3% of the TRR for fillet and liver. Diflubenzuron was metabolized and rapidly excreted, mainly via the bile. Six hours after administration, 39% of the radioactivity in bile was identified as diflubenzuron. One and 4 days after administration, most of the radioactivity in bile was attributed to polar metabolites.

Chromatographic analysis with radio-HPLC of fillet revealed three components. The major component was identified as parent diflubenzuron at concentrations of 389 µg/kg, 99.6 µg/kg and 21.4 µg/kg at 1, 4 and 7 days following repeat administration and 410 µg/kg at 1 day

following a single administration. Furthermore, one metabolite was identified as 4-chlorophenyl urea with a maximum concentration of 0.23 μg/kg at 4 days following repeat administration. The third component was not identified (less than 7 μg/kg) but the retention time was in the same range as for 4-chloroaniline. Base hydrolysis of solid residues in liver revealed at least five components at concentrations lower than 9 μg/kg. Three of the components were identified as diflubenzuron, 4-chloroaniline (less than 3 μg/kg) and 4-chlorophenyl urea (less than 9 μg/kg). The two unidentified metabolites were probably mono-hydroxylated products of diflubenzuron.

Comparative metabolism in animals

The metabolism studies indicated that diflubenzuron is metabolized in animals via two main routes (Figure 3.2). Reaction pathways A, B and C are hydroxylation reactions of the phenyl groups, which leaves the basic structure of diflubenzuron intact; the metabolites formed are 2,6-difluoro-3-hydroxydiflubenzuron, 4-chloro-3- hydroxydiflubenzuron, 4-chloro-2-hydroxydiflubenzuron and their conjugates. In the other pathway (Fig. 2, D), a cleavage between the carbonyl and amide groups takes place and 2,6-difluorobenzoic acid and 4-chlorophenyl urea are formed. Whereas pathways A, B and C are the major metabolic pathways in rat and cow, pathway D predominates in sheep, swine and chicken (IPCS 1981). Moreover, metabolism of diflubenzuron in laboratory animals was qualitatively similar to that in food-producing animals. In salmon, the second pathway appears to be the main metabolic pathway, with the metabolite 4-chlorophenyl urea identified in both fillet and muscle of salmon administered diflubenzuron.

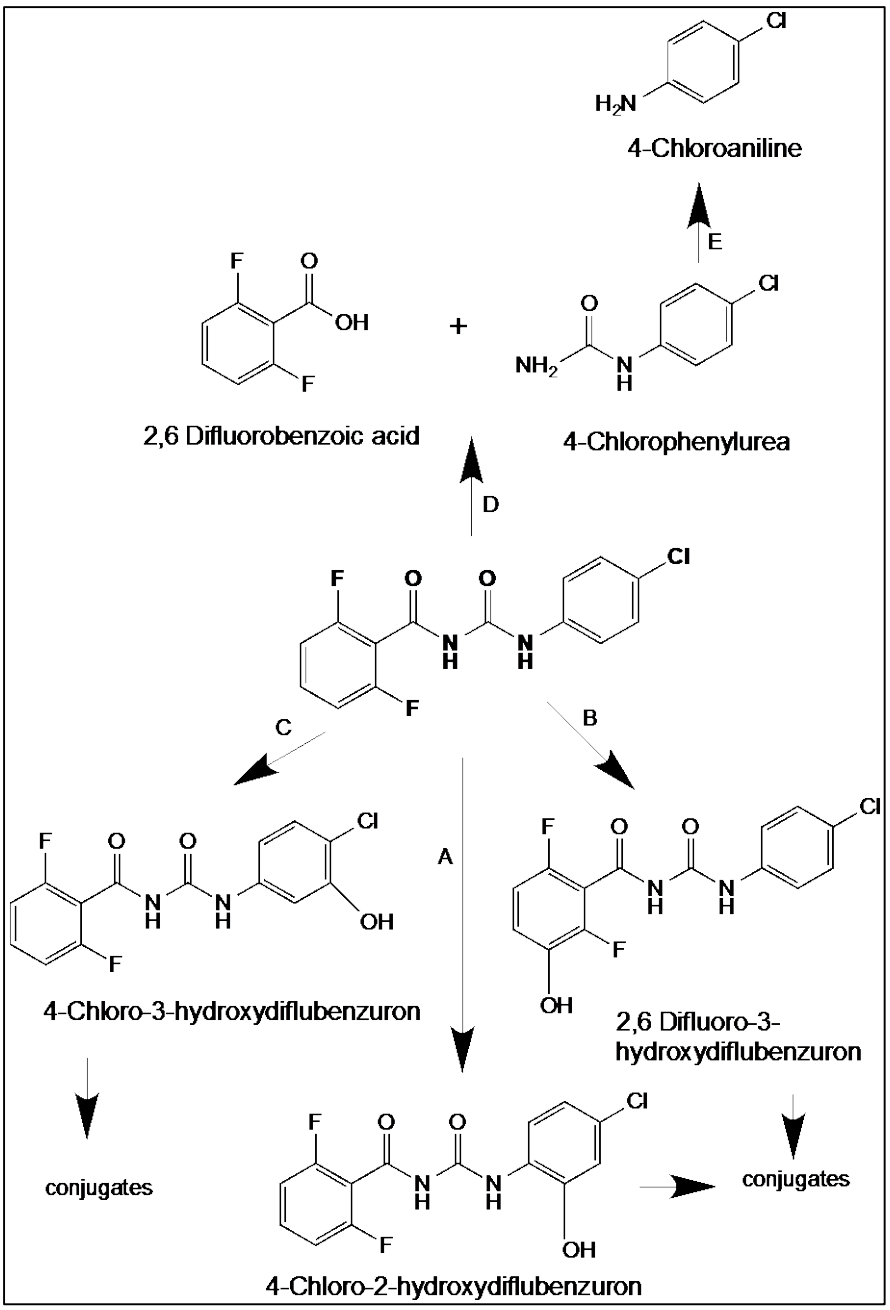


Figure 3.2. Metabolism of diflubenzuron in animals. (Adapted from JMPR, 1982).

Tissue residue depletion studies

Radiolabelled residue depletion studies

Salmon

The total radiolabelled residues were determined in two GLP-compliant studies in which Atlantic salmon (*Salmo salar*), held in sea water of approximately 15 °C, were dosed with [^{14}C]-diflubenzuron at two different regimes: (i) a single dose of 0.3% of bw equal to a dose of 3 mg/kg bw (2 MBq/kg) for one-day by gavage (*Study I*) and (ii) a repeated dose of 0.5% of bw/day equal to 3 mg/kg bw/day for 13 consecutive days using non-radiolabelled diflubenzuron followed by a single dose of radiolabelled [^{14}C]-diflubenzuron by gavage as

performed in the single dose trial (*Study II*) (Auger, 1997). In both studies the fish were treated with radiolabelled diflubenzuron at concentrations of 1.0 g/kg and 0.6 g/kg for the single dose and repeated dose, respectively. The higher concentration of the drug used in the single dose study was to reduce gavage to 0.3% of bw in order to minimize risk of stomach rupture.

Study I – Single oral dose of [^{14}C]-diflubenzuron (Auger, 1997).

Atlantic salmon (*Salmo salar*), weighing 440 to 851 g, were treated with a single dose of 3 mg/kg bw (2 MBq/kg) [^{14}C]-diflubenzuron by gavage. Liver, fillet (muscle and skin), gall bladder (including bile) and residual carcass were collected from 10 fish each at 1 and 7 days post final dose administration. Samples of tissues were collected for TRR determination using liquid scintillation counting. The limits of detection were 2 µg eq/kg for liver and 0.6 µg eq/kg for fillet and carcass. Acetonitrile and ethyl acetate tissue sample extracts were also analysed using reversed-phase HPLC coupled to a UV detector at 254 nm. Finally, fish fillet extracts were analysed by LC-MS.

Diflubenzuron was found as the main TRR both in fillet and in liver corresponding to 88.6% and 69.3% of the TRR for fillet and liver, respectively. The TRR concentrations in tissues are presented in Table 3.10 and the recovery proportions in Table 3.11.

Table 3.10. Change in concentration of radioactivity in tissues of Atlantic salmon (*Salmo salar*) with time following oral administration of a single dose of [^{14}C]-diflubenzuron of 3 mg/kg bw by gavage. Water temperature of 15 ºC (Auger, 1997)**.**

	Concentration of radioactivity (µg eq/kg) ± SD		
Time (days)	**Liver**	**Fillet**	**Carcass**
1	943 ± 106	447 ± 55	1930 ± 973
7	192 ± 51	21 ± 9	42 ± 17

SD = Standard Deviation (n = 10 fish).

Table 3.11. Change in recovery of radioactivity from tissues of Atlantic salmon (*Salmo salar*) with time following oral administration of a single dose of [^{14}C]-diflubenzuron of 3 mg/kg bw by gavage. Water temperature of 15 ºC (Auger, 1997)**.**

	Mean recovery of radioactivity (%) ± SD			
Time (days)	**Liver**	**Fillet**	**Carcass**	**Total recovery***
1	0.29 ± 0.03	9.27 ± 1.14	23.2 ± 12.1	32.8
7	0.06 ± 0.01	0.44 ± 0.19	0.51 ± 0.20	1.0

SD = Standard Deviation (n = 10 fish); * Sum of the average recoveries of liver, fillet and carcass.

Study II – Repeated dose of non-radiolabelled diflubenzuron for 13 consecutive days followed by a single dose of [^{14}C]-diflubenzuron (Auger, 1997).

Atlantic salmon (*Salmo salar*), weighing 514 to 863 g, were treated with diflubenzuron. Medicated feed containing non-radiolabelled diflubenzuron at a dose of 3 mg/kg bw per day was administered for 13 consecutive days. On day14 a single dose of 3 mg/kg bw radiolabelled [^{14}C]-diflubenzuron was administered by gavage. Liver, fillet (muscle and skin), gall bladder (including bile) and residual carcass were collected from 10 fish each at 1, 4 and 7 days post final dose administration. The analyses were carried out as described in *Study I.*

Diflubenzuron was found as the main TRR both in fillet and in liver corresponding to 94.8 and 72.2%, respectively, at day 1 after post-treatment. The TRR concentrations in tissues are presented in Table 3.12 and the recovery proportions in Table 3.13.

Table 3.12. Change in concentration of radioactivity in tissues of Atlantic salmon (*Salmo salar*) with time following repeated dosing. Salmon were administered non-radiolabelled diflubenzuron (3 mg/kg bw) via medicated feed for 13 consecutive days; on day 14 salmon received a single oral dose of [14C]-diflubenzuron (3 mg/kg bw) by gavage. Water temperature of 15 ºC (Auger, 1997)**.**

	Concentration of radioactivity (µg eq/kg) ± SD		
Time (days)	**Liver**	**Fillet**	**Carcass**
1	811 ± 100	466 ± 66	734 ± 118
4	334 ± 60	117 ± 33	181 ± 44
7	181 ± 33	26 ± 11	51 ± 22

SD = Standard Deviation (n = 10 fish).

Table 3.13. Recovery of radioactivity from tissues of Atlantic salmon (*Salmo salar*) with time following repeated dosing. Salmon were administered non-radiolabelled diflubenzuron (3 mg/kg bw) via medicated feed for 13 consecutive days; on day 14 salmon received a single oral dose of [14C]-diflubenzuron (3 mg/kg bw) by gavage. Water temperature of 15 ºC (Auger, 1997)**.**

	Mean recovery of radioactivity (%) ± SD			**Total recovery***
Time (days)	**Liver**	**Fillet**	**Carcass**	
1	0.25 ± 0.04	9.58 ± 1.16	8.78 ± 1.38	18.61
4	0.12 ± 0.02	2.35 ± 0.65	2.43 ± 0.58	4.90
7	0.06 ± 0.01	0.54 ± 0.23	0.62 ± 0.26	1.22

SD: standard deviation (n= 10 fish). * Sum of the average recoveries of liver, fillet and carcass.

The relationship between extractable (in acetonitrile) marker residue (diflubenzuron) and total residue in pooled liver and muscle homogenate samples from Atlantic salmon (*Salmo salar*) held in sea water at 15 ^{0}C is shown in Table 3.14.

Table 3.14. Concentrations (µg/kg) of total radioactive residues (TRR) and diflubenzuron residues (marker residue, MR) in liver and muscle of Atlantic salmon (*Salmo salar*) following repeated dosing. Salmon were administered non-radiolabelled diflubenzuron (3 mg/kg bw) via medicated feed for 13 consecutive days; on day 14 salmon received a single oral dose of [14C]-diflubenzuron (3 mg/kg bw) by gavage. Water temperature of 15 ºC (Auger, 1997).

Administration	Time post-dose (d)	Liver			Muscle		
		TRR (µg/kg)	MR (µg/kg)	MR/TRR ratio (%)	TRR (µg/kg)	MR (µg/kg)	MR/TRR ratio (%)
Single dose	1	922	703	76.3	463	421	91.0
	1	802	617	77.0	410	394	96.0
Repeated dose	4	324	185	57.1	114	100	88.0
	7	177	51.9	30.3	22.9	21.5	93.9

Four other minor metabolites were also detected, including 4-chlorophenylurea at a concentration of 0.23 µg/kg in liver on day 4 in the repeated dose group. The other three metabolites were not identified but were postulated to be mono-hydroxylated products of diflubenzuron. Basic hydrolysis of solid residues in liver revealed at least five components: diflubenzuron, 4-chlorphenyl urea (less than 9 µg/kg), 4-chloroaniline (less than 3 µg/kg) and two unknown substances.

The highest concentrations of [^{14}C]-diflubenzuron-equivalents found in all tissues analysed were at day 1 in both treatment groups (Tables 3.10 and 3.12). Excretion from the Atlantic salmon tissues was rapid with less than 20% of the radiochemical dose remaining in the liver, fillet and carcass 1 day following repeated administration and less than 33% remaining following a single dose administration (Table 3.11). The concentrations decreased to less than 1.5% by 7 days following both dosing regimens (Tables 3.11 and 3.13). The major metabolic pathway is excretion of the parent compound.

Residue depletion studies with non-radiolabelled drug

Salmon

Depletion of diflubenzuron in Atlantic salmon (*Salmo salar*), 600 to 987 g, was evaluated at a water temperature of 6 °C following 14 days of daily medication at a nominal concentration of 0.6 g/kg in feed (actual concentration 0.64 g/kg). This study (Todd, 1997a) was conducted according to EC Council Directive 87/18/EEC and 88/320/EEC and in compliance with GLP. The medicated feed was offered *at libitum* each day at a level of 0.5% of fish biomass per day, equivalent to an intended daily dose of diflubenzuron of 3 mg/kg bw (actual dose of 2.9 mg/kg bw). Livers (without gall bladder) and fillets (muscle and skin) of ten fish were analysed by HPLC-UV on days 1, 7, 14 and 21 post treatment.

The analytical method was validated over the range 0.05 to 5.0 mg/kg for both tissues. Recoveries from fillet ranged from 81 to 108%, with a coefficient of variation of 7.1%. Recoveries from liver ranged from 100 to 108%, with a coefficient of variation of 2.4%. A stability study using fortified tissues (1000 µg/kg) showed that diflubenzuron is stable at -18 °C in both fillet and liver over a storage period of 60 days.

The average concentrations of diflubenzuron in fillet were: 2240 µg/kg, 400 µg/kg, 100 µg/kg and below limit of quantification (LOQ, 50 µg/kg) on days 1, 7, 14 and 21 post-treatment, respectively. The average concentrations of diflubenzuron in liver were 3190 µg/kg, 730 µg/kg, 120 µg/kg and below LOQ on days 1, 7, 14 and 21 post-treatment, respectively. In this study, the withdrawal period was estimated (time where the upper one-side 95% tolerance limit is below the LOQ) to be 22 days for fillet and 21 days for liver. Considering a safety margin, a withdrawal period of 28 days was recommended.

The same protocol was used in a second study at a higher water temperature (15 °C), where Atlantic salmon (*Salmo salar*), 600-987 g, were fed diflubenzuron daily at an intended daily dose of 3 mg/kg bw (actual dose of 3.19 mg/kg bw) for 14 consecutive days (Todd, 1997b). Diflubenzuron was quantified in muscle and liver by a method using HPLC-UV, with a limit of quantification (LOQ) of 50 µg/kg. The average concentrations determined of diflubenzuron in fillet were: 1550 µg/kg and 200 µg/kg on days 1 and 7, respectively, and below LOQ on days 14 and 21 post-treatment. The average concentrations of diflubenzuron in liver were 2170 µg/kg and 260 µg/kg, on days 1 and 7 post-treatment, respectively, and less than 50 µg/kg (LOQ) after 14 days post-treatment. In this study the withdrawal period was estimated to be 18 days for fillet and 17 days for liver. Considering a safety margin, the same withdrawal period of 28 days recommended from the results of the study at 6 °C was recommended for the higher water temperature (15 °C).

In another GLP-compliant depletion study carried out at high temperature (14.6 to 15.6 °C), Atlantic salmon (*Salmo salar*) weighing 4.6 to 5.6 kg were fed *ad libitum* with diflubenzuron (0.63 g/kg) at a level of 0.5% of biomass per day for 14 consecutive days, equivalent to a daily dose of diflubenzuron of 2.66 to 3.2 mg/kg bw (Wallace *et al.*, 1997). Liver, muscle and skin samples collected during the treatment (days 3, 7 and 14) and on days 5, 14, 21 and 28 post-treatment were analysed using a validated HPLC-UV method. During the treatment, the highest average diflubenzuron concentration was found at day 14 in liver (1820 µg/kg) and muscle (2130 µg/kg). For skin, the highest diflubenzuron concentration of 1320 µg/kg was reached on day 7 during the treatment. The maximum diflubenzuron concentration of 3700 µg/kg was in one muscle sample on day 14 during the treatment with the medicated feed. In liver, the average diflubenzuron concentrations (10 fish) were 520 µg/kg (minimum < 50 µg/kg and maximum 890 µg/kg) and 70 µg/kg (minimum <50 µg/kg and maximum 150 µg/kg) on days 5 and 14, respectively. In muscle, the average concentrations were 900 µg/kg (minimum 530 µg/kg and maximum 1900 µg/kg) and 100 µg/kg (minimum <50 µg/kg and maximum 170 µg/kg) on days 5 and 14, respectively. In skin, the average concentrations were 320 (minimum <50 µg/kg and maximum 520 µg/kg) and less than 50 µg/kg (minimum <50 µg/kg and maximum 80 µg/kg), on days 5 and 14, respectively. At 21 days post treatment, all samples analysed had diflubenzuron concentrations lower than 50 µg/kg (LOQ).

Atlantic Cod

A non-GLP compliant residue depletion study of diflubenzuron in juvenile Atlantic cod (*Gadus morhua*), a fish species found near Atlantic salmon farms, was conducted at a water temperature of 7.7 ± 0.2 °C (Olsvik *et al.*, 2013). The fish (81 to 122 g) were fed at a nominal dose rate of 3 mg/kg bw (0.6 g/kg in feed), corresponding to a total dose of 42 mg/kg bw after the end of treatment. The highest concentrations of diflubenzuron in liver (181 ± 21 µg/kg) were observed 1 day after the end of the treatment (Day 15). The authors suggest that diflubenzuron is metabolized by phase I enzymes and particularly CYP3A after pregnane X receptor (PXR) activation in cod.

In another study conducted at a water temperature of 7.7 °C, Atlantic cod (65 – 165 g) were fed medicated pellets containing 0.6 g of diflubenzuron per kg for 14 consecutive days (Erdal, 2012). The feed was administered *ad libitum* for a nominal daily dose of 3 mg of diflubenzuron per kg bw. Samples of fillet and skin in natural proportions, liver and terminal colon, were taken during the treatment on days 4, 8, 12 and days 1, 4, 8, 15, 22 and 30 post treatment. At each time point, 10 fish were collected and analysed individually, with the exception of the bile samples, which were accumulated into one or two group samples for each sampling day. After extraction from the sample matrices, diflubenzuron was quantified by LC-MS using teflubenzuron as the internal standard. The LOQ of the validated method was 20 µg/kg. The calculated tissue concentrations in the samples showed high variability, attributed to individual differences in feed consumption and, to a lesser extent, in absorption. The median concentration determined in fillet and skin throughout the treatment period was 36.1 µg/kg, only 1.5% of the mean concentration determined in Atlantic salmon fillet after the same treatment, which indicates that diflubenzuron has a lower gastrointestinal uptake in Atlantic cod compared to Atlantic salmon.

The depletion half-lives for diflubenzuron in fillet and liver ranged from 0.8 to 0.9 days. The concentrations of 4-chloroaniline in all samples analysed by LC-MS/MS were below the detection limit of the method (2 µg/kg). However, these results do not rule out the possibility that 4-chloroaniline could be a metabolite of diflubenzuron in Atlantic cod because the tissue concentrations of the marker residue were so low that the fraction of PCA that might be formed probably would be below the detection limit of the method.

Method of analysis for residues in tissues

Many analytical methods for the determination of diflubenzuron in food, feed and biological matrices have been reported (Table 3.15). The Committee assessed the validation data available for these methods against the analytical requirements as published in CAC/GL71-2009 (FAO/WHO, 2014).Due to the high polarity and low volatility of diflubenzuron, liquid chromatography has been the method of choice. Most protocols use solvent extraction of diflubenzuron from the sample followed by clean-up steps, including solid phase extraction procedures and, more recently, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approach (Anastassiades, 2003). Chromatographic separation is commonly performed using reverse-phase chromatography. For the quantification of diflubenzuron, UV and, nowadays, tandem mass spectrometry detectors have been employed. In the latter case,

electrospray ionization is mostly employed in the positive ion mode using acquisition of ions in the selected reaction-monitoring mode (SRM).

Quantitative methods

Liquid chromatography (LC)

Single-residue methods employing HPLC-UV were used in depletion studies carried out in the mid-1990s for the quantification of diflubenzuron in fish tissues. The analytical method (Thus et al., 1995) consisted of extraction of diflubenzuron from the skin, muscle and liver samples (3 to 5 g) by solid-liquid extraction with acetonitrile (2 x 5 mL). The extract was evaporated to dryness at 50 °C, and dissolved in a solution of acetonitrile (1.5 mL), water (0.5 mL) and hexane (4 mL). The solution was vortexed, centrifuged and the hexane layer removed. An additional 4 mL of hexane, 1 mL of water and 4 mL of dichloromethane were added to the test tube. The mixture was vortexed, centrifuged and the dichloromethane layer separated. To the acetonitrile/water layer another 4 mL dichloromethane was added and the separation procedure repeated. The combined dichloromethane layers were mixed with sodium sulphate and the dried dichloromethane layer evaporated to dryness at 50 °C. The residue was dissolved in 4.0 mL of methylethylketone:petroleum ether, 2:25 v/v, with clean-up by solid phase extraction on a Florisil cartridge (500 mg). The chromatographic separation was performed on a C18 column (Zorbax, 250 x 4.6 mm, 7.5 µm particle size) at 35 °C, using acetonitrile:water, 1:1 v/v, as the mobile phase. Quantification was performed using a UV detector at 254 nm. The concentrations of diflubenzuron in the samples were calculated by comparing the peak height of the sample with the peak height of calibration solutions. The method was validated by analysing diflubenzuron fortified tissue samples, ranging from 0 to 3.3 mg/kg, with detection and quantification limits of 20 and 50 µg/kg, respectively. Average recoveries of 88% for liver, 91% for muscle (values corrected for blank) and 103% for skin were determined. Even though matrix effects are not relevant using a UV detector, it is important to consider that interferences could occur at low-concentration measurements in complex food matrices.

Gas chromatography (GC)

Gas chromatography has also been employed, to a lesser extent, for the determination of diflubenzuron in plant and animal products. Due to its thermal instability, high polarity and low volatility, derivatization processes are required. The method is based on hydrolysis of diflubenzuron to 4-chlorophenylurea and 4-chloroaniline followed by derivatization with heptafluorobutyric acid (HFBA) and determination of the N-(4-chlorophenyl)heptafluorobutanamide formed by GC-ECD or NPD (Stan and Klaffenbach, 1991). Mass spectrometry coupled to GC is necessary for confirmation purposes. It is worth emphasizing that any 4-chlorophenylurea or 4-chloroaniline in the sample will be determined as diflubenzuron if not separated before the hydrolysis step.

Confirmatory methods

Liquid chromatography – tandem mass spectrometry (LC- MS/MS)

In recent years, many multi-residue analytical methods for the determination of pesticides and veterinary drugs, including diflubenzuron, in food and biological matrices have been reported.

In general, the methods are based on solvent extraction (acetone, acetonitrile or methanol) with or without hexane liquid-liquid extraction to remove lipids, followed by clean-up over C18 or silica gel solid phase extraction cartridges and determination by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Matrix solid-phase extraction may be used as an alternative technique for the simultaneous extraction and clean-up (fat removal) of lipophilic chemicals. A simple and fast method for the determination and monitoring of eight pesticides, including diflubenzuron, in fish and shellfish by matrix solid-phase dispersion (MSPD) with anhydrous sodium sulphate and C18 as dispersants, silica as an adsorbent and LC-MS/MS quantification, has been reported (Carro *et al.*, 2012).

More recently, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approach (Anastassiades *et al.*, 2003), originally developed for the determination of pesticide residues in fruits and vegetables, has been modified and employed for the determination of multi-residues of pesticides, including diflubenzuron, in fish. The original approach is characterized by an extraction step using acetonitrile followed by a subsequent liquid-liquid partitioning of residues through the addition of salts and buffering agents and clean-up steps. Modifications in the original QuEChERS approach include the use of ethyl acetate instead of acetonitrile and incorporating two freezing steps for removal of lipids (Norli *et al.*, 2011). The use of a less polar solvent improves partitioning of analytes from the fatty lipid layer in fish tissues. The pigments that give salmon its trademark pink colour could cause interference with analytes of interests; these pigments could be removed by the use of primary-secondary amine and /or graphitized carbon black (Holmes *et al*., 2015).

Holmes and co-workers presented a single-laboratory ruggedness testing and validation of a modified QuEChERS approach to quantitate residues of 185 pesticides in salmon using UPLC-MS/MS (103 pesticides, including diflubenzuron) and GC-MS/MS (82 pesticides) for analysis (Holmes *et al*., 2015). The pesticides were extracted from the homogenized samples (20 g) with ethyl acetate and two freezing steps and a C18 dispersive solid phase extraction for removal of lipids were carried out. Briefly, 20 g of the homogenized samples were added to 30 mL ethyl acetate and extraction buffer (8 g of $MgSO_4$ and 2 g of NaCl). The samples were shaken and placed into a -20 °C freezer for 30 min, centrifuged and the upper layer decanted. The extracts were concentrated under a stream of nitrogen and diluted with acetonitrile to 15 mL and frozen for a second time at -40 °C for 30 min. After centrifugation, the upper layer was cleaned-up over C18 SPE cartridges prepared by adding 1 g of $MgSO_4$ on the top. The SPE columns were washed with 1% acetic acid in acetonitrile. Eluted extracts were concentrated and re-suspended with acetonitrile. Before LC-MS/MS quantification, an additional clean-up was performed that consisted of the addition of 150 mg of $MgSO_4$, 50 mg of primary-secondary amine (PSA) and 50 mg of C18 to a volume of 1 mL of the extract.

Analytes were separated on a silica-based bonded phase column (Acquity HSS T3 column, 50 x 2.5 mm, 1.8 µm particle size) at 40 °C using a binary mobile phase composed of 0.1% formic acid and 10 mM aqueous ammonium formate. Triphenylphosphate was used as internal standard. For the quantification, a matrix-matched standard of blank salmon extract was prepared at 1, 5 and 10 times the limit of quantification for each pesticide. The method was used in the analysis of 708 salmon samples collected as part of the U.S. Department of

Agriculture's Pesticide Data Program (USDA-PDP). Method validation was accomplished by assessing selectivity and conducting single-laboratory intra- and inter-day precision and accuracy studies and was conducted according criteria set by the USDA-PDP quality assurance program and based on EPA GLP.

Another simultaneous screening and confirmation procedure for multiple classes of drug residues in fish (salmon, trout, catfish and tilapia), including diflubenzuron, by liquid chromatography-ion trap mass spectrometry, was developed and validated (Smith *et al.*, 2009). Samples (2 g) were added to 2 mL of n-hexane and 10 mL of acetonitrile. After vigorous shaking and centrifugation, the hexane layer was aspirated and discarded. The acetonitrile phase was separated and the remaining tissue pellet was re-extracted with 10 mL acetonitrile and 2 mL n-hexane. The acetonitrile extracts were combined and evaporated just to dryness. Chromatographic separation was achieved using a Phenyl column (YMC, 50 x 4.0 mm, 3 µm particle size) and a mobile phase of 0.1% formic acid with 10 µm NaOH in water and acetonitrile. Analytes were detected on an ion trap mass spectrometer equipped with an ESI source operating at positive and negative mode. With this method, a lower confirmation limit for diflubenzuron of 100 µg/kg was achieved.

A rapid multi-residue screening method for the determination of 128 veterinary antiparasitic drugs and metabolites, including diflubenzuron, in chicken, porcine and bovine meat, was developed and validated according to the European Union Regulation 2002/657/EC for a quantitative screening method (Wei *et al*., 2015). The sample preparation procedure was based on the QuEChERS approach. The drugs were extracted from the chicken, porcine and bovine meat samples (2 g) using 10 mL acetonitrile:ethyl acetate 1:1 v/v and 1 g of $MgSO_4$. After sonication and centrifugation, the upper layer was separated and added to 1 mL of aqueous NH_3. The supernatant was cleaned-up over 200 mg ODS and 1.5 g anhydrous $MgSO_4$. The cleaned extract was evaporated and the residue dissolved in the mobile phase. Quantification was performed by LC-MS/MS, using a C18 chromatographic column (Hypersil, 150 x 2.1 mm, 5 µm particle size) at 40 °C, mobile phase of 12.5 mM ammonium formate at pH 4 in acetonitrile/methanol, 50:50 v/v, and an ESI source was used with both positive and negative ionization mode. The detection capabilities (CCβ) for diflubenzuron in chicken, swine and bovine meat were 2.15, 2.24 and 10.28 µg/kg, respectively.

Matrix solid-phase dispersion and liquid chromatography with UV or atmospheric pressure chemical ionization/mass spectrometry (APCI/MS) detection was reported for the determination of diflubenzuron and 4 other pesticides (hexaflumuron, aflufenuxuron, hexythiazox and benfuracarb) in orange samples from Spain (Valenzuela *et al.*, 2001). In 74.6% of the 150 samples analysed, the pesticide residues were below detection limits, which ranged from 2 to 50 µg/kg. Diflubenzuron residues exceeded 1000 µg/kg in 3 samples.

Several analytical methods used in supervised residue trials and in studies on storage stability in plant products, animal feeding or direct animal treatment were reported and considered by the JMPR in 2011. Most are single-residue methods for either diflubenzuron, 2.6-difluorobenzoic acid, *p*-chlorophenylurea, 4-chloroaniline or 4-chloroacetanilide in only a few substrates. HPLC methods for the determination of diflubenzuron consist of extraction, clean-up and determination with UV, MS or MS/MS detection. In addition, a great number of multi-

residue methods have been reported for residues of diflubenzuron in agricultural products and water (Martinez *et al.*, 2007).

Stability of residues

Residues of diflubenzuron are stable in frozen beef tissue, milk, poultry muscle and eggs at temperatures of at least -20 °C for up to 12 months (EPA, 1997). In Atlantic salmon fillet and liver matrices stored at approximately -18 °C, diflubenzuron was stable for a period at least 60 days (Todd, 1997b).

Table 3.15. Analytical methods for the determination of diflubenzuron in food and biological matrices.

Sample	Extraction	Clean-up	Analytical technique	Linear range	Recovery	LOQ	LOD	Reference
Fish	MSPD	-	LC-MS/MS	5-500 µg/kg	84.9-118% (salmon)	4.7 µg/kg	1.5 µg/kg	(Carro *et al.*, 2012)
Fish (salmon)	SLE1 (ethyl acetate + 2 freezing steps)	QuEChERS2	LC-MS/MS (IS3 triphenyl phosphate)	1, 5 and 10 xLOQ	70-120%	2 µg/kg	not stated	(Holmes *et al.*, 2015)
Fish (salmon, trout, catfish and tilapia)	SLE1 (hexane and acetonitrile)	-	LC-MS/MS	not stated	not stated	100 µg/kg	not stated	(Smith *et al.*, 2009)
Atlantic cod (muscle, fillet and liver)	SLE1 (acetone)	LLE4 (heptane); SPE5 (silica)	LC-MS/MS (IS3 teflubenzuron)	20-75 µg/kg	-	20 µg/kg	10 µg/kg	(Erdal, 2012)
Tilapia fillet	SLE1 (methanol)	SPE5 (C18)	HPLC-DAD	0.1 – 15 mg/L	not stated	110 µg/kg	32 µg/kg	(Luvizotto-Santos *et al.*, 2009)
Fish feed	SLE1 (acetone and THF)	-	HPLC-UV	0.3 – 2.0 g/kg	91.4-93%	0.25 g/kg	not stated	(Hormazabal and Yndestad, 1997)
Milk and cattle tissues	SLE1 (ACN)	QuEChERS2 and SPE5 (C18)	HPLC-DAD	0-25 mg/L	71.8-105.1%	50 µg/kg (kidney, liver,	15 µg/kg (kidney), 16 µg/kg (liver), 14 µg/kg (muscle),	(Tfouni *et al.*, 2013)

						muscle, fat) 10 µg/kg (milk)	6 µg/kg (fat and milk)	
Mushroom	SLE1 (ACN)	QuEChERS2	UPLC-MS/MS	5-5000 µg/L	78.1-107.6%	< 5 µg/kg	< 1.5 µg/kg	(Carro *et al.*, 2012, Du *et al.*, 2013)

[1] SLE = Solid-Liquid Extraction; [2] QuEChERS = Quick, Easy, Cheap, Rugged and Safe; [3] IS = Internal Standard; [4] LLE = Liquid-Liquid Extraction; [5] SPE = Solid Phase Extraction.

Appraisal

Diflubenzuron is a benzoylurea pesticide used in aquaculture for the treatment of sea lice in Atlantic salmon (*Salmo salar*) at a dose of 3-6 mg diflubenzuron per kg of fish biomass per day for fourteen consecutive days, with a withdrawal period in the range of 105–300 degree-days. It is also used in agriculture, horticulture and forestry to control a wide range of insect pests.

Diflubenzuron has not been previously reviewed by the Committee; however, it was evaluated as pesticide by JMPR in 1981, 1984 and 1985. An ADI of 0 – 0.02 mg/kg bw was established by JMPR in 1985.

Metabolism data are available for a variety of animal species, including rats, cattle, swine, sheep, goats, chicken and salmon. Diflubenzuron is predominantly unmetabolized and biliary excretion is the main path for elimination. The metabolic profiles indicated that diflubenzuron is metabolized in animals via two main routes: (i) hydroxylation of the phenyl groups and (ii) cleavage of the carbonyl and amide groups. In the second pathway 4-chloroaniline could be formed. In salmon, the second pathway seems to be the main route.

Metabolic profiling in salmon was available; two studies were carried out following single or repeated dose administration of radiolabelled diflubenzuron to salmon. Diflubenzuron was metabolized and rapidly excreted, mainly via the bile. Two compounds were identified in fillet, the parent drug and 4-chlorophenyl urea. A third compound was not identified, but it could not be confirmed that this compound was not 4-chloroaniline. In liver, diflubenzuron, 4-chlorophenyl urea and 4-chloroaniline were identified. Some metabolites remained unknown.

Radiolabelled residue depletion data are available for salmon at a water temperature of 15 ºC following single or repeated dose. Diflubenzuron was identified as marker residue in salmon muscle and liver.

The highest concentration (less than 500 µg/kg) of diflubenzuron in salmon muscle occurs 1 day after administration of the drug.

The Committee was informed that 4-chloroaniline is a potential hydrolysis product of 4-chlorophenyl isocyanate, which is one of the starting materials for the synthesis of diflubenzuron. Also, 4-chloroaniline could be formed through degradation of diflubenzuron at temperature higher than 100 ºC. Even if these two processes are controlled, it cannot be excluded that 4-chloroaniline is present in the drug used to formulate the medicated pellets. No data were available regarding contaminants and/or degradations products in formulated products. There were also no data available about the stability of diflubenzuron during feed processing, in particular regarding the presence or absence of 4-chloroaniline.

The residue depletion studies in salmon were conducted in the mid 90’s using HPLC-UV methods, which required complex sample preparation procedures for extraction and clean-up. The quantification limit was 50 µg/kg in salmon tissues. The state-of-the-art methods (LC-MS/MS) use simpler sample preparation procedures, based on the QuEChERS approach, and have a LOQ of 2 µg/kg. However, an analytical method (LC-MS/MS) for the determination of

diflubenzuron in salmon tissues (muscle and skin), validated according to the criteria described in CAC/GL 71-2009, is not available (FAO/WHO, 2014).

The Committee concluded that the HPLC-UV method provided by the Sponsor lacks in selectivity because of possible interferences from other components in the tissue extracts at the selected wavelength and cannot be recommended for regulatory monitoring of salmon tissues for diflubenzuron.

Maximum Residue Limits

The Committee noted that PCA is a potential hydrolysis product of 4-chlorophenyl isocyanate, which is one of the starting materials for the synthesis of diflubenzuron, and that PCA could be formed through degradation of diflubenzuron at high temperatures during processing of feed or food. The data available to the Committee at the time of the assessment were inadequate regarding the formation or presence of PCA in fish, as well as in processed food.

MRLs for diflubenzuron could not be recommended by the Committee, as the Committee was unable to establish an ADI for diflubenzuron.

The Committee also noted that there is no analytical method suitable for regulatory monitoring purposes.

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Addendum to the monographs prepared by the 36th, 40th, 54th, 58th and 78th Meetings of the Committee and published in FAO Food and Nutrition Papers 41/3, 41/5, 41/13 and 41/14, and FAO JECFA Monograph 15.

Background

Ivermectin (CAS No. 70288-86-7)2 is a macrocyclic lactone that is a member of the avermectin series and is widely used as a broad-spectrum antiparasitic endectocide against nematode and arthropod parasites in food-producing animals. In human medicine, ivermectin is used to treat onchocerciasis, lymphatic filariasis, strongiloidiasis and scabies. Ivermectin consists of two homologous compounds, 22,23-dihydroavermectin B1a (H2B1a or ivermectin B1a) and 22,23-dihydroavermectin B1b (H2B1b or ivermectin B1b), in the H2B1a:H2B1b ratio of 80:20. Ivermectin is used in cattle, sheep, goats, pigs, horses, reindeer and American bison at doses of 0.1–0.5 mg/kg bw given subcutaneously, topically or orally, as a single dose treatment only. Withdrawal periods range from 14 to 122 days where ivermectin is approved for use.

Ivermectin was previously considered by the Committee at its 36th (WHO, 1990), 40th (WHO, 1993), 58th (WHO, 2002), 75th (WHO, 2012a) and 78th (WHO, 2014) meetings. At its 40th meeting, the Committee established an ADI of 0–1 µg/kg bw based on the developmental toxicity of ivermectin in CF-1 mice and recommended MRLs of 40 µg/kg for fat and 100 µg/kg for liver for residues of ivermectin in cattle using the marker ivermectin B_{1a} (WHO, 1993). Subsequently, the 58th meeting of the Committee recommended an MRL of 10 µg/kg for ivermectin in milk from dairy cattle, determined as ivermectin B_{1a} (WHO, 2002). At its 78th meeting, the Committee recommended an MRL of 4 µg/kg for cattle muscle, determined as ivermectin B_{1a}, based on the depletion data contained in the residue monographs prepared by the 36th and 40th meetings of the Committee and on 2 × LOQ of the analytical method as validated for beef muscle (WHO, 2014).

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[2] (1'R,2R,4'S,10'E,14'E,16'E,21'R)-6-(butan-2-yl)-21',24'-dihydroxy-12'-{[(2R,4S,6S)-5-{[(2S,4S,6S)-5-hydroxy-4-methoxy-6-methyloxan-2-yl]oxy}-4-methoxy-6-methyloxan-2-yl]oxy}-5,11',13',22'-tetramethyl-3',7',19'-trioxaspiro[oxane-2,6' tetracyclo[15.6.1.1;{4,8}.0;{20,24}]pentacosane]-10',14',16',22'-tetraen-2'-one

Table 4.1. MRLs currently defined for ivermectin residues in cattle tissues.

Concentration in µg/kg									
Jurisdiction	**ADI (µg/kg bw)**	**Muscle**	**Liver**	**Fat**	**Milk**	**Kidney**	**Offal**	**Injection Site**	**Marker Residue**
Codex (as of 78th JECFA)	1	4*	100	40	10				[22, 23-dihydro-avermectin B_{1a}] (Ivermectin B_{1a})
US[1]	5	650	1600						
Canada	1	10	70	100		140	140		
EU	10	30	100	100		30		1300	
Japan	1	10	100	40	10	10	10	10	
Australia	1	40	100	40	50	10			
New Zealand	1	10	100	40	10				

* Retained at Step 4 by the 22nd Session of the CCRVDF (FAO/WHO, 2015). The 78th JECFA recommended an MRL for cattle muscle based on 2 x LOQ of the analytical method (WHO, 2014). The dietary intake calculation prepared by the 40th Meeting of the Committee included an estimate of the potential intake from muscle, based on concentrations of total residue reported from the radiolabel study (WHO, 1993). The numbers reported for the US are tolerances, which are derived using a different estimate of dietary intake than Codex MRLs.

At its 75th meeting, the Committee concluded that there was a need to evaluate the toxicological information on ivermectin with a view to identifying a critical effect other than in the CF-1 mouse for the establishment of an ADI (WHO, 2012a). At its 22nd Session, the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) requested that JECFA re-evaluate the ADI and the MRLs in all cattle tissues (FAO/WHO, 2015). The CCRVDF noted that the draft MRL for ivermectin in bovine muscle recommended at the 78th meeting was in some cases ≥ 2.5 times lower than the MRL established in some countries where ivermectin was used and thus did not reflect current Good Veterinary Practice (GVP). Furthermore, JECFA had not recommended an MRL for bovine kidney. Table 4.1 summarizes the MRLs that have been established by several national authorities and by the Codex Alimentarius Commission.

The current Committee received residue depletion studies from two sources, including six studies which had not been previously reviewed by JECFA. The original Sponsor also submitted some studies which had previously been reviewed at earlier meetings of the Committee. All studies considered in this report are GLP-compliant unless otherwise indicated.

Tissue residue depletion studies

Radiolabelled residue depletion studies

A non-GLP-compliant radiolabelled depletion study (Study CA-218) not previously evaluated by the Committee was conducted using [^{3}H]ivermectin pour-on formulation (Chiu *et al.*, 1986). Twelve steers were dosed at 0.5 mg/kg bw with a topical formulation of 0.5% (w/v) ivermectin at a specific activity of 300 μCi/mg and a 93:7 ratio of H_2B_{1a}:H_2B_{1b}. Three animals in each group were slaughtered at 7, 14, 28 or 42 days post-dose to collect edible tissues and excreta. The total radioactive residue (TRR) concentrations were determined by combustion analysis. The drug was excreted mainly in faeces, with a much lower percentage excreted in urine. The residue concentrations were the highest in liver, followed by fat, muscle adjacent to the dose site, kidney and, lastly, regular muscle (Table 4.2).

Table 4.2. Total radiolabelled residue (TRR) in μg/kg measured in edible tissues of steers dosed topically with [^{3}H]ivermectin (Chiu *et al*., 1986).

Days Post-Dose	Number of animals	TRR, Mean ± S.D. measured (μg/kg)				
		Liver	Kidney	Dose site Muscle	Regular Muscle	Fat
7	3	226±102	21±10	43±11	8±3	72±33
14	3	126±53	14±7	41±14	5±2	52±19
28	3	69±92	7±7	19±17	2±2	25±23
42	3	26±12	4±2	6±4	2±0	23±10
Detection Limits of Method (μg/kg)		0.2	0.2-0.3	0.2-0.3	0.2	0.3

The results of the determination of the total radiolabelled residues (TRR) from the topical dose study CA-218 were consistent with the results determined in the earlier SC dose study RN-190 using [^{3}H]ivermectin submitted previously to the 36th meeting of the Committee (FAO, 1991; see reference Baylis, F.P. *et al*., 1979b, in the residue monograph prepared by the 36th meeting of JECFA). The total radiolabelled residue concentrations in fat, kidney, liver and muscle from studies using a topical dose, study CA-218 (Chiu *et al*, 1986), and a subcutaneous dose, study RN-190 (Jacob *et al*., 1979[3]), are presented in Table 4.3.

The residue depletion half-lives by both dosing routes were calculated using linear regression analysis. Faster residue depletion was found with SC administration for all four tissues compared to topical administration, possibly due to slower absorption following a topical dose.

[3] A report on Study RN-190 is referenced in the residue monograph prepared by the 32nd meeting of the Committee as Baylis, F.P. *et al*. 1979b.

Table 4.3. Comparison of mean + S.D. of the total radioactive residue (TRR) in fat, liver, kidney and muscle tissue of steers dosed subcutaneously in study RN-190 (Jacob *et al*., 1979) and topically (Chiu *et al*., 1986) with [^{3}H]ivermectin.

Days post-dose	TRR, mean + S.D. (µg/kg)							
	Liver		Fat		Muscle		Kidney	
	SC	TOPICAL	SC	TOPICAL	SC	TOPICAL	SC	TOPICAL
Project	**CA-218**	**RN-190**	**CA-218**	**RN-190**	**CA-218**	**RN-190**	**CA-218**	**RN-190**
7	622±223	226±102	220±58	72±33	18±7	8±3	55±18	21±10
14	104±43	126±53	88±64	52±19	4±2	5±2	12±5	14±7
21	48±17		45±21		2±2		5±2	
28	32±19	69±92	34±9	25±23	1±1	2±2	4±2	7±7
42		26±12		23±10		2±0		4±2
$T_{1/2}$ (Days)	4.8	11	7.6	19	5.8	17	5.4	13
Correl. Coeff.	0.9	0.73	0.92	0.7	0.74	0.52	0.83	0.64
Detection Limits (µg/kg)	0.2		0.3		0.2		0.2-0.3	

The higher variation of residue concentrations among individual animals topically dosed is reflected in the lower correlation coefficients. On Day 7 post-dose, residue concentrations in all edible tissues are higher from cattle dosed subcutaneously than dosed topically, while the residue concentrations are comparable on Day 14 post-dose. On Day 28 post-dose following topical administration, the mean total radiolabelled residues are slightly higher in liver, kidney, and muscle, although the residue concentrations are lower in fat with the topical dose than with the SC dose.

The unaltered drug in the edible tissues (liver, fat, kidney, and dose-site muscle) from all animals in study CA-218 (Chiu *et al*., 1986) was quantified by a HPLC-reverse isotopic dilution assay method (RIDA) developed in study RN-190 (Jacob *et al*., 1979). H_2B_{1a} is considered a satisfactory marker residue and accounted for 46-70% and 77-86% of the total residue for liver and fat from individual animals, respectively. Metabolite profiling showed that a group of polar metabolites was present that accounted for 28-38% of the TRR. The major metabolite 24-OH-H_2B_{1a} was identified in liver.

A similar pattern of metabolite profiles with the same major metabolite was found in livers from cattle dosed subcutaneously in study RN-190 (Jacob *et al*., 1979). For fat, the most notable difference between the two dosing routes is that the unchanged drug remains as the major residue for the topically dosed steers, accounting for 61% of the total residue on 28 days post-dose, while it had decreased to 18% in the subcutaneously dosed steers. . The nonpolar metabolites from both dosing routes were characterized as conjugates of 24-OH-H_2B_{1a}. The percentages of H_2B_{1a} and H_2B_{1b} in TRR from both radiolabelled residue studies, CA-128 (Chiu *et al*., 1986.) and RN-190 (Jacob *et al*., 1979) are summarized in Table 4.4.

Table 4.4. Average % of the marker residue H_2 B_{1a} and the minor metabolite H_2B_{1b} in the TRR in edible tissues from Study CA-218 (Chiu *et al*., 1986),) compared with those in study RN-190 Jacob *et al*., 1979)

Days Post-Dose	**Marker residue H_2 B_{1a} and the minor metabolite H_2B_1 as fraction of the TRR (%)**							
	Muscle		**Liver**		**Kidney**		**Fat**	
	% *H_2B_{1a}	% H_2B_{1b}	% *H_2B_{1a}	% H_2B_{1b}	% *H_2B_{1a}	% H_2B_{1b}	% *H_2B_{1a}	% H_2B_{1b}
CA-218 (Chiu *et al*. 1986)								
7	81	6	61	6	69	5	80	6
14	86	8	56	6	-	-	73	5
28	77	5	49	3	-	-	61	4
42	-	-	-	-	-	-	-	-

RN-190 (Jacob *et al*., 1979)								
7	66	15	56	8	52	10	61	6
14	78	8	49	3	45	5	49	2
28	50	-	36	4	50	-	35	1
42	-	-	37	2	-	-	18	1

* Marker residue.

The ratios of marker residue H_2B_{1a} (MR) to TRR were 0.50, 0.36, and 0.69 for kidney, liver and muscle, respectively. The average marker residue (H_2B_{1a}) to TRR ratios declined from 0.61 at 7 days to 0.18 at 28 days. H_2B_{1a} accounted for 81%, 86%, and 77% of the total residue in dose-site muscle on Day 7, 14, and 28 post-dose, respectively, in study CA-218 (Chiu *et al*., 1986).

Residue depletion studies with non-radiolabelled drug

In a non-radiolabelled residue depletion study (Pollmeier *et al*., 2007), 40 cattle (20 females, 20 males) weighing 255–382 kg were administered a single subcutaneous injection of a combination product at a dose of 0.2 mg ivermectin/kg bw plus 2 mg clorsulon/kg bw. Four cattle (2 males and 2 females) were not treated and served as controls. Tissue samples (entire liver, both kidneys, perirenal fat, skeletal muscle, core injection site and concentric ring around the core injection site) were collected on days 3, 10, 17, 28, 45, 52, 60, 70, 79 and 80 post-dose. Tissue samples were assayed for ivermectin marker residue (ivermectin B_{1a}) using an HPLC method with fluorescence detection. The validated LOQ of the method for the marker residue was 5 µg/kg, and the LOD was 1 µg/kg. The injection site core muscle had the highest residues among all analysed tissues, followed by the liver, fat, kidney and regular muscle. The drug distribution pattern was the same as that observed in the earlier [^{3}H]ivermectin residue metabolism and depletion study (Jacob *et al*., 1979). Peak concentrations of the marker residue H_2B_{1a} in all tissues were observed on Day 10 post-dose, except for kidney where the residue concentration peaked on Day 3. Ivermectin residues depleted to concentrations below the LOQ by Day 28 post-dose for skeletal muscle. For other tissues, the residue concentrations decreased to below the LOQ by Day 45 in half or more of the animals in each group and only liver had residues above the LOQ in 3 out of 4 animals at Day 52. At 60 days post-dose, residues were still found in some liver and fat samples. Although sampling continued through 80 days post-dose, no samples were analysed beyond 60 days post-dose. The concentrations of ivermectin in all edible tissues from each individual animal and the average at each time point up to 60 days post-dose are summarized in Table 4.5. For the untreated control animals, the samples assayed did not have detectable residues.

In an earlier study using non-radiolabelled drug (Wallace *et al*., 1992), 36 castrated male and 36 female crossbred beef cattle weighing 297–401 kg were used. This depletion study was considered previously by the 40th meeting of the Committee in recommending MRLs for ivermectin in tissues from cattle (FAO, 1993). Six cattle (3 males and 3 females) were not

treated and served as controls. A 1% w/v ivermectin injectable formulation was administered subcutaneously at 1 mL per 50 kg. Animals were killed in groups of 12 at 21, 28, 35, 42, 49 and 56 days post-dose, and edible tissues, including injection site, were collected from each animal. The samples were analysed by a validated HPLC method with fluorescence detection. The limit of detection (LOD) and "limit of reliable measurement", assumed to be the limit of quantification (LOQ), were 1–2 µg/kg and 10 µg/kg, respectively.

Residues were highest in liver, followed by residue concentration in fat. Residues had depleted to below the LOQ of the method in liver by 49 days post-dose. In muscle and kidney, residue concentrations had depleted to below the LOQ by 21 days post-dose.

Table 4.5. Mean ± S.D. of ivermectin concentrations measured in the depletion study after a single subcutaneous administration of a combination product at a dose of 0.2 mg ivermectin/kg bw plus 2 mg clorsulon/kg bw (Pollmeier *et al*., 2007)

5	Concentration of ivermectin B_{1a}, mean ± S.D. (µg/kg)						
	Animal ID	Liver	Kidney	Muscle			Fat
				Inner IS	Outer IS	Regular Muscle	
3	895	717	97.1	9 610	125	5.81	196
	107	387	76.5	54.2	38.3	11.7	180
	681	59	6.2	2 910	31.9	BLQ	13.2
	307	183	49.8	569	95.2	9.75	102
	Mean	337	57.4	3 290	72.6	6.8	123
10	572	541	33.4	32.9	27.6	15.3	215
	872	298	22.3	21.8	9.55	9.69	71.2
	272	271	30.1	56 200	108	18.2	193
	317	314	58.8	8760	760	19.2	170
	Mean	356	36.2	16 300	226	15.6	162
17	350	185	23.4	527	10.4	8.73	134
	603	97.2	19.9	25.8	14.4	7.97	103
	242	186	9.85	BLQ	8.5	BLQ	28.6
	284	263	27.6	4 180	13.1	7.21	87.5
	Mean	183	20.2	1 180	11.6	6.74	88.3

	627	23.6	9.26	340	BLOD	BLQ	52.2
	267	89.2	7.65	1260	5.36	BLQ	44.8
28	557	40.7	5.73	BLQ	BLQ	BLOD	36.5
	180	103	7.19	215	BLQ	BLOD	35.5
	Mean	64.1	7.46	455	BLQ	BLQ	42.3
	18	BLQ	BLOD	BLOD	BLOD	BLOD	BLQ
	592	19.3	BLQ	BLQ	BLQ	BLQ	14
45	228	18.9	BLOD	BLQ	BLOD	BLOD	5.7
	312	BLQ	BLOD	BLOD	BLOD	BLOD	BLQ
	Mean	11.1	BLQ	BLQ	BLQ	BLQ	6.46
	820	13.2	BLOD	5320	77.1	NA	BLQ
	708	110	6.27	BLQ	BLQ	NA	37.5
52	295	BLOD	BLOD	BLQ	BLOD	NA	BLOD
	618	12.7	BLOD	BLOD	BLQ	NA	6.11
	Mean	33.9	BLQ	1 330	20.9	NA	11.8
	935	6.39	BLOD	BLOD	BLQ	NA	BLQ
	508	52.9	BLOD	BLOD	BLOD	NA	7.47
60	326	BLOD	BLOD	BLOD	BLOD	NA	BLOD
	332	BLOD	BLOD	BLOD	BLOD	NA	BLOD
	Mean	15.1	BLOD	BLOD	BLQ	NA	BLQ

NA = Not Assayed; IS = Injection Site; BLOD = Below Limit of Detection (if 0 < BLOD < 0.99 ng/g, 0.50 ng/g was used in calculations; BLQ = Below Limit of Quantification (if 0.99 < BLQ < 5.12 ng/g, 3.06 ng/g was used in calculations); Note that the Sponsor had excluded day 3 results in the statistical analysis on the basis that its addition leads to unacceptable distribution of variance.

The marker residue H_2B_{1a} concentrations (µg/kg) in tissues of steers after subcutaneous administration determined in this study (Wallace *et al*., 1992) are presented in Table 4.6.

Table 4.6. Concentrations of ivermectin residues measured after a single dose subcutaneous administration of Ivomec (1% w/v ivermectin, 1 mL per 50 kg) to steers (Wallace *et al*., 1992).

	Group	Animal ID	Days Post-dose	Concentration of ivermectin B_{1a} residues (µg/kg)			
				Liver	Kidney	Fat	Muscle
1	2	81	21	23.0	5.0	37.0	6.0
2	2	85	21	18.0	5.0	28.0	2.0
3	2	89	21	8.0	2.0	12.0	1.0
4	2	400	21	42.0	5.0	33.0	4.0
5	2	402	21	24.0	6.0	42.0	6.0
6	2	415	21	68.0	11.0	37.0	4.0
7	2	417	21	9.0	3.0	22.0	3.0
8	2	435	21	46.0	2.0	14.0	3.0
9	2	440	21	95.0	2.0	52.0	7.0
10	2	443	21	80.0	5.0	25.0	4.0
11	2	453	21	120.0	ND	31.0	4.0
12	2	570	21	21.0	5.0	19.0	2.0
13	3	44	28	25.0	2.0	11.0	2.0
14	3	83	28	24.0	4.0	18.0	2.0
15	3	334	28	51.0	3.0	12.0	2.0
16	3	405	28	14.0	2.0	10.0	1.0
17	3	410	28	5.0	1.0	6.0	ND
18	3	414	28	44.0	2.0	16.0	2.0
19	3	419	28	4.0	ND	1.0	ND
20	3	433	28	34.0	5.0	23.0	3.0
21	3	439	28	55.0	ND	15.0	4.0
22	3	445	28	25.0	ND	5.0	ND
23	3	455	28	22.0	2.0	8.0	1.0
24	3	564	28	22.0	2.0	11.0	ND
25	4	309	35	7.0	2.0	9.0	ND
26	4	408	35	10.0	ND	6.0	ND
27	4	413	35	3.0	ND	5.0	ND
28	4	416	35	7.0	ND	6.0	2.0

29	4	424	35	8.0	ND	4.0	ND
30	4	432	35	38.0	4.0	15.0	2.0
31	4	436	35	3.0	ND	1.0	ND
32	4	442	35	18.0	2.0	9.0	1.0
33	4	448	35	4.0	ND	ND	ND
34	4	543	35	3.0	ND	ND	ND
35	4	589	35	2.0	ND	3.0	ND
36	4	597	35	12.0	4.0	16.0	2.0
37	5	86	49	ND	ND	ND	ND
38	5	98	49	ND	ND	3.0	ND
39	5	401	49	8.0	ND	5.0	1.0
40	5	421	49	ND	ND	ND	ND
41	5	428	49	ND	ND	ND	ND
42	5	4239	49	7.0	ND	2.0	ND
43	5	430	49	11.0	ND	2.0	ND
44	5	434	49	ND	ND	ND	ND
45	5	450	49	5.0	ND	2.0	ND
46	5	456	49	2.0	ND	ND	ND
47	5	557	49	ND	ND	ND	ND
48	5	588	49	7.0	ND	2.0	ND

ND = Not Detected; Residues were <LOQ in all tissues at day 56 and were therefore not included in the table.

In this study, the residue concentrations in the four edible tissues of animals treated in Study ASR 13527 were converted to total residue according to the expected proportion of marker to total residue determined from the radiolabelled study RN-190 shown in Table 4.7.

The calculations were based on the total residue concentrations and the H_2B_{1a} percentage of total residue with the exception that H_2B_{1a} residue concentrations at 42 days post-dose were calculated using the JECFA marker to total ratio of 0.67 for muscle since the percentage of H_2B_{1a} in total residue was not available for that day. The 0.67 ratio follows the decreasing trend for the marker to total ratio starting from Day 14 post-dose and is, therefore, considered an appropriate substitute.

Table 4.7. Total radioactive residues and marker residue H_2B_{1a} concentrations (µg/kg) in tissues of steers after a single dose subcutaneous administration of Ivomec (1% w/v ivermectin, 1 mL per 50 kg bw) to steers (Wallace *et al*., 1992) using marker to total residue correction factors determined in the radiolabelled Study RN-190 (Jacob *et al*., 1979).

Day Post-Dose	Animal ID	Concentrations in µg/kg							
		Liver		Fat		Kidney		Muscle	
		Total Residue TRR**	*Marker Residue MR	Total Residue TRR**	*Marker Residue MR	Total Residue TRR**	*Marker Residue MR	Total Residue TRR**	*Marker Residue MR
7	1981	717	352	232	140	63	31	20	14
7	1991	368	168	157	101	35	NA	10	NA
7	1994	782	300	270	160	68	40	23	15
14	1987	55	28	83	48	6	NA	2	NA
14	1992	135	51	95	30	14	5.3	5	3.1
14	2001	122	45	85	50	16	8.2	5	4.7
21	1989	68	21	69	12	7	3.7	4	2.1
21	1993	39	12	39	5.8	5	NA	1	NA
21	2006	37	10	28	6.1	4	NA	2	NA
28	1988	37	11	44	8.0	4	NA	1	NA
28	1997	47	16	28	5.0	5	NA	2	NA
28	2005	11	2	29	NA	2	NA	0	NA

* Denotes concentrations of the Marker residue H_2B_{1a} measured by HPLC/Fl; Detection Limits: RIDA -3.1 µg/kg for Liver, 3.3 µg/kg for Fat, 1.1 µg/kg for Kidney, 0.7 µg/kg for Muscle; Detection Limits HPLC/FL: 1-2 µg/kg, Limit of Reliable measurement – 10 µg/kg; NA = Not Assayed; ** From Report by Wood (1980). "Ivermectin (MK-0933): Tissue Residue in cattle Subcutaneous Injection. Study CA-129 [0.3 mg/kg Formulation B]".

The concentration of ivermectin residues determined in cattle fat, kidney, liver and regular muscle tissues from these two GLP-compliant studies using non-radiolabelled studies submitted for consideration by the current meeting of the Committee (Pollmeier *et al*., 2007; Wallace *et al*., 1992) are summarized in Table 4.8.

Table 4.8. Concentrations of ivermectin residues determined in cattle liver, kidney, fat and regular muscle tissue (Pollmeier *et al*., 2007; Wallace *et al*., 1992).

	PR&D 0127201 (Pollmeier *et al*., 2007)				ASR13527 (Wallace *et al*., 1992)			
Days Post-Dose	**Liver**	**Muscle**	**Kidney**	**Fat**	**Kidney**	**Fat**	**Liver**	**Muscle**
3	337±287	6.8±5.2	57.4±39.2	123±83.8				
10	356±125	15.6±4.3	36.2±15.8	162±63.5				
17	183±68	5.98±NA	20.2±7.6	588.3±44.2				
21					4.3±2.8	29.3±11.8	46.2±36.6	4.2±1.9
28	64.1±38	BLQ	7.46±1.5	42.3±7.8	2.6±1.2	11.3±6.1	27.1±16.4	2.1±0.99
35					BLQ	7.4±4.9	9.6±10.1	BLQ
45	11.1±NA	BLQ	BLQ	6.46				
49					BLQ	BLQ	BLQ	BLQ
52	33.9±NA	NA	BLQ	11.8				
60	15.1±NA	NA	BLOD	BLQ				

BLQ = Below Limit of Quantification; BLOD = Below Limit of Detection.

In a non GLP-compliant study (Errecalde. and Mestorino, 2007)) sixteen Hollando Argentino calves weighing 100-150 kg were subcutaneously administered an oil based formulation of ivermectin 3.15%, developed by INCAM S.A. for Brouwer S.A., at a dose of 1 mL per 50 kg live body weight. The animals were assigned to 4 groups of 4 animals and killed 50, 70, 90 and 110 days post-dose. Fat, kidney, liver, skeletal muscle and injection site muscle tissues were collected at slaughter.

For the method of analysis, 5 g samples were homogenized in an Ultra-Turrax with acetonitrile and ultrasonicated. The homogenate was centrifuged for 5 min at 3000 rpm. The supernatant was transferred to a clean tube. The procedure was repeated with the base of the tube, and the supernatant was added to same tube. 1.6 mL of water at 4° C was added to the tube, then vortex mixed and placed in a previously conditioned Bakerbond cartridge. The cartridge was eluted, the eluate was evaporated, derivatized and injected into the HPLC system with fluorescence detection.

The limit of quantification of the method for ivermectin in tissues was 2 µg/kg and the limit of detection was 0.5 µg/kg. The percentage recoveries for ivermectin in fat, kidney, liver and muscle were 82.3%, 61.5%, 71.8% and 82.5%, respectively, with corresponding coefficients of variation (CV) of 17%, 18.4%, 9.5% and 3.9%, respectively. The percentage recovery for ivermectin at the injection site was 80.2% with a CV of 11.1%.

Highest concentrations of ivermectin residues were found in the injection site tissues and were similar to the concentrations measured in liver at 50 days post-dose (Table 4.9). These concentrations depleted slowly with time until about 90 days post-dose where low levels of ivermectin residues were still measurable. Another finding was the elevated concentration of ivermectin residues in liver and fat, which appears logical and consistent with the characteristics of ivermectin (high lipid solubility).

A GLP-compliant study was conducted using twenty five healthy bovines (13 males and 12 females) of British breed or their crossbreeds with body weights between 250 and 400 kg (Formentini, 2010). One animal was not treated and served as control. Twenty animals were allotted to 5 groups of 4 each and each animal was given a single subcutaneous injection at the base of the neck with Bagomectina LA Star/Ivergen Platinum 3.15 at a rate of 1 mL per 50 kg of live body weight (equivalent to 630 µg/kg). The 5 treated groups were killed at 20, 40, 60, 90 and 130 days post-dose, respectively.

One hundred and fifty (150 g) grams of fat, kidney, liver, back muscle and 500 g from the injection site were collected and analysed using a HPLC-MS/MS method with a LOQ of 18 µg/kg reported for liver. The LOQs of the method for the other tissues were not reported**.** The results of the analysis of ivermectin residue concentrations measured in the fat, kidney, liver and injection site muscle tissues after a single subcutaneous injection of ivermectin are presented in Table 4.10.

Table 4.9. Concentrations of ivermectin in various tissues and plasma between 50 and 110 days post-administration of MR11 3.15% in samples from animals treated with 1 ml per 50 kg of weight by the subcutaneous route (Errecalde and Mestorino, 2007).

	Concentration of ivermectin B_{1a} residues in tissues									
	Injection Site (µg/kg)		**Regular muscle (µg/kg)**		**Liver (µg/kg)**		**Kidney (µg/kg)**		**Fat (µg/kg)**	
Rec. Factor	**1.198**		**1.198**		**1.392**		**1.385**		**1.215**	
Days Post-Dose	**OC**	**RCC**	**OC**	**RCC**	**OC**	**RCC**	**OC**	**RCC**	**OC**	**RCC**
	25.4	30.4	25.4	30.4	20.2	28.1	15.7	21.8	25.4	30.9
	24.7	29.5	24.7	29.5	42.1	58.6	4.4	6.1	15.1	18.4
	31.3	37.5	31.3	37.5	30.3	42.1	7.2	9.99	10.7	13.0
	40.2	48.2	40.2	48.2	50.5	70.3	8.1	11.2	22.2	26.9
Mean±S.D.	30.4±7.2	36.4±8.6	8.8±3.6	10.5±4.3	35.8±13.3	49.8±18.5	8.9±4.8	12.3±6.6	18.4±6.6	22.3±8.0
	8.3	9.9	8.3	9.9	12.2	16.9	0.5	0.69	14.6	17.8
	24.6	29.5	24.6	29.5	18.4	25.6	3.1	4.3	4.1	5.0
70	15.1	18.0	15.1	18.0	9.6	13.4	6.3	8.7	6.4	7.8
	12.3	14.7	12.3	14.7	4.5	6.3	4.0	5.6	9.3	11.3
Mean±S.D.	15.1±6.9	18.1±8.3	2.1±1.4	2.5±1.7	11.2±5.8	15.6±8.1	3.5±2.4	4.8±3.3	8.6±4.5	10.4±5.5

90	3.1	3.7	3.1	3.7	9.9	13.7	0.25	0.35	4.8	5.8
	10.7	12.8	10.7	12.8	8.4	11.7	0.25	0.35	0.25	0.30
	6.2	7.4	6.2	7.4	3.2	4.4	2.5	3.5	3.58	4.3
	12.6	15.1	12.6	15.1	13.6	18.9	1.0	1.4	4.23	5.13
Mean±S.D.	8.2±4.3	9.8±5.2	1.2±0.8	1.4±0.96	8.8±4.3	12.2±5.9	1.3±0.99	1.8±1.4	3.3±1.9	4.0±2.3
110	0.25	0.299	0.25	0.299	7.3	10.2	0.25	0.35	0.25	0.30
	0.26	0.311	0.26	0.31	0.25	0.35	0.26	0.36	0.25	0.30
	0.25	0.299	0.25	0.299	3.7	5.2	0.25	0.35	3.4	4.13
	0.26	0.311	0.26	0.31	4.95	8.9	0.25	0.35	4.1	4.99
Mean±S.D.	0.5±0	0.6±0	0.5±0	0.6±0	4.1±2.8	5.7±3.9	0.5±0	0.7±0	2.1±1.9	2.5±2.3

OC = Original measured Concentration; RCC = Recovery Corrected Concentration; the limit of quantification for ivermectin in tissues was 2 ng/g and the limit of detection was 0.5 ng/g; * When concentrations were not detected, the detection limit of the technique is recorded; ** When the measurement of concentration was below the limit of quantification, it is marked with double asterisks.

Table 4.10. Concentration of ivermectin B_{1a} in fat, kidney, liver and injection site muscle tissue from experimental animals a single subcutaneous injection with Bagomectina LA Star/Ivergen Platinum 3.15 (Formentini, 2010).

Days Post-dose	Concentration of ivermectin B_{1a} residues in tissues				
	Animal ID	Injection Site(µg/kg)	Kidney (µg/kg)	Liver (µg/kg)	Fat (µg/kg)
20	068	396.3	38.6	159.2	110.1
	079	183.3	19.4	157.4	40.9
	083	102.0	39.2	110.7	31.2
	084	791.2	41.2	146.1	23.4
	Mean±S.D.	368.2±308.1	34.6 ±10.2	143.4± 22.5	51.4±39.8
40	065	28.5	10.4	91.5	ND
	087	134.7	18.0	69.0	ND
	125	75.2	12.3	95.9	ND
	686	166.6	12.4	86.7	ND
	Mean±S.D.	101.3±61.5	13.3± 3.3	85.8±11.8	ND
60	086	7.7	ND	26.7	ND
	088	25.5	ND	ND	ND
	089	ND	ND	ND	ND
	3193	30.1	ND	18.6	ND
	Mean±S.D.	21.1±11.9	ND	22.7±5.7	ND
90	064	20.7	ND	24.3	ND
	073	36.1	ND	ND	ND
	074	52.0	ND	ND	ND
	078	ND	ND	ND	ND
	Mean±S.D.	36.3±15.7	ND	24.3	ND
130	063	16.9	ND	ND	ND
	069	5.8	ND	ND	ND
	071	34.2	ND	ND	ND
	125	5.8	ND	ND	ND
	Mean± S.D.	15.7±13.4	ND	ND	ND

ND = Not Detected.

Although the final report of the study submitted for review claimed that the analytical method was validated, no method validation report was provided to JECFA upon request to the Sponsor. Therefore, relevant information that would have enabled JECFA to determine whether the method used was suitable and fit-for-purpose was not provided. The relevant information would have included the accuracy and precision, the selectivity, sensitivity, interference tests, and stability of ivermectin standards in solution and under frozen storage conditions.

A study of unknown GLP status was conducted to determine the pre-slaughter withdrawal time for a 1% Ivermectin formulation with AD3E Vitamins {Bagomectina AD3E Forte®/Ivergen Plus AD3E®} (Formentini., 2012). Twenty cattle were administered a single subcutaneous dose at a rate of 1 mL per 50 kg of body weight (equivalent to 630 µg/kg bw). The animals were allocated into groups of 4 and killed 7, 14, 21, 28 and 35 days post-dose. Liver and fat samples were collected from each animal at slaughter and analyzed using a method with reported LOQs of 13.6 and 3.3 µg/kg for liver and fat, respectively. Kidney and muscle tissue samples from the study were not collected for analysis. The results of the liver and fat tissue residue analysis are shown in Table 4.11.

It is to be noted that the final report of this study (Formentini, 2012) on the "clinical trial to determine the pre-slaughter withdrawal time for a 1 % ivermectin formulation with AD3E vitamins after subcutaneous administration to cattle" was scant in detail. The report did not provide details about the nature of the experiments conducted (whether under GLP or not) and did not provide any validation reports on the analytical methods used for the analysis of the tissue matrices to enable JECFA to evaluate whether the method was suitable and fit-for-purpose. Additional supporting information could not be provided by the Sponsor in response to a request from JECFA.

In another study of unknown GLP status (Boggio, 1998), 21 cattle were treated with a single dose subcutaneous injection of a slow release formulation at 630 µg ivermectin/kg bw. In this study, three animals were killed on each of days 21, 42, 49, 56, 63, 70, 77 and 84 days post-dose. Samples of fat, kidney, liver, regular muscle and injection site muscle were collected from each animal and analysed using an HPLC method with fluorescence detection and a reported limit of detection of 0.5µg/kg. The results of the depletion study are presented in Table 4.12.

It is to be noted that the final report (Boggio, 1998) did not provide details about the nature of the experiments conducted (whether under GLP or not) and did not provide any validation reports on the analytical method used for the analysis of the tissue matrices to enable the JECFA Experts to evaluate whether the method was suitable and fit-for-purpose. Additional supporting information requested by JECFA could not be provided by the Sponsor.

No raw data were provided for the 84 day sampling but the indicated average results were presented in a summary page provided by the study author.

Table 4.11. Concentration of ivermectin B_{1a} residues in cattle liver and fat tissues after a single dose subcutaneous administration of a 1% ivermectin formulation with AD3E vitamins (Bagomectina AD3E Forte/Ivergen Plus AD3E) to cattle (Formentini, 2012).

		Concentration of ivermectin B_{1a} residues in tissues	
Days Post-dose	**Animal ID**	**Liver (µg/kg)**	**Fat (µg/kg)**
7	431	41.1	369.1
	432	123.5	264.9
	433	95.3	231.5
	434	97.0	218.1
	Mean±S.D.	89.2± 34.6	270.9± 68.4
14	435	89.1	75.7
	436	33.0	106.9
	437	39.1	133.6
	438	57.5	116.5
	Mean±S.D.	54.7± 25.2	108.2± 24.3
21	439	16.9	33.7
	440	54.1	445.5
	441	27.5	70.0
	442	48.9	69.1
	Mean±S.D.	38.6± 17.6	154.6± 194.7
28	443	-	-
	444	-	1.6
	445	-	6.2
	446	-	4.1
	Mean±S.D.	-	4.0 ±2.3
35	447	-	-
	448	-	-
	449	-	-
	450	-	-
	Mean±S.D.	-	-

Table 4.12. Distribution of ivermectin residues in cattle after administration of a single subcutaneous dose of ivermectin slow release formulation at 630 µg/kg body weight. (Boggio, 1998).

Day Post Drug Administration	Concentration of ivermectin B1a residues in tissues (µg/kg)				
	Injection Site	Regular muscle	Liver	Kidney	Fat
21	106	26	156	14	223
	89	31	191	26	189
	121	18	215	20	134
Mean ± S.D.	105±16	25±7	187±30	20±6	182±45
42	78	12	19	7	79
	77	18	28	11	88
	53	27	46	7	38
Mean ± S.D.	69±14	19±8	31±14	8.3±2.3	68.3±27
49	37	2	19	U	28
	56	10	17	3	41
	87	9	31	7	28
Mean ± S.D.	60±25	7±5	22.3±8	5±2.8	32.3±7.5
56	12	2	6	U	4
	57	U	19	U	13
	62	5	8	2	16
Mean ± S.D.	43.7±27.5	3.5±2.1	11±7	2±NA	12.3±4
63	39	2	6	U	11
	52	3	8	U	19
	27	U	U	U	6
Mean ± S.D.	39.3±12.5	2.5±0.71	7±1.4	±	12.0±6.6
70	14	U	U	U	2
	32	U	1	U	6
	24	U	5	U	7
Mean ± S.D.	23.33±9.0	±	3.0±2.8	±	5.0±2.7

77	21	U	2	U	3
	19	U	U	U	4
	7	U	U	U	U
Mean ± S.D.	16±8	U	U	U	3.5±0.7
84					
Mean ± S.D.	12.0				1.0

U = Undetected; Analysis conducted using LC/FL method with a detection limit of 0.5 µg/kg; No raw data were provided for the 84 day sampling but the indicated mean results for the injection site and fat were presented in a summary page; NA = Not Available.

While lacking sufficient information to be considered suitable in the development of MRL recommendations on their own, these 4 studies provided supporting information that were consistent with those presented in the more well documented studies (Chiu *et al*., 1986; Pollmeier *et al*., 2007; Wallace *et al*., 1992).

On the basis of the deficiencies identified by the Committee in these four new depletion studies, the data from those studies were not included in the elaboration of MRLs for ivermectin. Only two non-radiolabelled depletion studies (Wallace *et al*., 1992; Pollmeier *et al*., 2007) together with the two radiolabel studies (Jacob *et al*., 1979; Chiu *et al*., 1986) were used in the development of MRL recommendations.

Residues at the injection site

Significantly high concentrations of ivermectin residues resulting from the subcutaneous administration of ivermectin following label instructions were measured at the injection sites in two of the studies (Pollmeier *et al*., 2007; Chiu *et al*., 1986). These injection site residue concentrations are summarized in Table 4.13.

Table 4.13. Marker residue H_2B_{1a} concentrations (Mean + S.D.) in injection-site muscle samples (Chiu *et al*., 1986; Pollmeier *et al*., 2007).

Days Post Drug Administration	Concentration of ivermectin B1a residues in tissues (µg/kg)		
	CA-218 (Chiu *et al*., 1986)	PR&D 0127201 (Pollmeier *et al*., 2007)	
		Inner IS	Outer IS
3		3 290±4 395	72.6±45.0
7	34.8±8.5		
10		16 300±26 947	226±358
14	35.3±11.8		
17		1 180±2 013	11.6±2.7
28	14.4±12.7	455±556	BLQ
42	4.0±2.4		
45		BLQ	BLQ
52		1 330	20.9
60		BLOD	BLOQ

BLQ = Below Limit of Quantification; BLOD = Below Limit of Detection.

Methods of Analysis for Residues in Tissues

Validation data were provided for the reversed-phase HPLC method with fluorescence detection used to determine the marker residue (ivermectin B_{1a}) in bovine edible tissues in one of the depletion studies considered by the Committee (Pollmeier *et al*., 1997). After tissue homogenization in acetone–water, the marker residue is extracted with isooctane. Following evaporation, fat is removed from the sample with acetonitrile/hexane binary mixture. The solvent is again removed by evaporation, and a fluorescent derivative is formed by on-line derivatization with trifluoroacetic anhydride/*N*-methylimidazole (Figure 4.1). The derivatized residue is assayed using HPLC/fluorescence with an excitation wavelength of 365 nm and an emission wavelength of 470 nm. No internal standard is used. The method quantitatively measures the B_{1a} component of ivermectin by comparison with a series of derivatized ivermectin external standards.

The Committee assessed the validation data against the analytical requirements as published in CAC/GL71-2009 (FAO/WHO, 2014).The method has been validated for selectivity, precision and accuracy, LOD and LOQ. No interfering peaks were observed at the retention time of

ivermectin B_{1a} in any of the non-fortified tissue samples, attesting to the selectivity of the method. The response of the method was linear over the range 5–1000 μg/kg. Calculated LODs were 0.10 μg/kg for fat, 0.10 μg/kg for kidney, 0.10 μg/kg for liver and 0.05 μg/kg for muscle. The LOD of the method was set at 1 μg/kg (lowest analysed concentration). The LOQ (the lowest concentration validated for ivermectin B_{1a} with an acceptable precision and accuracy) was set at 5 μg/kg for all tissues.

The selectivity (interference caused by metabolites or homologues of ivermectin) has been studied (Wood, 1980; Wood, 1981); interferences caused by ivermectin's homologues have not been observed. Supporting data are available from other studies, including application of the method to bovine, ovine and swine liver (Markus and Sherma, 1992), to bovine liver, kidney, fat and muscle (Kvaternick, 1992), to swine liver (Wood, 1981; Kvaternick, 1995).

Accuracy of the method was assessed by measurement of recovery of the analyte from tissues fortified at known concentrations, calculating a percent recovery. Various observations from different sources involving bovine tissue were provided. For liver, recoveries were within the range 72 to 89%, at concentrations from 3.6 to 1000 μg/kg. The reported values covered a total of 77 replications from 6 different studies. For muscle, recoveries were within the range 81 to 100%, again calculated at concentrations from 3 to 1000 μg/kg. The reported values covered a total of 48 replications from 5 different studies. For kidney, recoveries ranged from 71 to 98 % at concentrations from 5 to 1000 μg/kg, with reported values covering 45 replications collected in 5 different studies. For fat, recoveries of 73 to 92% were calculated at concentrations ranging from 5 to 1000 μg/kg, based on reported values which covered in total 37 replications collected in 4 different studies. As the grand average recovery for all tissues was within the range of 70 to 110% for ivermectin H_2B_{1a} (concentration ranging from 5 to 1000 μg/kg), no correction is applied for recovery in the method. These values meet the requirements for method recovery in CAC/GL 71-2009 (FAO/WHO, 2014).

A convenient measure for determining the precision is the coefficient of variation (%CV or %RSD). Observations with the ivermectin method have shown acceptable precision for edible tissue. The precision of the method generally meets the current VICH guideline requirements (VICH GL48, 2015), i.e. %CVs better than 25% for concentration values below 10 μg/kg, better than 15% for concentrations within 10-100 μg/kg range and better than 0.15 for values above 100 μg/kg. These values also meet the requirements for method precision in CAC/GL71-2009 (FAO/WHO, 2014).

Linearity of the method validation external standards was assessed by calculating the coefficient of correlation of sets of six standards each run before and after the analytical samples. The coefficient of correlation (r) was greater than 0.985 for ivermectin standards.

The limit of detection (LOD) is the concentration at which the smallest possible amount of analyte can be differentiated from background with acceptable statistical certainty. For this method, the LOD was determined by the signal to noise ratio in the presence of matrix. The signal to noise ratios (S/N) ranged from 30 to 69 for approximately 1 μg/kg ivermectin H_2B_{1a}. Theoretical calculated LOD (S/N>3) would be 0.05 μg/kg for muscle, 0.10 μg/kg for fat,

0.10 µg/kg for kidney and 0.10 µg/kg for liver. The LOD of the method was set (by the Sponsor) at 1 µg/kg, the lowest concentration analyzed.

The limit of quantification (LOQ) is the concentration at which the smallest amount of analyte can meet the requirements of precision and accuracy. The Limit of Quantification (LOQ), the lowest concentration validated for ivermectin H_2B_{1a}, was set at a concentration of 5 µg/kg (Kvaternick, 1992 and 1995, and Wehner, 1990 and 2004). The method, as described, did not include a suitable internal standard.

With the exception of some passing comments in the Sponsor's dossier (Merial Inc., 2015) on the stability of stock solutions, glassware cleaning and noting the instability in water for ivermectin during HPLC separation, there was no indication of any systematic study of the stability of the analytes in solution, under frozen storage conditions or under freeze-thaw storage conditions.

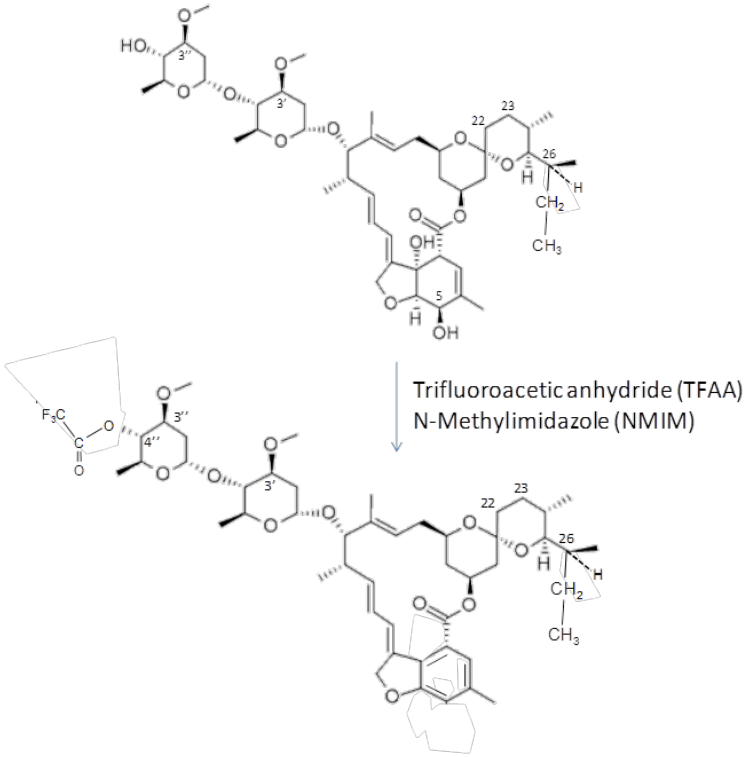

Figure 4.1. Reaction mechanism for the formation of the fluorescent ivermectin derivative.

The Committee considered the quantitative HPLC/fluorescence method submitted by the Sponsor to be suitably validated to support the MRLs recommended by the present meeting of the Committee.

The Committee also noted that a validated LC-MS/MS method (Danaher, 2013) submitted for review by the 78th meeting of the Committee (WHO, 2014) meets the requirements of guideline CAC/GL 71-2009 (FAO/WHO, 2014) and was also available for regulatory analysis.

Appraisal

Ivermectin has been previously reviewed by the Committee. Ivermectin is a chemically modified-fermentation product belonging to the macrocyclic lactone class of endectocides.

Ivermectin consists of a mixture of two homologous compounds, 22,23-dihydroavermectin B_{1a} (H_2B_{1a}, not less than 80%) and 22,23-dihydroavermectin B_{1b} (H_2B_{1b}, not more than 20%). In veterinary medicine, ivermectin is used in cattle, sheep, goats, pigs, horses and reindeer at doses of 0.1-0.5 mg/kg body weight, given subcutaneously, topically or orally as a single dose treatment only. Two radio-labelled studies in cattle, one after topical administration and one after subcutaneous administration, demonstrated that ivermectin B_{1a} (22,23-dihydro-avermectin B_{1a}), the principal component of parent drugs is the marker residue.

On the basis of the deficiencies identified by the Committee in four new depletion studies submitted for consideration by the current meeting, the data from those studies were not included in the elaboration of MRLs for ivermectin. Only two non-radiolabelled depletion studies (Pollmeier *et al.*, 2007; Wallace *et al.*, 1992) together with the earlier studies with radiolabelled drug (Chiu *et al.*, 1986; Jacob *et al.*, 1979) were used in the development of MRL recommendations. Two routes of administration of ivermectin were used to perform these studies: the subcutaneous route (Jacob *et al.*, 1979, Pollmeier *et al.*, 2007, Wallace *et al.*, 1992) and a pour-on application (Chiu *et al.*, 1986). Two different ivermectin formulations were used for the non-radiolabelled studies.

The Committee confirmed that ivermectin B_{1a} is the marker residue and that liver and fat are the target tissues for the use of ivermectin in cattle.

The Committee used the ratio of the marker residue (ivermectin H_2B_{1a}) to the total residues in cattle previously defined by the 40th meeting of the Committee. The ratios were 0.67 in muscle, 0.37 in liver, 0.54 in kidney and 0.18 in fat.

All the data reported above the limit of detection (1 µg/kg) from the two studies with non-radiolabelled ivermectin were pooled together to estimate the depletion curves (Pollmeier *et* al., 2007; Wallace et al., 1992) with a large number of measurements for each tissue and timepoint.

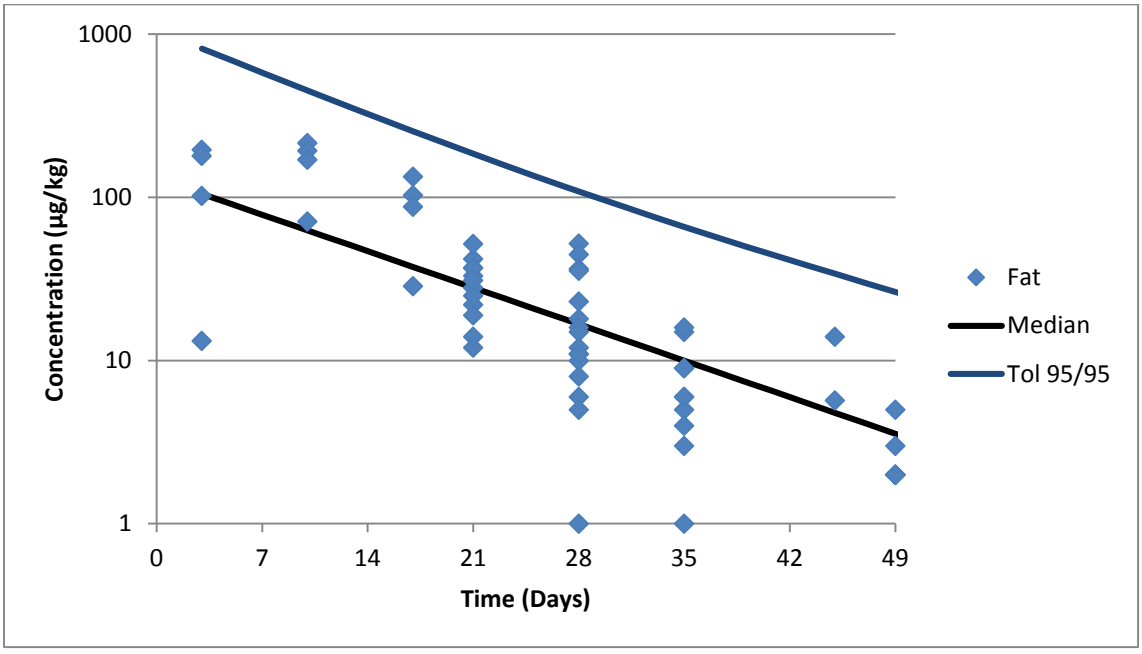

Figure 4.2. Median concentrations and upper tolerance limits of ivermectin B_{1a} in fat.

MRLs derived from the two studies were graphically compared to the data obtained from all data reported to confirm that they are compatible with good veterinary practices (withdrawal times ranged between 14 and 122 days).

Figures 4.2-4.5 show the distribution of the median concentrations and upper tolerance limits of ivermectin B_{1a} in fat, kidney, liver and muscle, respectively, versus days post-dose generated from the 2 well documented non radiolabelled depletion studies.

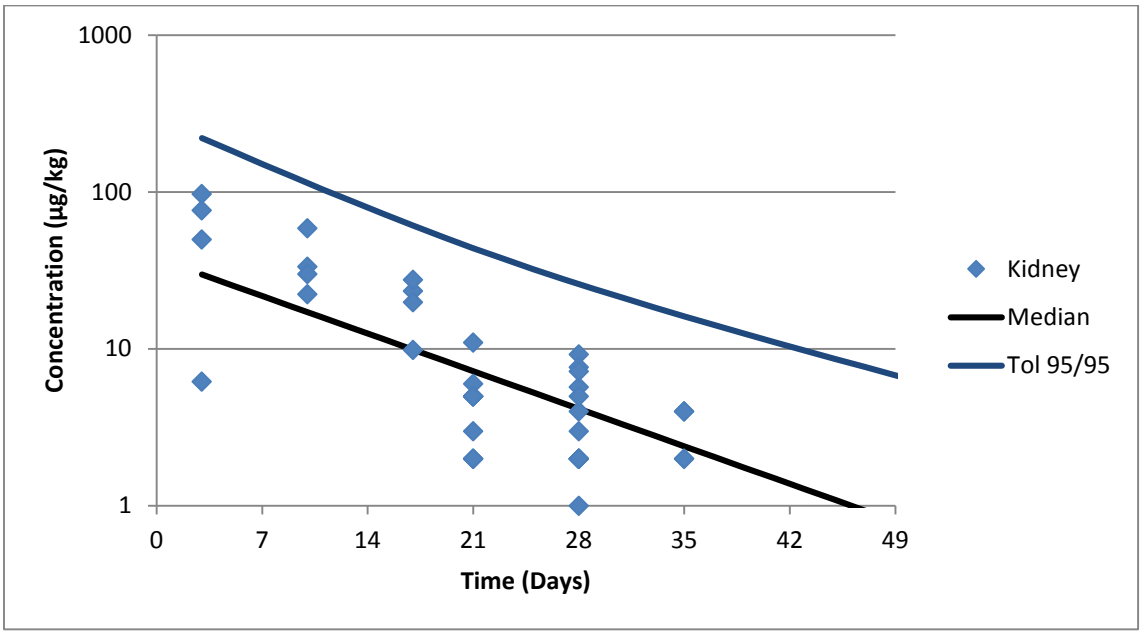

Figure 4.3. Median concentrations and upper tolerance limits of ivermectin B_{1a} in kidney.

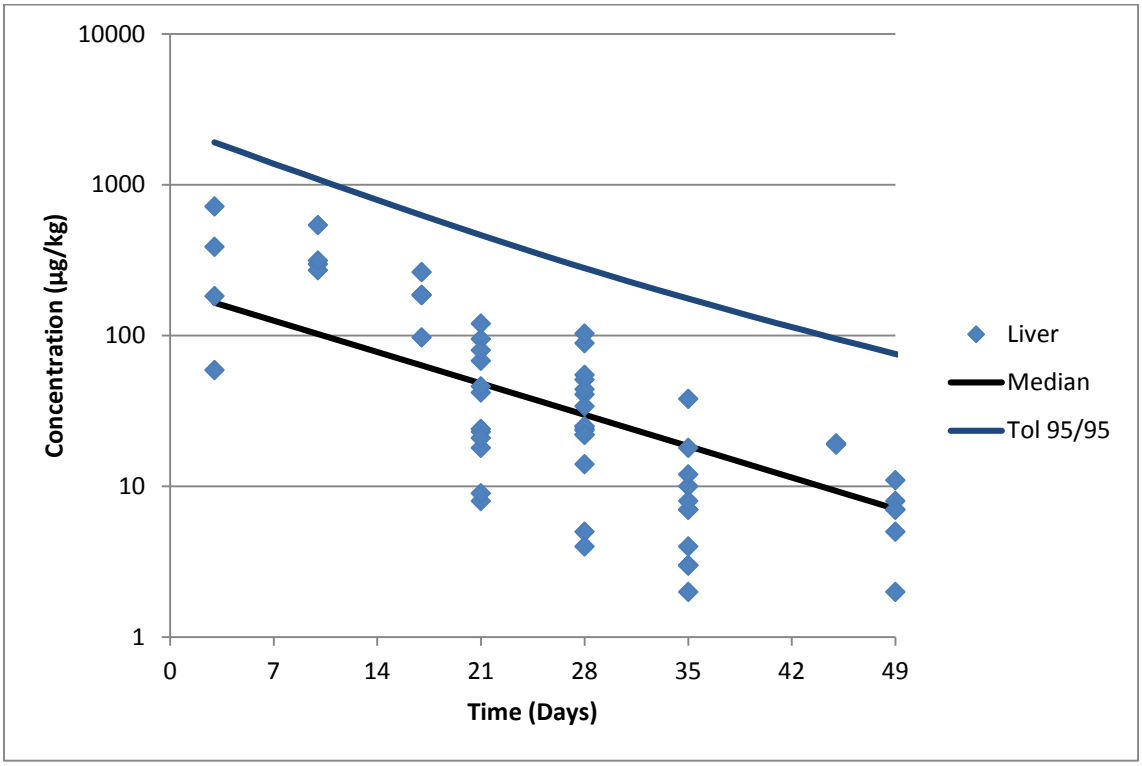

Figure 4.4. Median concentrations and upper tolerance limits of ivermectin B_{1a} in liver.

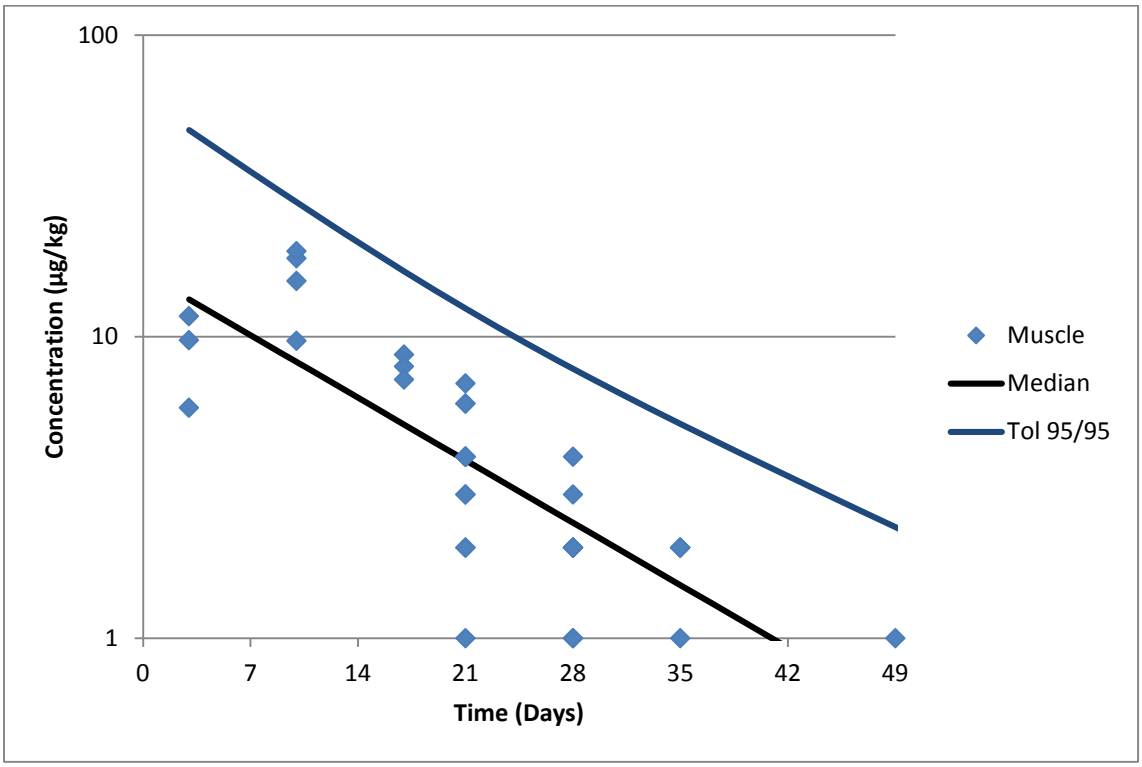

Figure 4.5. Median concentrations and upper tolerance limits of ivermectin B_{1a} in muscle.

Figures 4.6-4.9 show the distribution of the pooled data of all the non-radiolabelled depletion studies for fat, kidney, liver and muscle tissue data versus days post-dose submitted for consideration by the current JECFA.

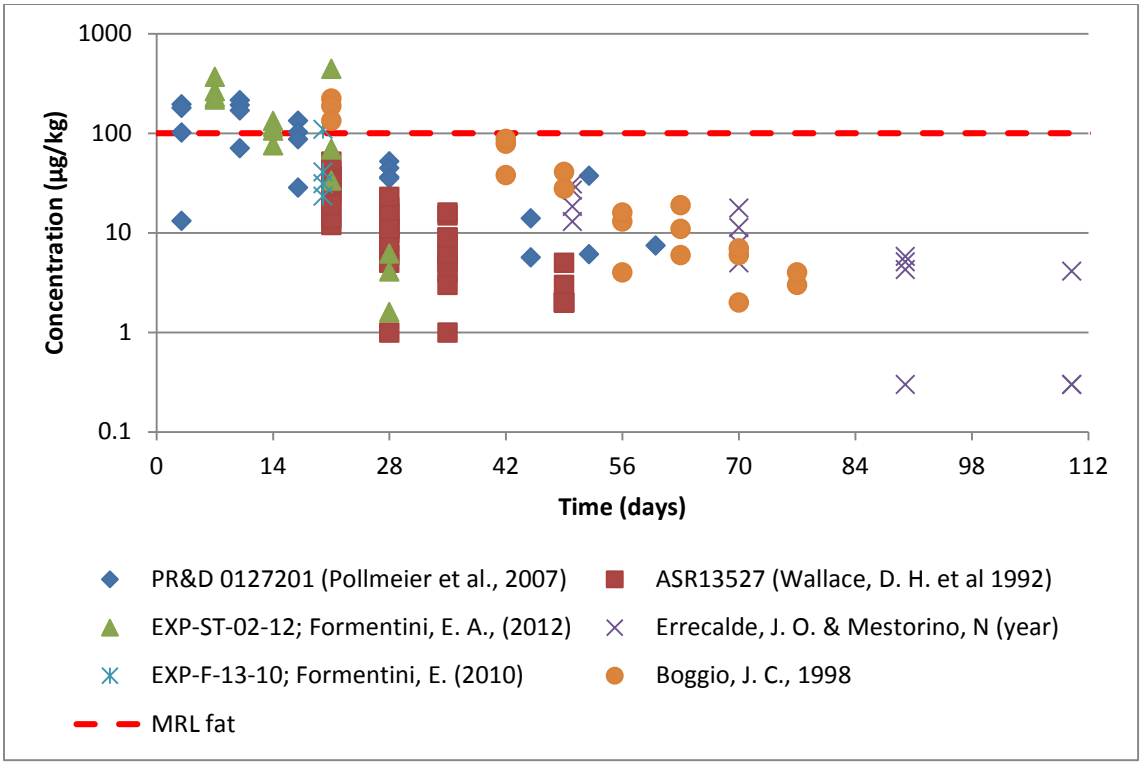

Figure 4.6. Derivation of MRLs from data provided for residues in fat.

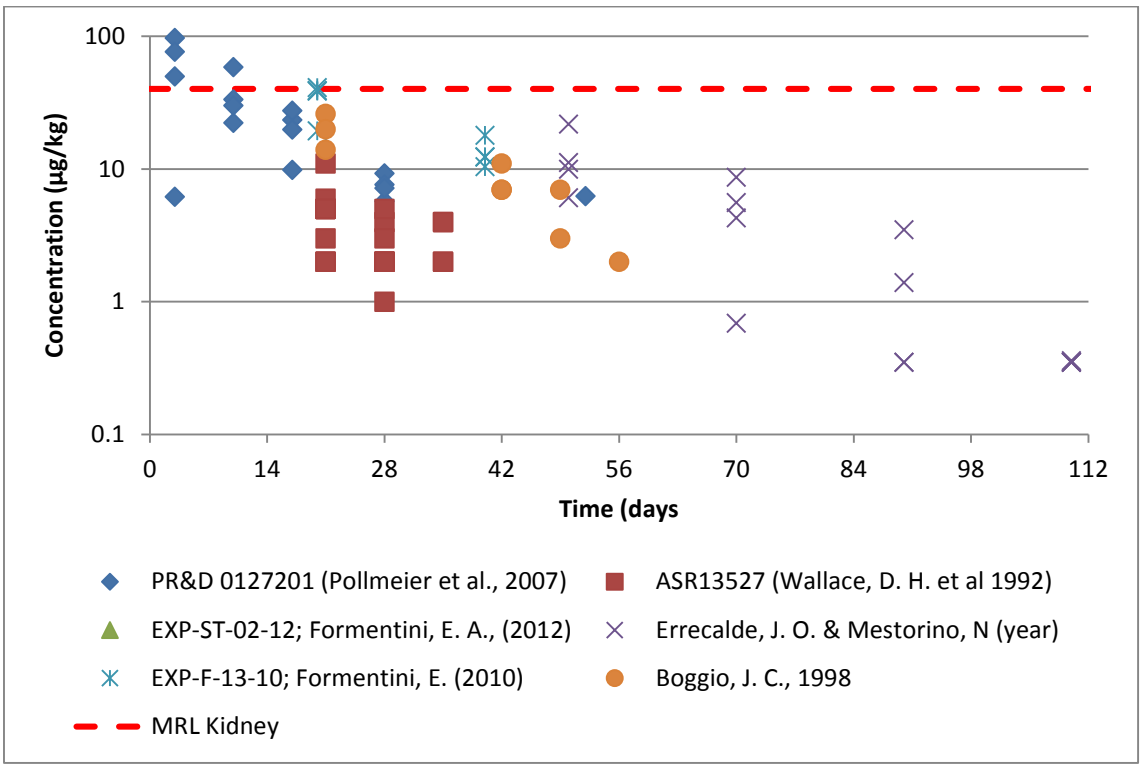

Figure 4.7. Derivation of MRLs from data provided for residues in kidney.

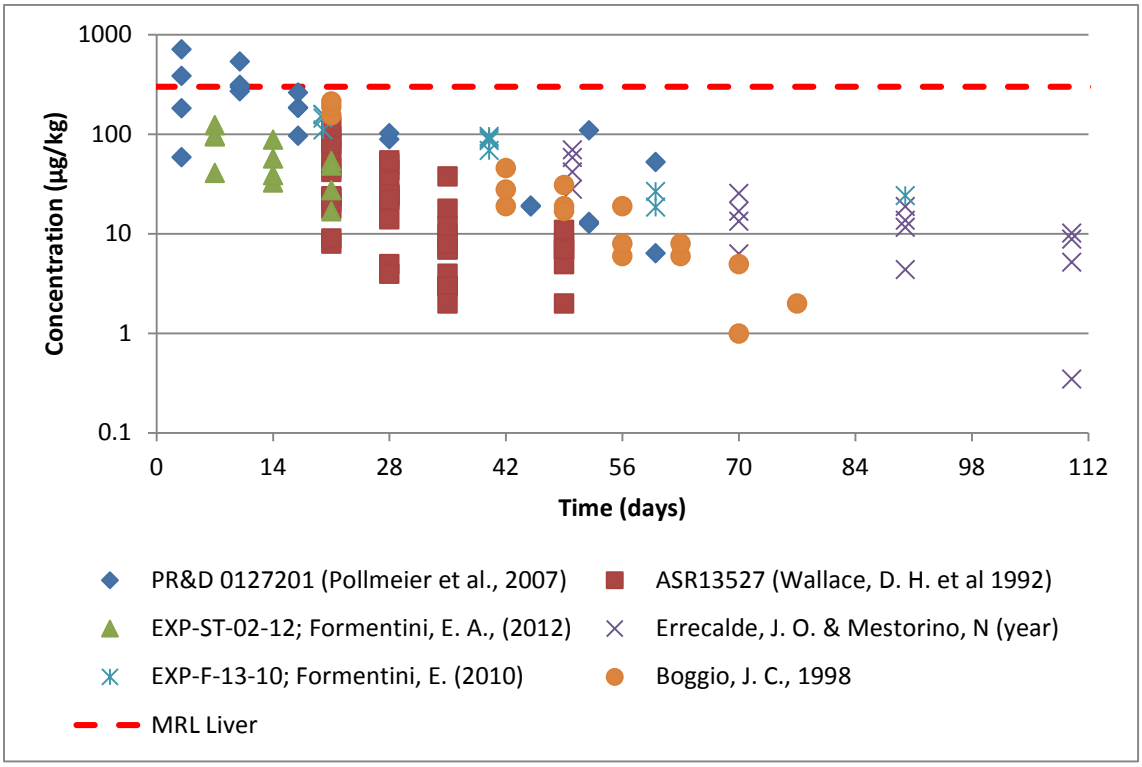

Figure 4.8. Derivation of MRLs from data provided for residues in liver.

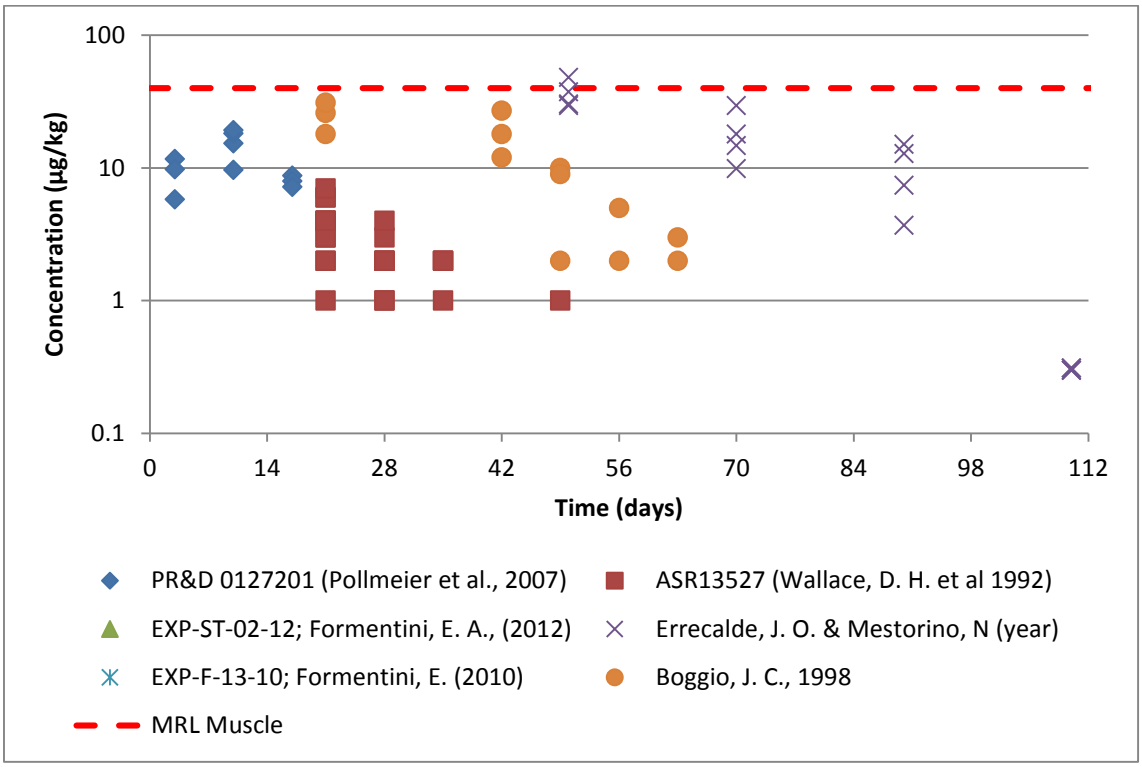

Figure 4.9. Derivation of of MRLs from data provided for residues in muscle.

Residues at the injection site

To study the depletion curves of ivermectin residue at injection sites, the data obtained from the 6 studies from inner core and outer ring samples from injection sites (Pollmeier *et al*., 2007; Wallace et *al*., 1992; Boggio, 1998; Formentini, 2010; Errecalde and Mestorino, 2007; Formentini, 2012) were pooled. While they represent different product formulations and different sampling procedures, they were considered to reflect the variability of exposure scenarios.

Acute dietary exposure assessment: injection site residues

For the purpose of undertaking the acute dietary exposure assessment of ivermectin residues, up-to-date individual food consumption database of animal tissues and food of animal origin expressed on a large portion (LP) sizes values based on the 97.5th percentile of food consumption were used by the Committee (Table 14). The Committee used data derived from records of individual consumer days (i.e. survey days on which the food or foods of interest were consumed) reported in individual-level survey data from 25 countries (Australia, Brazil, China and 22 European countries) and summarized in the EFSA Comprehensive European Food Consumption Database. Those data were previously collected following a request to Member countries as part of the Joint FAO/WHO Expert Meeting on Dietary Exposure Assessment Methodologies for Residues of Veterinary Drugs (WHO, 2012b). Dietary exposure was compared with the acute reference dose of 200 µg/kg bw established by the current meeting of the Committee.

Table 4.14. Estimated acute dietary exposure to ivermectin (GEADE) occurring at injection sites.

Category	Type	95/95 UTL[1] conc. (µg/kg)	97.5th Consumption[2] µg/kg bw/day	MR:TR ratio[1]	GEADE[3]	
					µg/kg bw/day	% ARfD
General Population						
Mammalian muscle	Beef and other Bovines (Injection Site)	5 447	7.7	0.8	52	27
Children						
Mammalian muscle	Beef and other Bovines (Injection Site)	5 447	12.7	0.8	87	43

[1]95/95 UTL concentration at the injection site after 14 days; [2]highest 97.5th food consumption figures considered from the available dataset; [3]GEADE is the product of the 97.5th level of consumption multiplied with the highest residue.

A combined analysis of all studies submitted showed that after 14 days, the maximum concentrations of residues found at injection sites led to a Global Estimate of Acute Dietary Exposure (GEADE) of 52 µg/kg bw for the general population and 87 µg/kg bw for children,

corresponding, respectively, to 27% and 43% of the ARfD (Table 4.14) as illustrated in Figure 4.10.

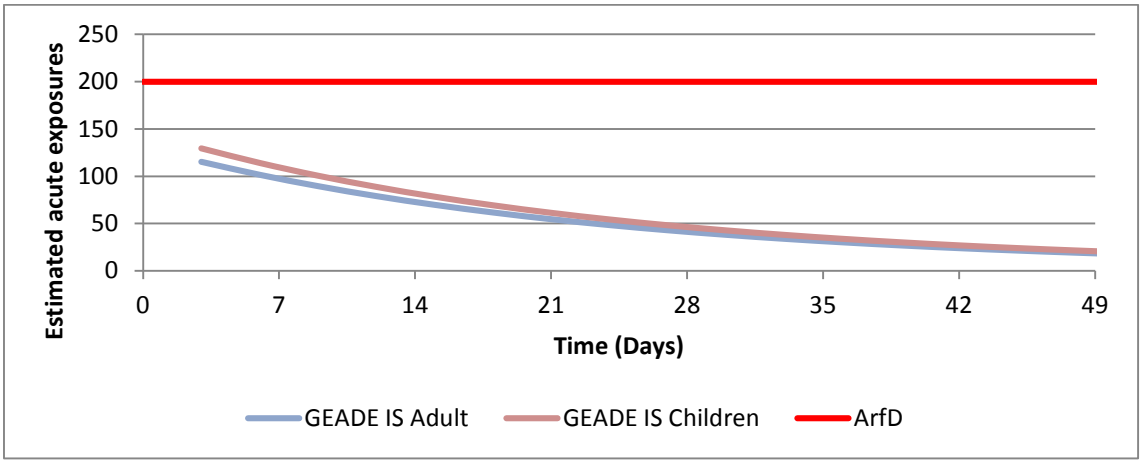

Figure 4.10. Acute reference dose and Global Estimate of Acute Dietary Exposure for total population and children.

The Committee considers that the presence of high concentrations of ivermectin residues at the injection site is product dependent and must be assessed on a case-by-case basis during marketing authorization by comparison of suitable acute dietary exposure estimates with the ARfD.

Chronic dietary exposure assessment

The estimated daily intake (EDI) is 38 µg/person per day, based on a 60 kg individual, which represents 6% of the upper bound of the ADI of 0–10 µg/kg bw established by the current meeting of the Committee (Table 4.15).

Table 4.15. Estimated chronic dietary exposure to ivermectin (EDI).

Tissue	Median concentration* (µg/kg)	Standard Food Basket (kg)	MR:TR ratio[1]	Daily intake (µg)
Muscle (Beef and other Bovines)	6.3	0.3	0.67	2.7
Liver (mammalian)	78.0	0.1	0.37	21.1
Kidney (mammalian)	12.5	0.05	0.54	1.2
Fat (mammalian)	46.7	0.05	0.18	13.0
TOTAL				38

*Median concentration 14 days after treatment.

The Global Estimate of Chronic Dose Exposure (GECDE) for the general population is 0.9 µg/kg bw per day, which represents 9% of the upper bound of the ADI.

The GECDE for children is 1.5 µg/kg bw per day, which represents 15% of the upper bound of the ADI. The GECDE for infants is 1.3 µg/kg bw per day, which represents 13% of the upper bound of the ADI (Table 4.16).

Table 4.16. Estimated chronic dietary exposure to ivermectin (GECDE).

Category	Type	Median concentration[1] µg/kg	Mean consumption[2] whole population, g/d	97.5th consumption[3] consumers only, g/d	MR:TR ratio[1]	Exposure µg/kg bw/day		GECDE[4]	
						Mean	97.5th	µg/kg bw/day	%ADI
General Population									
Mammalian muscle	Beef and other Bovines	6	63.0	291	0.67	0.01	0.05	0.01	0.1
Mammalian trimmed fat, skin and added fat	Mammalian trimmed fat, skin and added fat excluding butter	47	14.0	125	0.18	0.06	0.54	0.06	0.6
Mammalian offal	Mammalian liver	78	2.0	237	0.37	0.01	0.83	0.83	8.3
Mammalian offal	Mammalian kidney	13	0.5	166	0.54	0.00	0.06	0.00	0.0
TOTAL						0.0	0.8	0.9	9
Children									
Mammalian muscle	Beef and other Bovines	6	37.0	159	0.67	0.02	0.10	0.02	0.2

Mammalian trimmed fat, skin and added fat	Mammalian trimmed fat, skin and added fat excluding butter	47	1.7	29	0.18	0.03	0.50	0.03	0.3
Mammalian offal	Mammalian liver	78	3.0	103	0.37	0.04	1.45	1.45	14.5
Mammalian offal	Mammalian kidney	13	0.5	150	0.54	0.00	0.23	0.00	0.0
TOTAL						0.1	1.4	1.5	15
				Infants					
Mammalian muscle	Beef and other Bovines	6	2.5	68	0.67	0.00	0.13	0.00	0.0
Mammalian trimmed fat, skin and added fat	Mammalian trimmed fat, skin and added fat excluding butter	47	-	-	0.18	-	-	-	-
Mammalian offal	All mammalian offal	78	0.1	31	0.37	0.00	1.31	1.31	13.1
TOTAL						0.0	1.3	1.3	13

[1]Median concentration at14 days; [2]Highest mean consumption figures based on whole population considered from the available dataset; [3]Highest 97.5th food consumption figures based on consumers only considered from the available dataset; [4]GECDE is the sum of the highest exposure at the 97.5th percentile of consumption for a food and the mean dietary exposures of the other foods.

A graphical plot of the estimated daily intake based on median and upper tolerance limits and global estimated chronic dietary exposure for the general population, children and infants (expressed as µg/kg bw) versus days post-dose compared to the acceptable daily intake is shown in Figure 4.11.

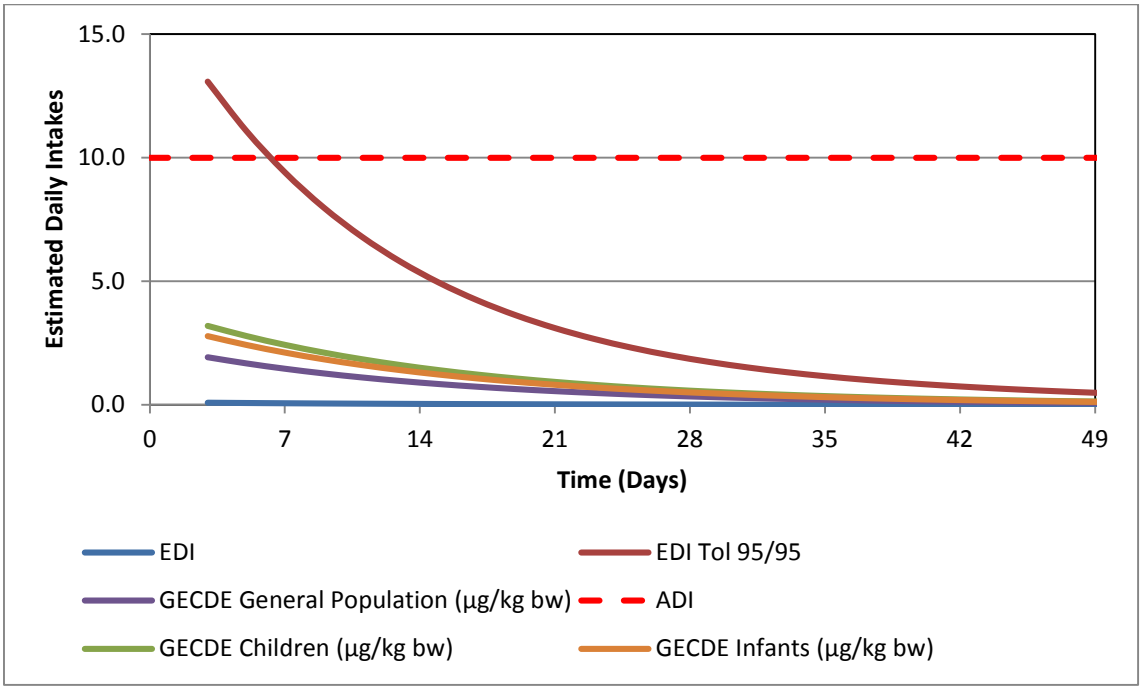

Figure 4.11. Acceptable daily intake, estimated daily intake based on median and upper tolerance limits and global estimated chronic dietary exposure for the general population, children and infants (expressed as µg/kg bw).

Maximum residue limits

In recommending MRLs for ivermectin in cattle, the Committee considered the following factors:

- The ADI established by the Committee was 0–10 µg/kg bw.
- An ARfD of 200 µg/kg bw was established by the Committee.
- Ivermectin B_{1a} (synonym for 22,23-dihydroavermectin B_{1a}) is confirmed as the marker residue.
- The ratios of the marker residue to total residues of 0.18 in fat, 0.54 in kidney, 0.37 in liver and 0.67 in muscle defined by the fortieth JECFA were confirmed.
- Two studies were used for deriving the MRLs and represent different formulations and routes of administration of ivermectin to cattle.
- The analysis of all data in cattle shows comparable residue depletion profiles.
- A validated quantitative analytical method for all edible tissues is available and is suitable for regulatory monitoring.

- The MRLs recommended for cattle tissues are based on the upper limit of the one-sided 95% confidence interval over the 95th percentile of residue concentrations (95/95 UTL) for the day 14 post-treatment data from the non-radiolabelled residue depletion studies. The time point chosen is consistent with approved uses (GVP).

Based on the new assessment, the Committee recommended the following revised MRLs in cattle tissues: 400 µg/kg for fat, 100 µg/kg for kidney, 800 µg/kg for liver, and 30 µg/kg for muscle.[4]

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5. Lasalocid sodium

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Addendum to the monograph prepared by the 78th meeting of the Committee and published in the FAO JECFA Monograph 15.

Background

Lasalocid sodium (hereafter, lasalocid), a divalent polyether ionophore antibiotic, produced by *Streptomyces lasaliensis*, is included as a medicinal additive in feed for continuous use to control coccidiosis in poultry species. It is a broad spectrum anticoccidial agent approved to protect against the *Eimeria* species in broilers and replacement pullets, turkeys, pheasants and quails.

The mechanism of action of lasalocid and other ionophores has been extensively investigated and reported. Like other carboxylic polyether ionophores, lasalocid disturbs ionic homeostasis, leading to osmotic lysis of coccidia.

Lasalocid is not approved for use in laying birds as it partitions into fat (egg yolks) at high concentrations.

Lasalocid was previously reviewed by the Committee at its 78th meeting (FAO, 2013), which established an ADI of 0–5 µg/kg bw, corresponding to an upper bound of acceptable intake of 300 µg/day for a 60 kg person. The ADI is the toxicological ADI, based on the NOAEL of 0.5 mg/kg bw per day from the developmental toxicity study in rabbits and the multigenerational reproductive toxicity study in rats, with application of an uncertainty factor of 100 for interspecies and intraspecies variability. The Committee also recommended MRLs, determined as lasalocid A, in tissues of chickens, turkeys, and quail of 400 µg/kg in muscle, 600 µg/kg in kidney, 1200 µg/kg in liver and 600 µg/kg in skin/fat. Because sufficient data were available to calculate median residue values and the ADI is based on a chronic endpoint, the EDI approach was used to assess exposure. Using the model diet and marker to total residue ratio, based on total residue of toxic concern on “day 0”, the ratios are 22% in liver, 41% in kidney, 55% in muscle, and 52% in skin/fat of chicken. The EDI calculated is 80 µg/person *per* day, which represents 27% of the upper bound of the ADI.

At the 22nd Session of the Codex Committee on Residue of Veterinary Drugs in Food (CCRVDF), two conference room documents (CRD) were presented raising concerns for lasalocid. The first CRD form, provided to the CCRVDF session from the European Union, CRD 13 (FAO/WHO, 2015a), was formatted as a Concern Form and considered that the EDI approach does not adequately address disruption of the colonization barrier and proposed that the use of a microbiological ADI end-point and the TMDI approach were the more appropriate

basis for assessing exposure. The concern form also noted that applying the TMDI approach to the recommended MRLs would result in an estimate of human exposure of 882.11 μg/person, which represents 175% of the JECFA microbiological ADI, 504 μg/person. The second conference room document, CRD 27 (FAO/WHO, 2015b), was prepared by Canada and contained comments on Agenda Item 6(c). The comments were subsequently submitted, with minor revisions, in the Concern Form format (FAO, 2015) and considered 1) that the EDI approach may be inappropriate given the variability in the residue depletion data for lasalocid; 2) that the marker to total radiolabelled residue ratio (MR:TRR) from day 0 data was used but the MRLs were based on depletion data from day 1. As the MR:TRR decreases significantly between day 0 and day 1, the use of the MR:TRR for day 0 may underestimate total exposure and it was suggested that using the MR:TRR data from day 1 would be more appropriate; 3) that, if the EDI approach was not applicable, the TMDI approach would result in an estimate of daily human exposure in excess of the ADI; and 4) that using the MR:TRR at day 1 (and, by extension, day 0) would result in exposure exceeding the global estimate of chronic dietary exposure (GECDE) and that the use of day 2 depletion data would be more appropriate. In summary, although the proposed MRLs are based on one of the approaches that JECFA uses, given the potential limitations of the EDI approach when working with highly variable data, Canada expressed concern that the proposed MRLs might expose consumers to residues of lasalocid that are higher than the ADI. CRD 27 also had requested that the JECFA recommend appropriate risk management recommendations to ensure food safety based on unintended exposure of lasalocid to laying hens but this request was not included with the formal Concern Form submission.

The 22nd Session of the CCRVDF requested that JECFA re-evaluate the basis for the ADI and MRLs for lasalocid.

Current evaluation

No new data or studies were provided for the current evaluation. Concerns from two member states, CRD 13 (FAO/WHO, 2015a) and CRD 27 (FAO/WHO, 2015b), plus the resultant Concern Form (FAO, 2015), were evaluated. A comment from the sponsor relating to the toxicological evaluation also was submitted for consideration. Additionally, a numerical error made in the evaluation conducted by the 78th Meeting of JECFA in the entry of day 0 residue depletion data into a spreadsheet (one value was omitted and a second value was reported twice) was discovered and corrected. However, the day 0 residue depletion data are not used to recommend MRLs and this correction does not affect the previous calculations.

Concern from the European Union summarized in CRD 13

This CRD relates to the assignment of the ADI and was not addressed in the residue assessment. The issue has been addressed in a re-assessment of the toxicology of lasalocid by the present meeting of the Committee.

Concern from Canada summarized in CRD 27 and the resultant Concern Form

This CRD (FAO/WHO, 2015b) and the resultant Concern Form (FAO, 2015) relate to the recommended MRLs and have been addressed in the residue evaluation conducted by the present meeting of the Committee:

"Canada would like to raise the following scientific points for further consideration by JECFA:"

Comment 1. "The MRLs proposed for this compound were calculated based on the estimated daily intake (EDI) approach. Canada had earlier expressed the concern that there would be limitations with using the EDI approach when residue depletion data are highly variable. In the case for lasalocid residues in chicken tissues (see Table 7.5 of the monograph) the standard deviations of residues in each tissue on 1-day withdrawal period (WP) (time for which exposure estimates were evaluated) were much higher than the mean of the residues (i.e., the coefficient of variation was > 100%). Mean and standard deviations of lasalocid A residues at 1-day WP were respectively, 65 ppb and 103 ppb in muscle, 244 ppb and 329 ppb in liver, 128 ppb and 194 ppb in kidney, and 106 ppb and 165 ppb in skin/fat of chickens. Given the highly variable nature of the data used to derive the MRLs, Canada considers that this approach may not be robust enough for the establishment of lasalocid MRLs in order to ensure safety to consumers."

JECFA response: The Committee considered the concern expressed by the Member State. In developing the EDI procedure, the 66th meeting of the Committee (FAO/WHO, 2006) concluded that "the TMDI was no longer the most suitable estimate of chronic intake, because the MRL was a single concentration representing the estimated upper limit of a high percentile of the distribution of marker residue present in a given tissue of the treated animals". The 66th meeting of the Committee concluded that "it was not realistic to use an extreme value of the distribution in a scenario describing chronic intakes. In such a scenario, all concentrations of the distribution of residues should be considered. The median concentration represents the best point estimate of a central tendency over a prolonged period of time, because the concentrations of residues in a given tissue consumed varies from day to day, as reflected in the distribution. Therefore, the Committee decided to use the median of the residue distribution to substitute for the MRL in the intake estimate." While acknowledging that the lasalocid data are variable, the current Committee noted that the EDI approach has been applied in other assessments where residue data were variable. Additionally, the Committee noted that the median is not unduly affected by outliers. Finally, the Committee noted that variability in residue values is not uncommon in studies involving poultry or when dosing via feed. The observed variability associated with lasalocid residue values does not appear to be the result of a systematic bias. The current Committee concluded that the lasalocid residue depletion data are robust, were collected in a GLP-compliant study and can be used with the EDI approach.

Comment 2. "JECFA monograph indicates that the residue data from 1-day WP was used to derive the proposed MRLs. However, marker to total residue (MR:TR) ratios based on data for 0-day WP were used instead. There is significant reduction in MR:TR between the 0-day and 1-day WP (see Appendix below). After 1-day WP, the MR:TR remains fairly stable. Hence, the MR:TR ratio at 0-day would likely under-estimate the total exposure. Canada therefore

considers that MR:TR based on 1-day WP of <25% for muscle, 8.8% for liver, 14.2% for kidney and 29.2% for skin/fat (see Table 7.2 of the monograph) should perhaps be used along with the residue depletion data in the exposure assessment."

JECFA response: As noted in the monograph prepared for the 78th JECFA (FAO, 2014; see Table 7.2, footnote), the withdrawal times for the radiolabelled residue depletion study are actually 16 hours post last dose relative to their designation (i.e. "0" withdrawal is actually 16 hours post last dose). For the current assessment, all the withdrawal times are restated to clearly indicate the elapsed time from the final dosing. Following this re-presentation of the withdrawal times in the radiolabelled residue depletion study, it is clear that the withdrawal times in that study align more closely to the withdrawal times in the residue depletion study using non-radiolabelled drug than was apparent from Table 7.2 in the monograph prepared by the 78th Meeting of JECFA. The MR:TRR ratios at 16 hours post last dose are 55% (muscle), 52% (skin/fat), 22% (liver) and 41% (kidney).

Using a different approach, interpolated MR:TRR values were developed. For muscle, where there was no MR:TRR at 40 hours post last dose (formerly designated 24 hours withdrawal), the hypothetical 25% MR:TRR for muscle proposed by the requestor was used. The formula ($MR:TRR_{16}$ - $MR:TRR_{40}$)/3 was used to calculate the change-over-time in the MR:TRR ratio between 16 and 40 hours post last dose in 8-hour increments, and this value was then subtracted from $MR:TRR_{16}$ to give $MR:TRR_{24}$. The interpolated MR:TRR ratios at 24 hours post last dose are 45% (muscle), 44% (skin/fat), 18% (liver) and 32% (kidney).

Using either the experimentally derived MR:TRR ratios or those MR:TRR ratios developed through the interpolation, both the EDI and the GECDE remain below the ADI for the general population (Tables 5.1, 5.9), children and infants (Table 5.9). However, because the adjusted sample collection times in the radiolabelled residue depletion study align well with the sampling times in the depletion study using unlabelled drug, the experimentally derived MR:TRR ratios at 16 hours post last dose are used in conjunction with MRLs derived from the 1-day withdrawal residues in the residue depletion study using unlabelled drug in the exposure assessment for lasalocid in chicken tissues.

Comment 3. "When the data are insufficient or of quality not suitable for the EDI approach, the JECFA has historically used the theoretical maximum daily intake (TMDI) approach to establish MRLs. Based on our calculation using the same data but using the TMDI approach, if the exposure was estimated using the proposed MRLs and the marker to total residue ratios at 1-day WP, the daily human exposure to lasalocid residues would be 2157.6 µg per person which is 7 times higher than the ADI value of 300 µg per person (see Table 6 of Appendix for detailed calculation)."

Table 5.1. Comparison of EDIs and GECDEs calculated using various data sets and MR:TRR ratios and the median values indicated.

Calculation parameters			Estimated Exposure
1 day WP medians and 0 day (now designated 16-hour) MR:TRR (from 78th JECFA)	EDI, general population	µg/person/day	80.0
	ADI, general population	%ADI	27
1 day WP medians and 0 day (now designated 16-hour) MR:TRR (from 78th JECFA)	GECDE, general population	µg/person/day	114
	ADI, general population	%ADI	37
1 day WP medians and interpolated 24-hour MR:TRR (from the current assessment)	EDI, general population	µg/person/day	98
	ADI, general population	%ADI	33
1 day WP medians and interpolated 24-hour MR:TRR (from the current assessment)	GECDE, general population	µg/person/day	138
	ADI, general population	%ADI	45

JECFA response: The Committee has concluded that when data are sufficiently robust to support the use of the EDI approach, that approach will be used, because it is more representative of actual exposure from the consumption of tissues derived from treated animals. The lasalocid residue depletion data are robust, were collected in a GLP-compliant study and can therefore be used with the EDI approach (see also the response to #4).

Comment 4. "While Canada understands that the new dietary exposure assessment approach piloted by the JECFA in its 78th meeting is still being verified, the global estimate of chronic dietary exposure (GECDE) using the MR:TR on 1-day WP for lasalocid would have exceeded the ADI. The GECDE represents 92% of ADI for adults, 168% of ADI for children and 149% of ADI for infants (see Appendix for calculations). JECFA's conclusion that the GECDE is below the ADI was because of considering the MR:TR for 0-day WP which we believe underestimates the exposure. Given that 1-day WP residue data does not support the safety to consumers based on GECDE approach, perhaps the residue data from 2-day WP would have been ideal to establish MRLs for this compound. The 95th percentile (upper 95% CI) of residue

data at 2-day WP would have yielded the MRLs of 100 ppb in muscle, 500 ppb in liver, 250 ppb in kidney and 200 ppb in skin and fat (see Appendix, Table 7)."

JECFA response: Following adjustment of the sampling times in the radiolabelled residue depletion study to clearly reflect the actual time post last dose at which the samples were collected, it is clear that the sampling times in that study and the sampling times in the residue depletion study using non-radiolabelled drug align well and can be used to derive MRLs for the use of lasalocid in chickens. Using the MR:TRR at 16 hours post last dose, both the EDI and the GECDE remain below the upper bound of the ADI for adults, children and infants.

An EDI of 1.33 µg/kg bw (80 µg/60 kg person per day) was calculated, based on median residues for a 1-day withdrawal in chicken, and is equivalent to 27% of the upper bound of the ADI.

The GECDE for the general population is 1.9 µg/kg bw per day, which represents 37% of the upper bound of the ADI. The GECDE for children is 3.4 µg/kg bw per day, which represents 67% of the upper bound of the ADI. The GECDE for infants is 3.0 µg/kg bw per day, which represents 60% of the upper bound of the ADI.

In addition to the numbered questions, the Member State raised the additional concern that they were not able to reproduce the results contained in Table 7.2 of the residue monograph prepared by the 78th meeting of the Committee(FAO, 2014).

JECFA response: The values in Table 7.2 of the JECFA monograph (FAO, 2014) are correctly calculated. For complete transparency, the individual residue values for each animal and each tissue assayed in both the radiolabelled residue depletion study and the residue depletion study using unlabelled drug are presented in the current addendum, Tables 5.2 and 5.4.

Appraisal

No new data or studies were provided for the current evaluation. Two conference room documents (CRDs) were presented at the 22nd Session of the CCRVDF raising concerns for lasalocid. The first CRD form, from the European Union, CRD 13 (FAO/WHO, 2015a), was formatted as a Concern Form. This Concern Form and a comment from the sponsor, relate to the assignment of the ADI, and have been addressed in a re-assessment of the toxicology information available for lasalocid by the present meeting of the Committee. The second conference room document, CRD 27 (FAO/WHO, 2015b), was prepared by Canada; a resultant Concern Form (FAO, 2015) was submitted to the current Committee. The concerns identified by Canada have been addressed in a re-assessment by the present meeting of the Committee of the residue information available for lasalocid and are provided below.

The monograph prepared for the 78th JECFA used data from the day 0 in the radiolabelled study (MacLellan *et al.*, 2003) to calculate the MR:TRR used in the exposure assessment. The mean MR:TRR values presented are correct; however, because mean values were presented in Table 7.2 (FAO, 2014), it is not possible to reproduce the calculated results. In the footnote to Table 7.2, it is stated that 0 hours withdrawal is actually 16 hours after the final dose. All of the MR:TRR ratios in that monograph therefore are for time points 16 hours later than the stated

withdrawal times. Thus, the day 1 MR:TRR data are identified as 24 hours withdrawal but are, in fact, 40 hours after the final dosing. All of the individual data from the radiolabelled residue depletion study (MacLellan *et al.*, 2003) are presented in Table 5.2. All times in Table 5.2 are re-presented to show the correct elapsed time from the last dose.

CRD 27 (FAO/WHO, 2015b) and the related Concern Form (FAO, 2015) from Canada correctly note the significant decrease in MR:TRR between these two sampling points, 16 and 40 hours post-last-dose. However, the MR:TRR ratios are variable and, in fact, increase again at later sampling times. For muscle, there was only one time at which the data were available to calculate the MR:TRR ratio.

Although the 16-hour MR:TRR data remain the most relevant to the exposure assessment, it is possible to use the 16- and 40-hour MR:TRR data to interpolate a hypothetical MR:TRR at 24-hour post dosing. In this alternative approach, interpolated MR:TRR values were determined using the difference between the 16-hour MR:TRR ratio and the 40-hour MR:TRR ratio (including using the 25% value for the muscle MR:TRR proposed in CRD 27 and the related Concern Form at 40 hours (previously identified as 24 hours) for each tissue. The difference was then divided by three to approximate the linear decline over 24hours in 8-hour intervals (*i.e.*, 24 hours/3 = 8 hours). Finally, the 8-hour difference in MR:TRR ratio was subtracted from the 16-hour MR:TRR value to represent an interpolated estimate of the 24-hour MR:TRR value to fully align with the residue depletion data sampling points used to recommend MRLs. Using this linear interpolation, the interpolated MR:TRR values are shown in Table 5.3.

The monograph prepared for the 78th JECFA used the combined residue depletion data from Croubels (2010) and McLellan and King (2006) to calculate the MRLs. This was not clearly identified in the monograph prepared by the 78th Meeting of JECFA (FAO, 2014). While increasing the number of data points available for the MRL determination, this approach lacks transparency and creates a slight disparity between the values used to calculate the recommended MRLs and the values used to calculate the EDI. For the current evaluation, only the depletion data from Croubels (2010) were used (Table 5.4). While using only the Croubels (2010) data set reduced the number of total samples in the assessment, the difference is small. The Croubels (2010) study provides a robust data set of 191 quantifiable residue values from 12 animals from all 4 tissues and 4 withdrawal times; one skin/fat sample at 3 days withdrawal contained residues below the method limit of quantification (LOQ). The McLellan and King (2006) data set contains only 35 residue values above the LOQ, including 24 residue values (6 animals X 4 tissues) at 0 withdrawal. However, at 1-day withdrawal, the McLellan and King (2006) data provide only 11 additional samples (6 liver samples, 3 kidney samples and 2 skin/fat samples). Samples from 2 and 3 days withdrawal are all below the method LOQ. Because the 0-day withdrawal samples are not considered for calculating the MRLs, a total of 72 samples (4 tissues X 12 animals from Croubels (2010) + 4 tissues X 6 animals from McLellan and King (2006)) are not used in the MRL calculation. At 1-day

Table 5.2. Concentrations (µg/kg) of total radiolabelled residues (TRR) and lasalocid residues and resulting MR:TRR (%) (MacLellan *et al.*, 2003).

Time	Tissue	TRR	Lasalocid	MR:TRR	Tissue	TRR	Lasalocid	MR:TRR	Tissue	TRR	Lasalocid	MR:TRR	Tissue	TRR	Lasalocid	MR:TRR
16	Liver	1255	294	0.23	Kidney	403	125	0.31	Skin/Fat	643	342	0.53	Muscle	91	51	0.56
		819	175	0.21		237	119	0.50		145	73	0.50		57	31	0.54
			mean	0.224			mean	0.406			mean	0.518			mean	0.55
40		779	91	0.12		150	27	0.18		166	65	0.39				
		1064	89	0.08		245	28	0.11		122	31	0.25				
		855	41	0.05		158	20	0.13		92	25	0.27				
		609	31	0.05		217	32	0.15		101	22	0.22				
		691	58	0.08						121	34	0.28				
		1030	134	0.13												
			mean	0.086			mean	0.143			mean	0.283				
88		608	94	0.15		115	31	0.27		115	39	0.34				
		840	20	0.02												
		564	60	0.11												
		431	58	0.13												
		392	35	0.09												
		514	31	0.06												
			mean	0.09			mean	0.27			mean	0.34				
136		396	23	0.06		49	70	1.43		70	24	0.34				
		499	37	0.07		97	28	0.29		81	35	0.43				
		402	106	0.26												
			mean	0.13			mean	0.86			mean	0.39				

withdrawal, Croubels contributes 48 quantifiable data points (4 tissues X 12 animals) but McLellan and King (2006) contributes only 11 quantifiable data points, as noted above. Considering all available data points from 1-day withdrawal onward, using the data from Croubels (2010) provides 143 quantifiable data points (*vs.* 154 when the data are combined with the 11 quantifiable data points from McLellan and King (2006)). Tissue medians, means, and upper tolerance limits based on the data from Croubels (2010) are presented in Table 5.5.

Table 5.3. Interpolated MR:TRR values (%) between 16- and 40-hours post last dose sampling (MacLellan *et al.*, 2003).

Tissue	Time	MR:TRR	8 h interval change in MR:TRR
Liver	16	22.4	4.6
	24	17.8	
	32	13.2	
	40	8.6	
Kidney	16	40.6	8.8
	24	31.8	
	32	23.0	
	40	14.3	
Skin/Fat	16	51.8	7.8
	24	44.0	
	32	36.1	
	40	28.3	
Muscle	16	55.0	10
	24	45.1	
	32	35.1	
	40	25.0	

* $(MR:TRR_{16}-MR:TRR_{40})/3$; this value is then subtracted from $MR:TRR_{16}$ to give $MR:TRR_{24}$.

Table 5.4. Residue depletion data (µg/kg)(Croubels, 2010).

Withdrawal Time (d)	Kidney	Muscle	Liver	Skin/Fat
0	810.27	337.47	1628.35	947.6
0	1667.45	627.25	2801.57	1462.62
0	1180.53	402.28	1917.63	1056.72
0	1354.99	538.1	2015.38	1211.63
0	1432.27	533.76	2092.25	1491.66
0	851.54	345.1	1360.06	1129.46
0	663.5	281.58	1640.54	576.51
0	883.08	414.77	1810.6	977.57
0	737.24	372.83	1564.45	677.15
0	1414.19	774.2	2051.58	1216.14
0	792.7	335.48	1769.49	828.49
0	815.96	400.21	1430.63	905.03
1	17.93	13.5	50.17	42.72
1	54.04	32.4	168.48	28.78
1	73.41	25.25	145.15	49.62
1	44.86	24.77	102.6	30.49
1	68.4	32.65	165.24	40.86
1	45.96	16.09	79.85	29.48
1	427.68	294.35	832.17	334.31
1	23.11	8.26	40.36	16.88
1	33.7	14.83	60.13	42.57
1	44.35	14.58	82.84	31.25
1	73.77	26.21	156.74	69.7
1	633.11	276.79	1038.88	554.3
2	90.78	54.62	351.47	89.07

2	27.64	10.89	73	12.24
2	28.96	14.83	76.81	14.17
2	43.4	20.57	92.49	16.11
2	79.7	15.38	218.46	34.73
2	274.12	83.8	444.18	191.88
2	28.86	10.35	47.32	14.82
2	44.7	16.74	91.87	19.6
2	22.26	10.94	79.28	10.96
2	24.16	8.86	38.63	9.03
2	35.9	13.91	85.04	14.85
2	34.97	14.64	57.71	11.43
3	19.95	8.14	47.46	8.4
3	23.29	8.77	45.46	9.29
3	33.36	12.07	160.98	20.2
3	43.82	14.06	120.72	14.98
3	17.1	9.25	41.54	6.78
3	16.05	5.12	29.12	10.09
3	30.99	8.17	71.21	9.72
3	45.29	10.53	194.4	8.97
3	32.11	6.39	68.53	9.11
3	15.78	6.26	27.8	7.44
3	15.69	5.9	22.71	<LOQ
3	23.77	6.52	36.75	10.27

LOQ = 5 µg/kg.

Table 5.5. Upper tolerance limits (µg/kg) based on Croubels (2010).

	Time (day)	Kidney	Muscle	Liver	Skin/Fat
Median	0	867.31	401.25	1790.05	1017.15
Mean		1050.31	446.92	1840.21	1040.05
SD		338.84	144.06	385.42	281.75
N		12.00	12.00	12.00	12.00
K		2.71	2.71	2.71	2.71
UTL		1967.21	836.74	2883.15	1802.46
Median	1	50.00	25.01	123.88	41.72
Mean		128.36	64.97	243.55	105.91
SD		193.66	103.39	329.30	165.40
N		12.00	12.00	12.00	12.00
K		2.71	2.71	2.71	2.71
UTL		652.39	344.75	1134.62	553.47
Median	2	35.44	14.74	82.16	14.84
Mean		61.29	22.96	138.02	36.57
SD		70.43	22.71	130.99	53.66
N		12.00	12.00	12.00	12.00
K		2.71	2.71	2.71	2.71
UTL		251.88	84.40	492.49	181.77
Median	3	23.53	8.16	46.46	9.29
Mean		26.43	8.43	72.22	10.48
SD		10.62	2.70	56.39	3.85
N		12.00	12.00	12.00	11.00
K		2.71	2.71	2.71	2.78
UTL		55.18	15.74	224.82	21.19

UTL = Upper one-sided 95% Tolerance Limit.

Using the MR:TRR from MacLellan *et al.*, 2003, at withdrawal time 16 hours (previously designated 0 hours), and the median values for the tissues from Croubels (2010), at day 1 (24 hours) withdrawal, the EDI provided in the residue monograph prepared by the 78th meeting of the Committee (FAO, 2014; see Table 7.10) is as shown in Table 5.6.

Table 5.6. EDI (µg/kg) provided in the 78th JECFA (Table 7.10) (FAO, 2014).

Tissue	Median	MR:TRR	Consumption	Exposure
Liver	123.9	0.22	0.100	56.3
Kidney	50.0	0.41	0.050	6.1
Muscle	25.0	0.55	0.300	13.6
Skin/Fat	41.7	0.52	0.050	4.0
Total				80.0
% ADI				26.7

Using the interpolated MR:TRR at 24 hours post-last-dose from Table 5.3 above, and the median values for the tissues from Croubels (2010) alone at day 1 withdrawal, the EDI is shown in Table 5.7.

Table 5.7. EDI (µg/kg) using interpolated MR:TRR at 24 hours post-last-dose from Table 5.3 above and the median values for the tissues from Croubels (2010) alone at day 1 withdrawal.

Tissue	Median	MR:TRR	Consumption	Exposure
Liver	123.9	0.18	0.100	68.8
Kidney	50.0	0.32	0.050	7.8
Muscle	25.0	0.45	0.300	16.7
Skin/Fat	41.7	0.44	0.050	4.7
Total				98.0
% ADI				33

A comparison of the EDIs for the general population, using various data sets, is shown in Table 5.8.

Table 5.8. Comparison of EDIs calculated using various data sets and MR:TRR ratios and the median values indicated.

Calculation parameters			Estimated Exposure
1 day WP medians and 0 day (now designated 16-hour) MR:TRR (from 78th JECFA)	EDI, general population	µg/person/day	80.0
	ADI, general population	%ADI	27
1 day WP medians and extrapolated 24-hour MR:TRR (from the current assessment)	EDI, general population	µg/person/day	98
	ADI, general population	%ADI	33
0 day WP medians and 0 day (now designated16-hour) MR:TRR (from CRD 27 and Concern Form)*	EDI, general population	µg/person/day	1373.4
	ADI, general population	%ADI	458
1 day WP medians and MR:TRR (from CRD 27 and Concern Form)	EDI, general population	µg/person/day	195.5
	ADI, general population	%ADI	65

* The 0-day withdrawal data were not considered appropriate for establishing MRLs or determining exposure. They are included here because they were presented in CRD27 and in the Concern Form.

In recommending MRLs for lasalocid in poultry food commodities, the 78th Meeting of the Committee considered the following factors:

- An ADI of 0-5 µg/kg of body weight was established by the Committee. The upper bound of the ADI is equivalent to 300 µg lasalocid sodium for a 60 kg person.
- Where information on approved veterinary uses was provided, withdrawal times were in the range 0-7 days.
- Lasalocid sodium is extensively metabolized in poultry, although the metabolites were not identified.
- Lasalocid A is a suitable marker residue in all edible tissues of poultry.
- Lasalocid A represents conservatively 22% of lasalocid sodium in liver, 41% in kidney, 55% in muscle, and 52% in skin/fat in chicken;
- Extension of MRLs to turkey and quail and the extrapolation of MRLs to pheasant are appropriate as depletion data were available, the marker residue was demonstrated and information was available on authorized uses.

- Validated LC-MS/MS and HPLC methods were provided and considered suitable for routine monitoring of lasalocid A as marker residue in poultry tissues.

The MRLs recommended for chicken, turkey, quail and pheasant tissues were based on the upper limit of the one-sided 95% confidence interval over the 95th percentile (UTL 95/95) for the 1-day post-treatment data from the unlabelled residue depletion study in chicken.

The MRLs recommended for chicken, turkey, quail and pheasant by the 78th Meeting of the Committee were 1200 µg/kg in liver, 600 µg/kg in kidney, 400 µg/kg in muscle and 600 µg/kg in skin plus fat.

An EDI of 1.33 µg/kg body weight per day (80 µg/60 kg person per day) was calculated, based on median residues for a 1-day withdrawal in chicken, equivalent to 27% of the upper bound of the ADI. The GECDE for the general population based on median residues for a 1-day withdrawal was 1.9 µg/kg body weight per day, which represents 37% of the upper bound of the ADI; the GECDE for children and infants was 3.4 µg/kg body weight per day and 3.0 µg/kg bw per day resp., which represents 67% and 60% of the upper bound of the ADI.

The current Committee reviewed these MRL recommendations, based on the dietary exposure evaluation in the following section and the decision by the present Committee to retain the ADI of 0-5 µg/kg of body weight established by the 78th Meeting of the Committee. The question of residue carry over into eggs was deferred pending the outcome of an electronic working group established by the 22nd Session of the CCRVDF.

Dietary Exposure Assessment

An EDI of 80 µg/person per day was calculated, based on median residues for a 1-day withdrawal in chicken, and are equivalent to 27% of the upper bound of the ADI (see Table 5.7).

In addition, the Committee calculated GECDE values to be compared with the EDI. In this additional dietary exposure assessment, poultry muscle, fat and skin and offal were contributors to dietary exposure. Calculated GECDE values for lasalocid for the general population, children and infants are shown in Table 5.9.

Using the median residue as inputs, the GECDE for the general population was 1.85 µg/kg bw/day, which is equivalent to 37% of the upper bound of the ADI. In children the GECDE was 3.38 µg/kg bw/day which represents 68% of the upper bound of the ADI. Exposure of infants was estimated to be lower at 2.99 µg/kg bw/day, 60% of the upper bound of the ADI.

Maximum Residue Limits

Following consideration of the issues raised in the concern forms, the ADI established and MRLs recommended at the seventy-eighth meeting of JECFA remain unchanged.

Table 5.9. Calculated GECDE values for lasalocid for the general population, children and infants.

Category	Type	Median concentration[1] (µg/kg)	Mean consumption[2] whole population, g/d	97.5th consumption[3] consumers only, g/d	MR:TR ratio[1]	Exposure (µg/kg bw/day)		GECDE[4]	
						Mean	97.5th	µg/kg bw/day	%ADI
				General Population					
Poultry	Poultry muscle	25.0	118.0	352	0.55	0.09	0.27	0.09	1.8
Poultry	Poultry fat and skin	41.7	1.0	23	0.52	0.00	0.03	0.00	0.0
Poultry	Poultry offal	123.9	5.0	188	0.22	0.05	1.76	1.76	35.2
TOTAL						0.1	1.8	1.85	37
				Children					
Poultry	Poultry muscle	25.0	35.0	207	0.55	0.11	0.63	0.11	2.2
Poultry	Poultry fat and skin	41.7	0.1	3	0.52	0.00	0.02	0.00	0.0
Poultry	Poultry offal	123.9	0.4	87	0.22	0.02	3.27	3.27	65.4
TOTAL						0.1	3.3	3.38	68
				Infants					

Poultry	Poultry muscle	25.0	6.3	77	0.55	0.06	0.70	0.06	1.1
Poultry	Poultry fat and skin	41.7	-	-	0.52	-	-	-	-
Poultry	Poultry offal	123.9	0.1	26	0.22	0.01	2.93	2.93	58.6
TOTAL						0.1	2.9	2.99	60

[1]Median concentration at 1 day; [2]highest mean consumption figures based on whole population considered from the available dataset; [3]highest 97.5th food consumption figures based on consumers only considered from the available dataset; [4]GECDE is the sum of the highest exposure at the 97.5th percentile of consumption for a food and the mean dietary exposures of the other foods.

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Identity

International Non-proprietary name (INN; proposed): Sisapronil

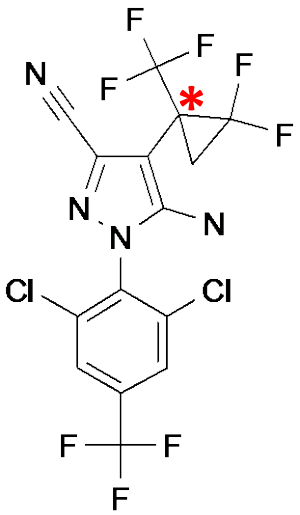
Synonyms: PF-00241851, PF-0241851, PF-241851, BRIN PF-241851, Arylpyrazole

IUPAC name: 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[2,2-difluoro-1-(trifluoromethyl) cyclopropyl]-1H-pyrazole-3-carbonitrile

Chemical Abstract Service Number: 856225-89-3

PubChem number: 172232505

Structural formula:



Molecular formula: $C_{15}H_6Cl_2F_8N_4$

Molecular weight: 465.1282 (average), 463.98416 (monoisotopic)

Other information on identity and properties

Pure active ingredient: Sisapronil is a racemic mixture containing one asymmetric carbon atom (*)

Appearance: White to off-white solid

Melting point: 185-186°C (Form A and Form B)

Log P: 5.1

Solubility: 0.002 g/L water, 20 g/L long-chain triglyceride oils, 100 g/L medium-chain triglyceride oils, 400 g/L short-chain triglyceride oils, 150 g/L benzyl benzoate, 130 g/L ethanol

UV_{max}: > 220 nm

Residues in food and their evaluation

Conditions of use

Sisapronil is a member of the phenylpyrazole class of antiparasitics. It is a long-acting subcutaneous injectable ectoparasiticide for control of cattle ticks. It also aids in the control of bot fly larvae, hornfly and screwworm. Sisapronil binds tightly to ligand-gated chloride channels, in particular those gated by the neurotransmitter gamma-aminobutyric acid (GABA), blocking the pre- and post-synaptic transference of chloride ions through cell membranes in insects or mites, exposed through ingestion or contact. This mechanism of action results in hyperexcitability of the central nervous system and death of the parasites. Sisapronil has been registered for use in Brazil with a withdrawal period of 120 days.

Dosage

The recommended dose is a single subcutaneous injection of 1 mL *per* 50 kg body weight (BW), equivalent to 2 mg sisapronil/kg BW. The label includes a warning that the product is not indicated for use in dairy cattle.

Pharmacokinetics and metabolism

Test material used in radiolabel pharmacokinetic and metabolism studies

Pharmacokinetic and metabolism studies were conducted with [^{14}C]-sisapronil, which was synthesized using [^{14}C] diazomethane, incorporating the radiolabel into the cyclopropyl ring of the molecule.

Specific activity: 39mCi/mmol

Purity: 99.2% (by HPLC)

Pharmacokinetics in laboratory animals

Studies examining the pharmacokinetics of sisapronil in laboratory animals were conducted as part of the toxicology program, therefore doses were administered primarily *via* the oral route.

Rats

In a non-GLP compliant dose range finding study (Gagnon, 2012a), six groups of 2 male Wistar rats/group received a single oral dose of vehicle or 100, 250, 500, 1000, or 1500 mg/kg sisapronil. Sisapronil concentrations in plasma increased with increasing dose from 100 to 500 mg/kg and began to plateau from 500 to 1500 mg/kg. Mean concentrations in plasma at 6 days post dose were 3380, 6865, 33800, 27600, 30900 ng/mL for doses of 100 (n=2), 250 (n=2), 500 (n=1), 1000 (n=1) and 1500 (n=1) mg/kg BW, respectively.

In a single dose study (Ryan, 2011), rats were administered a single oral dose of 100, 500 or 1000 mg/kg sisapronil. Mean concentrations in plasma at 3 days post dose were 1210 ± 233, 9440 ± 5670, and 20500 ± 5250 ng/mL for 100 (n=20), 500 (n=20), and 1000 (n=14) mg/kg BW, respectively.

In a non-GLP compliant study (Hu, 2009), Sprague Dawley rats were dosed orally with 0.1, 1, or 10 mg/kg BW/day for 28 days. Blood samples were taken at 4, 8, and 24 hours post dose on days 1, 14 and 28. An additional group was given a single dose (0.5 mg/kg BW) with samples collected at 4, 8, and 24 hours after the first dose and on study days 4, 7, 14, 21, 28, 35, 38, and 42. In the 28-day treatment groups, substantial accumulation was observed in all dose groups with C_{max} 5-7 times higher at day 14 than at day 1; however, accumulation had reduced substantially between day 14 to 28 with C_{max} approximately 1.3 times higher at day 28 than at day 14. After the first dose, the AUC_{0-24h} was 142, 1460, and 10920 h ng/mL and C_{max} was 9.35, 77.4, and 580 ng/mL for doses 0.1, 1 and 10 mg/kg, respectively. Concentration of sisapronil in plasma appears to have increased in a slightly less than dose proportionate manner. The mean C_{max}/dose values at day 1 were 93.5, 77.4 and 58.0 ng/mL for doses 0.1, 1, and 10 mg/kg BW respectively. In the single dose animals, sisapronil was rapidly absorbed achieving a C_{max} of 43.5± 6.3 ng/mL at 8h post dose. The terminal disposition phase appeared to begin on study day 6 post dose and the calculation of $T_{1/2}$ was performed using data for study days 6-42, resulting in a plasma elimination half-life of 13.1 days and a mean residence time (MRT) of 19.0 days. Exposure was high in the single dose rats, with an $AUC_{0-\infty}$ of 583 ng day/mL.

In a GLP compliant study (Rodríguez Gómez, 2012), 10 Wistar rats/sex/group were dosed orally once daily with 0.1, 0.3, 1, or 10 mg sisapronil/kg BW/day for 13 weeks. Blood samples were collected 4, 8, and 24 hours after the first dose and analysed using LC-MS/MS. At day 90 of the study, blood was collected at a single time point from 6 animals/sex/group 4 hours post final dose. Due to the limited data available, the pharmacokinetic parameters were calculated combining data of male and female rats. Exposure increased with dose with the exception of the 0.1 and 0.3 mg/kg BW/day dose groups on day 1. Mean AUC_{0-24h} was 129, 103, 339, and 3,496 ng h/mL, for the 0.1, 0.3, 1.0, and 10 mg/kg BW/day dose groups, respectively. Mean C_{max} was 8.9, 6.4, 17.8, and 208.7 ng/mL for the 0.1, 0.3, 1.0, and 10 mg/kg BW/day dose groups, respectively. The sisapronil concentrations in plasma at 4 hours after the last dose (90^{th} day group) were 53, 110, 268, and 1046 ng/mL for the 0.1, 0.3, 1.0, and 10 mg/kg BW/day dose groups, respectively. These concentrations in plasma were 6, 17, 18 and 11 times higher than those at 4 hours after the 1st dose.

In another repeated dose study (non-GLP compliant), rats were administered 0.1, 0.3, 1, or 10 mg sisapronil/kg BW/day orally for 52 weeks (Rodríguez Gómez, 2013a, 2013b). In general, sisapronil concentrations in plasma increased with dose and treatment duration. At day 1, average concentrations at 4-hour post dose were 8.81, 7.53, 23.8, and 248 for 0.1, 0.3, 1, and 10 mg/kg BW/day dosing groups, respectively, and at day 362, the means were 333, 539, 1088, and 2203 ng/mL, respectively.

A strain comparison study was conducted to examine the observed study-to-study variability in concentrations of sisapronil in plasma (Gagnon, 2012b). No difference in C_{max} or AUC_{0-4d}

was found between sonicated and non-sonicated formulations or between Sprague Dawley and Wistar rats after oral dosing at 10 mg/kg. Mean C_{max} values ranged from 669 to 808 ng day/mL and t_{max} ranged from 4 to 8 hours.

Dog

Two studies were conducted examining the oral pharmacokinetics of sisapronil in dogs. In one study (Heward, 2011), beagle dogs (4/sex/group) were dosed orally with unlabelled sisapronil daily for 28 days at 0, 1, 5, or 25 mg/kg BW/day. Single blood samples were collected from all animals on study days 1, 8, 15, and 28 at 8 hours post dose, and plasma samples were analysed for unchanged sisapronil using a LC-MS/MS method (Table 6.1).

Table 6.1. Mean sisapronil concentration in plasma in ng/mL (%CV) determined 8 hours after oral dosing on days 1, 8, 15, and 28 (Heward, 2011).

Dose (mg/kg BW)	Concentrations of sisapronil in plasma (ng/ml) by study day			
	1	8	15	28
1	14.9 (115)	141 (48)	230 (46)	479 (57)
5	35.5 (95)	424 (62)	730 (67)	1500 (57)
25	375 (112)	2050 (47)	3980 (51)	7020 (41)

In the second study (Heward, 2012), beagle dogs (4/sex/group) were dosed orally with unlabelled sisapronil daily for 90 days at 0, 0.3, 1, or 10 mg/kg BW. Single blood samples were collected from all animals on study days 1, 30, 60, and 90 at 8 hours post dose. Plasma samples were analysed for unchanged sisapronil using a validated LC-MS/MS method, with a validated range of 0.500 to 500 ng/mL (Table 6.2).

Table 6.2. Mean sisapronil concentration in plasma in ng/mL (%CV) determined 8 hours after oral dosing on days 1, 30, 60, and 90 (Heward, 2012).

Dose (mg/kg BW)	Concentrations of sisapronil in plasma (ng/mL) by study day			
	1	30	60	90
0.3	5.92 (91)	166 (27)	345 (36)	485 (36)
1	15.1 (85)	503 (49)	920 (56)	1230 (47)
10	120 (110)	4670 (16)	8800 (17)	11000 (18)

Both studies showed a dose dependent increase in sisapronil concentrations in plasma over time, which did not appear to reach steady state.

Monkey

A non-GLP compliant study investigated the pharmacokinetics of sisapronil in monkeys following intravenous (IV) or oral administration (Stuhler, *et al*., 2012). Fasted male and female monkeys (2 of each sex *per* group) were administered sisapronil once either IV (0.5 mg/kg) or orally (2 mg/kg). Blood samples were taken at 1, 2, 4, 8, 24, 96, 168, 240, 336, 504, 672, 840, 1008, 1344, 1680, 2160, and 2880 hours following dosing. The terminal elimination half-life following IV dosing was 12.4 days. Absorption was moderately slow following oral dosing with mean T_{max} of 24 hours, and the oral bioavailability was low (6.5%). Following the 2 mg/kg oral dose, C_{max} was 16.8 ng/mL and $AUC_{0\text{-}70d}$ was 7152 h ng/mL.

Pharmacokinetics in food-producing Animals

Cattle

In a non-GLP compliant study (Boucher, 2012), four male and four female cross-bred cattle were administered a single subcutaneous injection of 2 mg/kg unlabelled sisapronil. Blood samples were collected prior to administration and at 1, 3, 5, 7, 14, 21, 56, 70, 84, 98, 112, 126, and 140 days post dose. In plasma, sisapronil reached a mean peak concentration of 74.8 ng/mL at 15.8 days post dose. The mean terminal half-life was 23.0 days, mean residence time was 48.3 days, and the extent of exposure (AUC) was 3950 day ng/mL.

In another study, groups of 10 cattle were treated with a single injection of sisapronil at 2.0 mg/kg SC or 2.0 mg/kg IV (Merritt, 2011 ref?). Following a single IV dose, mean clearance of sisapronil was very low (0.87 L/kg/d, or 0.6 mL/kg/min), mean volume of distribution was very high (24 L/kg), and the mean terminal half-life was 19 days. Following a single SC dose, mean C_{max} was 72 ng/mL at a mean T_{max} of 12 days. Based upon parallel comparison of mean AUCs for SC and IV treatments, the bioavailability after SC administration was near 100%. The mean terminal half-life was 19 days, and the mean residence time (MRT) was 32 days.

A GLP-compliant study with nine groups of three beef cattle (2 male and 1 female or 1 male and 2 female) averaging 207 kg BW received a single dose of [^{14}C]-sisapronil *via* subcutaneous injection, at a target dose rate of 2.0 mg/kg BW on study day zero (Walker, 2011). Plasma samples were collected from the final two sacrifice groups at study days 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, and 90 (final group only) post dose.

Radioactivity in plasma peaked at 5 days post-dose reaching a mean of 343 µg eq/kg (80 days post-dose), and 300 µg eq/kg (90 days post-dose). Radioactivity in plasma declined to a mean of 42 µg eq/kg at 80 days post-dose and 26 µg eq/kg at 90 days post-dose.

Metabolism in Laboratory Animals

In a GLP-compliant study, two groups of Sprague Dawley rats were administered [14C]-sisapronil by oral dose (Lineham, 2012). One group of eight rats (4/sex; group 1) received a daily oral dose of [^{14}C]-sisapronil at a dose rate of 50 mg/kg BW for four consecutive days. A second group of eight rats (4/sex; group 2) received a single oral dose of [^{14}C]-sisapronil at a dose rate of 50 mg/kg BW. Excreta and cage wash samples were collected daily for four days post first dose for group 1 (multiple dose) rats and daily for 6 days post-dose for group 2 (single

dose) rats. Livers were collected 24 hours post last dose (96 hours post 1st dose) for group 1 rats and 144 hours post dose for group 2 rats. Samples were analysed for total radioactive residues (TRR), and the nature of the radioactivity present in the excreta and liver tissues was investigated by HPLC profiling with off-line radioactivity detection using liquid scintillation counting (LSC).

The primary route of excretion of [^{14}C]-sisapronil derived radioactivity was in the faeces, with approximately 9% (group 1, multiple dose) and 28% (group 2, single dose) of the administered radioactivity excreted within the first 24 hours post the first dose. The percent recovery of total radioactivity over the entire study period was higher in the single dose group, with approximately 49% compared to approximately 31% in the multiple dose group.

The excretion of radioactive residues was gradual with the majority of radioactivity excreted via the faeces. For group 1 (multiple dose) male rats, 27.3 % of total dosed radioactivity was excreted *via* the faeces and 0.8% of total dosed radioactivity was excreted via the urine. For group 1 female rats, 22.6% of total dosed radioactivity was excreted via the faeces and 1.7 % of total dosed radioactivity was excreted via the urine. For group 1 rats, g> 97% of the excreted radioactivity from males and >93% from females partitioned into the faeces. For group 2 (single dose) male rats, 46.5% of total dosed radioactivity was excreted via the faeces and 0.87% of total dosed radioactivity was excreted via the urine. For group 2 female rats, 41.2% of total dosed radioactivity was excreted via the faeces and 2.3% of total dosed radioactivity was excreted via the urine. For group 2 rats, > 98% of the excreted radioactivity from males and >94% from females partitioned into the faeces.

Profiling results demonstrated that intact sisapronil was the primary residue in faeces from both treatment groups, representing >91% of TRR in males and females in both groups from 0-24 hours. The percentage of sisapronil gradually decreased over time, with a more rapid decline in group 2 rats.

Mean residues in liver represented 4.86 and 3.85% of the total radioactive dose administered, respectively, for multiple dose group 1 male and female rats and 3.25 and 3.41% of the total dose, respectively, for single dose group 2 male and female rats. [^{14}C]-sisapronil was the primary residue from group 1 and group 2 rats. One metabolite designated in the test site report as metabolite E correlates with the significant metabolite observed in bovine liver.

Metabolism in food-producing animals

Cattle

In a GLP-compliant study with nine groups of three beef cattle (2 male and 1 female or 1 male and 2 female) averaging 207 kg BW, the cattle each received a single dose of [^{14}C]-sisapronil *via* subcutaneous injection, at a target dose rate of 2.0 mg/kg BW on study day zero (Walker, 2011). Urine and faeces were collected on a total of 12 days, study days 10-12, 30-32, 60-62 and analysed for total radioactive residues (TRR. Bile was also collected at slaughter from each of the nine groups over the 90 day in-life period.

Radioactivity was excreted primarily via the faeces with greater than 97% of excreted residues present in the faeces. For study days 10-12, 2-4% of total dosed radioactivity was excreted

daily via the faeces with ≤0.1% of total dosed radioactivity excreted daily via the urine. By study days 60-62, ≤0.45% of total dosed radioactivity was excreted daily via the faeces with ≤0.01% of total dosed radioactivity excreted daily via the urine. For all days over which excreta and cage wash samples were collected, 14.58% (day 80 animals) and 16.29% (day 90 animals) of total dosed radioactivity was excreted via the faeces representing 97.3 and 97.5%, respectively, of total excreted radioactively for the two treatment groups collected during these study intervals. In bile, a maximum mean TRR concentration of 2409 µg eq/kg was measured at 10 days withdrawal, with a steady decrease in concentration to 84 µg eq/kg at 90 days post-dose.

HPLC fractionation with off-line radioactivity detection was performed for analysis of tissue extracts (Zielinski, 2010; Lu and Wang, 2012). Intact sisapronil was the primary residue in faeces, and typically represented < 40% of the residues in urine. Results in urine showed two significant co-eluting metabolites accounting for 15-62% of the TRR, with intact sisapronil representing 3-42% of the TRR. These two metabolites in urine were characterized as having undergone both an oxidation as well as a conjugation with glucuronic acid.

Tissue residue depletion studies

Radiolabelled residue depletion studies

Cattle

In a GLP compliant study, 27 beef cattle (14 male and 13 female) were treated with the recommended label dose consisting of a single subcutaneous injection of 2.0 mg/kg [^{14}C]-sisapronil (Walker, 2011). Cattle were killed starting at study day 10 and every ten days through study day 90. Loin muscle, injection site muscle (injection site core), surround injection site muscle (injection site ring), fat (omental & renal), small intestine (contents removed), liver (gall bladder removed), bile, kidneys, diaphragm, heart and lungs were collected and analysed for total radioactive residues (TRR).

For edible tissues, fat samples contained the highest concentrations of TRR at all time points, followed by liver, small intestine, kidney, and loin muscle. Concentrations were highest at 10 days withdrawal, and were detected in each of these tissues through 90 days withdrawal (Figures 6.1 and 6.2, Table 6.3). It was noted that homogenization of injection site samples proved difficult, which likely contributed to the inconsistent analytical results from the injection sites.

HPLC fractionation with off-line radioactivity detection was performed for analysis of tissue extracts (Zielinski, 2010; Lu and Wang, 2012). Intact sisapronil was the primary residue in fat, liver, kidney, loin muscle, and injection site muscle. Parent sisapronil accounted for 94-99.6, 86.0-99.6, and 90.2-100% of the TRR in fat, kidney, and loin muscle, respectively. One significant metabolite accounting for 19-45% of the TRR was observed in liver but was not identified. This metabolite comprised a smaller percentage of the TRR (around 20%) at the earlier withdrawal times and increased in percentage over the withdrawal times. Based on

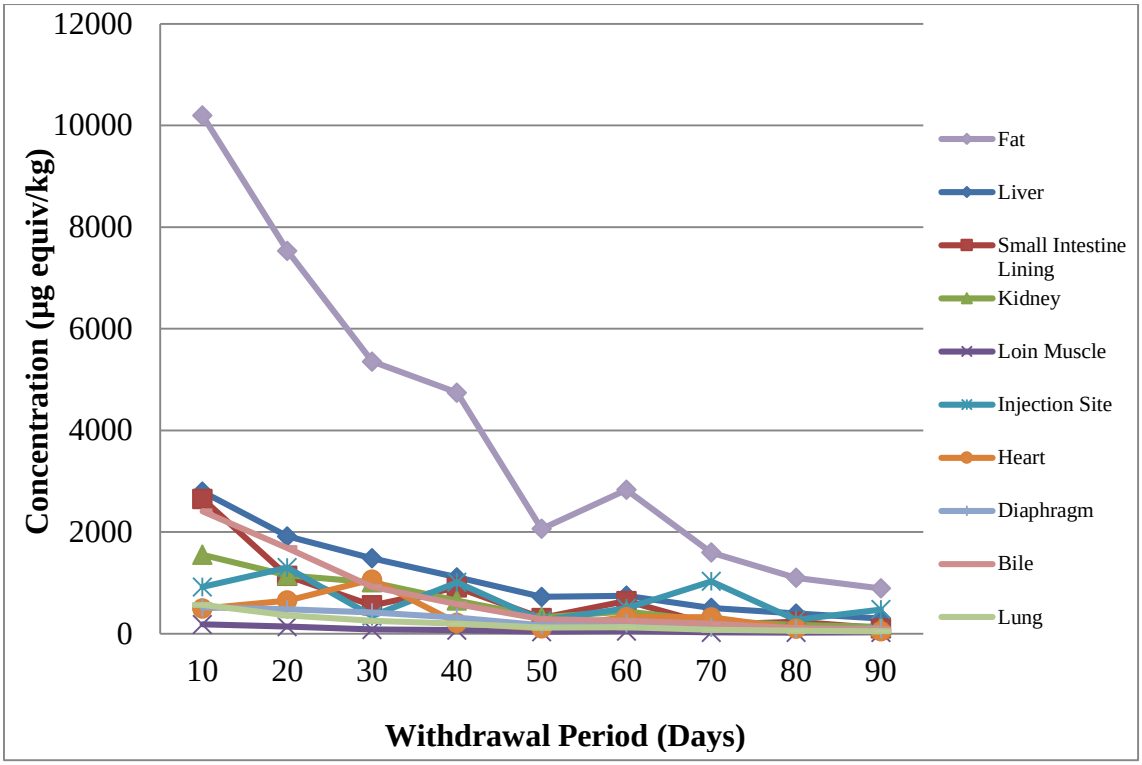


Figure 6.1. TRR depletion (group mean values) in tissues of cattle (n=27) following a single subcutaneous dose of [^{14}C] sisapronil at a rate of 2.0 mg/kg (Walker, 2011).

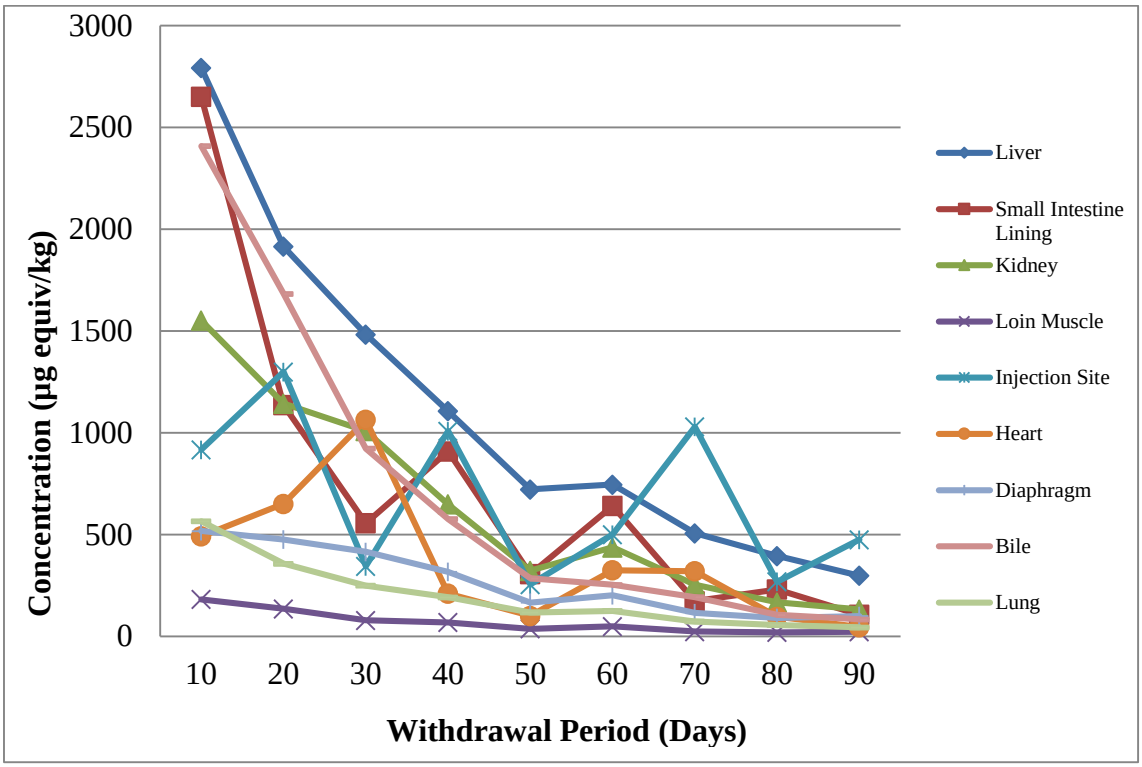


Figure 6.2. TRR depletion (group mean values) in tissues (with the exception of fat) of cattle following a single subcutaneous dose of [^{14}C] sisapronil at a rate of 2.0 mg/kg (Walker, 2011).

Table 6.3. Mean concentration of total radioactivity in tissues following subcutaneous administration of 2.0 mg/kg [^{14}C] sisapronil to cattle (Walker, 2011).

Withdrawal Time	Concentration of sisapronil in tissues reported as µg eq/kg				
	Fat	Liver	Small Intestine Lining	Kidney	Loin Muscle
10 days	10195 ± 763	2793 ± 226	2651 ± 291	1552 ± 564	183 ± 47.1
20 days	7534 ± 1234	1915 ± 588	1137 ± 314	1144 ± 329	136 ± 4.7
30 days	5355 ± 877	1484 ± 182	557 ± 110	1010 ± 357	79.5 ± 2.2
40 days	4743 ± 996	1107 ± 104	910 ± 80.9	650 ± 190	69.6 ± 11.8
50 days	2061 ± 592	723 ± 53.4	307 ± 138	323 ± 70.9	37.8 ± 11.9
60 days	2832 ± 730	746 ± 6.2	642 ± 281	438 ± 114	50.0 ± 13.2
70 days	1595 ± 418	507± 153	174 ± 36.5	255 ± 127	24.3 ± 4.8
80 days	1097 ± 693	396 ± 56.0	231 ± 242	168 ± 83.8	20.8 ± 12.8
90 days	891 ± 517	299 ± 55.0	107 ± 75.9	133 ± 67.3	< LOD

LOD = 30 dpm above background.

These results, parent sisaspronil was identified as the marker residue The mean ratios of parent sisapronil to TRR in liver at various timepoints following administration are presented in Table 6.4.

Table 6.4. Mean ratio of parent sisapronil marker residue (MR) to total radioactive residues (TRR) in liver tissue following subcutaneous administration of [^{14}C] sisapronil to cattle (Zielinski, 2010; Lu and Wang, 2012).

Withdrawal Time (days)	Sample	Mean MR / TRR
10	Liver	0.73
20	Liver	0.64
30	Liver	0.61
40	Liver	0.63
50	Liver	0.57
60	Liver	0.57
70	Liver	0.50
80	Liver	0.64
90	Liver	0.56

Residue depletion studies with unlabelled drug

Cattle

In a GLP-compliant study (Zielinski, 2011), 36 cattle (18 males, 18 females), weighing 236-342 kg at dosing, were treated with a single subcutaneous injection of sisapronil at a mean dose rate of 2.1 mg/kg BW. Two male and two female animals were killed after 30, 60, 90, 120, 150, 180, 210 and 240 days withdrawal post-dose. Hind quarter muscle, primary injection site muscle, surround injection site muscle, liver, kidney, fat (peri-renal) and small intestine (contents removed) were collected from each animal and submitted for analysis of sisapronil using a validated LC-MS/MS method with a Limit of Quantitation (LOQ) of 5 µg/kg.

Sisapronil residues depleted gradually from all tissues over the 240 day study period. Measurable concentrations were still detectable at greater than the LOQ at 240 days withdrawal in all tissues. Fat samples declined from a mean value of 7520 µg/kg at 10 days withdrawal to a mean of 564 µg/kg at 240 days withdrawal. Residues in all individual animal fat residues were below 1900 µg/kg by 120 days withdrawal. All hind quarter muscle, small intestine and kidney samples contained residues which were below 125 µg/kg by 120 days withdrawal, and all liver samples were below 225 µg/kg by 120 days. Table6.5 summarizes the mean tissue residue data.

Table 6.5. Mean concentration of sisapronil (parent drug, marker residue) in edible tissues of beef cattle (n=36) administered sisapronil by subcutaneous injection at a dose rate of 2.0 mg/kg BW (Zielinski, 2011).

Withdrawal Time (days)	Mean Concentration of Sisapronil Parent Drug (marker residue) in µg/kg				
	Fat	Liver	Kidney	Hind Quarter Muscle	Small Intestine
30	7520 ± 1240	759 ± 136	465 ± 136	172 ± 15	232 ± 36
60	3760 ± 741	385 ± 123	249 ± 150	110 ± 40	143 ± 42
90	2450 ± 782	264 ± 92	120 ± 39	93.9 ± 33	132 ± 24
120	1450 ± 429	158 ± 40	97.1 ± 19	49.4 ± 17	40.8 ± 9.2
150	1240 ± 158	133 ± 30	50.1 ± 8.7	61.5 ± 26	55.4 ± 16
180	1160 ± 308	117 ± 27	54.9 ± 13	62.0 ± 45	45.8 ± 12
210	825 ± 284	89.6 ± 30	42.4 ± 14	38.6 ± 15	86.3 ± 54
240	564 ± 211	60.3 ± 21	43.2 ± 22	32.4 ± 18	45.5 ± 24

Residues in the core injection site (~500 g) were greater than residues from their respective samples of tissue ringing the injection site samples (~300 g) for all individual animals through withdrawal day 90. At day 120, residues from 3 of 4 injection site muscle samples were greater than their corresponding surrounding tissue samples and at day 150, 2 of 4 injection site muscle samples had residue concentrations greater than their corresponding samples of surrounding tissue. Beginning at withdrawal day 180 and through the end of the study (day 240), sisapronil concentrations were higher in the samples of tissue surrounding the core injection site for all individual animals. Table 6.6 summarizes residue concentrations in core injection site muscle and concentrations in tissue surrounding the injection site, with an additional column summarizing results for injection site muscle with inclusion of concentrations in tissue surrounding the injection site at days 180, 210 & 240.

Table 6.6. Mean concentration of sisapronil residues for injection site muscle and muscle tissues immediately surrounding the injection site of beef cattle administered sisapronil by subcutaneous injection at a targeted dose rate of 2.0 mg/kg BW (Zielinski, 2011).

Withdrawal Tine (days)	Mean Concentration of Sisapronil Parent Drug (marker residue) in µg/kg	
	Primary Injection Site	Muscle Surrounding Injection Site
30	29650 ± 29662	4007 ± 4223
60	5376 ± 8386	350 ± 206
90	574 ± 625	313 ± 219
120	129 ± 24	159 ± 148
150	277 ± 360	107 ± 30
180	87.4 ± 43	165 ± 92
210	70.7 ± 27	99.5 ± 44
240	76.4 ± 49	119 ± 77

Methods of analysis for residues in tissues

Main principles of the analytical method

A high performance liquid chromatography tandem mass spectrometry method (Zielinski *et al*., 2012) was used to determine the marker residue (parent sisapronil) in bovine edible tissues. The target residue is extracted from 1 g tissue twice with 1% trifluoroacetic acid in (9:1 CH_3CN:H_2O, v/v) (1:7, v/v). After agitation and centrifugation, the supernatant is transferred to a HPLC vial. No additional purification step is performed on the extract. All reagents used

during the analysis were analytical grade or better. The mobile phase was 0.027% formic acid in 2 mM ammonium acetate (v/v) (A) and acetonitrile (B). Injected volume was 5 µL and flow rate was set at 0.5 mL min-1. The stationary phase was a 5 µm C18 100A 2 x 50 mm column equipped with a guard column (2 x 4 mm C18). The gradient was set as follow: 45%A at 0 min, 5%A from 0.6 to 2.4 min, 45%A from 2.5 to 6.5 min. The internal standard used (PF241851) was sisapronil labeled at three positions (13C2-15N). Standard curves were generated using simple linear regression. A 1/x weighting was required during validation in order to span the 5 to 1000 µg/kg analytical range.

Validation of the analytical method

The Committee assessed the validation data against the analytical requirements as published in the Codex guidelines for analytical methods for residue control, CAC/GL 71-2009 (FAO/WHO, 2014).

The method selectivity was proved by comparison of control samples with spiked samples. The non-presence of significant interfering substances eluting at or near the retention time of sisapronil was used as a criteria to demonstrate the method specificity.

The intra-day mean accuracy (defined as the % recovery of the actual concentration) for bovine muscle was 84.9-105%, 98.0-109% for liver, 96.2-108% for kidney, 97.0-110% for fat and 104-107% for small intestine. The inter-day mean accuracy for bovine muscle was 94.1-102%, 103-105% for liver, 100-101% for kidney and 103-105% for fat, meeting the criteria in CAC/GL 71-2009. Small intestine was assayed on just one day and thus inter-day accuracy was not applicable (Zielinski *et al*., 2012).

The intra-day precision (expressed as a coefficient of variation) for bovine muscle was 1.5-11.8%, 1.9-12.5% for liver, 2.9-17.5% for kidney, 1.1-14.9% for fat and 0.8-4.2% for small intestine. The inter-day precision for bovine muscle was 3.4-12.4%, 4.6-8.7% for liver, 5.1-11.8% for kidney and 3.4-9.3% for fat, meeting the criteria in CAC/GL 71-2009. Small intestine was assayed on just one validation day and thus inter-day precision was not applicable (Zielinski *et al*., 2012).

The calculated assay limit of detection (LOD) was established by analysing 20 samples of each control bovine matrix (from each of 6 different cattle) and determining the level of mean background noise in each sample. The LOD was calculated by determining the response of the peak or background at the retention time of the analyte peak and expressing this as the mean plus 3x standard deviations of the background data, meeting the criteria in CAC/GL 71-2009. The calculated assay LODs (rounded to 1 digit) were 0.2, 0.6, 0.6, and 0.3 µg/kg, for bovine muscle, liver, kidney, and fat, respectively (Zielinski, 2013).

The calculated assay limit of quantitation (LOQ) was first established by determining the response of the peak or background at the retention time of the analyte peak and expressing this as the mean plus 10x standard deviations of the background data, meeting the criteria in CAC/GL 71-2009. The calculated assay LOQs (rounded to 1 digit) were 0.9, 0.9, 0.8, and 0.7 µg/kg, for bovine muscle, liver, kidney, and fat, respectively (Boner, 2011). However, after implementation of the validated method, frequent failures of QC samples prepared near the method LOQ were observed. The LC system has thus been changed from an HPLC system to

an UPLC system prior to MS/MS measurement. A second validation study was then initiated (Zielenski, 2013) with main aim to re-evaluate the method performance (accuracy and precision in terms of repeatability and reproducibility) at an estimated LOQ of 5 µg/kg. Calculated assay LOQs (rounded to 1 digit) were 0.6, 1.6, 1.7 and 0.8 µg/kg for bovine muscle, liver, kidney, and fat, respectively. The new assay limit of quantification for sisapronil in bovine edible tissues was set at 5 µg/kg as precision and accuracy fitted within expected ranges at this concentration level.

- The stability of sisapronil was demonstrated in the following experiments:
- In spiking solutions, e.g. acetonitrile/water (50:50, v/v) stored at *ca* 1-8 °C for at least 104 d and 1% trifluoroacetic acid in acetonitrile/water (90:10, v/v) stored at *ca* 1-8 °C for at least 76 d.
- In fortified tissues at -10 °C for at least 80 d in fat/liver, and at least 91 d in muscle/kidney.
- In incurred tissue samples during up to 3 freeze/thaw cycles, as well as at least 18 h in all tissues at ambient temperature, -10°C for at least 193 d for all edible tissues.
- In final sample extracts stored at controlled room temperature for at least 3 d for all edible tissues.

Appraisal

Sisapronil has not been previously reviewed by the Committee. Sisapronil is a long-acting injectable phenylpyrazole ectoparasiticide for control of cattle ticks, and aids in the control of bot fly larvae, hornfly and screwworm. It is registered for use in cattle at a recommended dose of a single subcutaneous injection of 2.0 mg/kg BW. Sisapronil accumulates primarily in fat and is slowly released through the circulatory system and skin, providing prolonged ectoparasitic control.

A radiolabelled study in cattle demonstrated that parent sisapronil is the marker residue and that it remains predominantly unmetabolized (Walker, 2011; Zielinski, 2010). The ratios between the marker residue and the total residues remained steady through 90 days withdrawal, and have been determined in cattle as 0.90 in muscle, 0.50 in liver, 0.96 in kidney, 0.97 in fat. Fat and liver have been identified as prinicpal target tissues. Marker to total ratios in fat, kidney and muscle were farily consistent over time. However, it was noted that there were some fluctuations in recovery of TRR. Therefore, the Committee concluded that a conservative approach to assignment of the marker residue: total residue of pharmacological concern (MR:TR) would be appropriate, and chose the lowest values reported for each tissue.

Residue data were obtained using a validated HPLC-MS/MS method to quantify sisapronil in bovine edible tissues – muscle, liver, kidney, fat and small intestine. The method is applicable in the range of 5.00-1000 µg/kg for all tissues.

Maximum Residue Limits

MRLs could not be recommended by the Committee, as an ADI could not be established.

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7. Teflubenzuron

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Identity

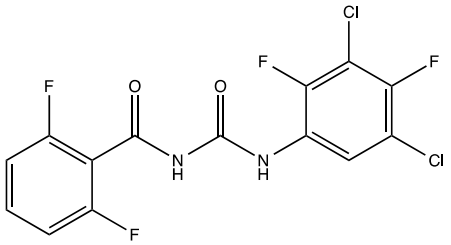
International Non-proprietary Name (INN): Teflubenzuron

Synonyms: AC 291898, CME 134, CME 134-01, CME 13406, Calicide, Ektobann, HOE 522, MK 139, Nomolt, Nomolt agro, OMS 3009, Tefluron

IUPAC Name: 1-(3,5-dichloro-2,4-difluorophenyl)-3-(2,6-difluorobenzoyl)urea.

Chemical Abstract Service Number: 83121-18-0

Structural formula:



Molecular formula: $C_{14}H_6Cl_2F_4N_2O_2$

Molecular weight: 381.1 g mol^{-1}

Other information on identity and properties

Pure active ingredient:	Teflubenzuron (purity ≥ 95%)
Appearance:	White to off-white crystalline solid
Melting point:	222.5 °C
Solubility in water:	0.6 g/L at 20 °C
Solubility in methanol:	1.8 g/L at 20 °C
Solubility in acetonitrile:	1.1 g/L at 20 °C
Solubility in dichloromethane:	1.8 g/L at 20 °C
Vapour pressure:	8 x 10^{-10} Pa at 20 °C
Log $K_{o/w}$:	4.56

Residues in food and their evaluation

Teflubenzuron is used for the treatment of sea lice in Atlantic salmon, as well as in agriculture to control codling moth, leaf miners, whiteflies and caterpillars on fruit trees, vines, vegetables, potatoes, soybean, tobacco and cotton.

Teflubenzuron (CAS No. 83121-18-0) is an acyl urea insecticide used as a veterinary drug in aquaculture for the treatment of sea lice (*Lepeophtheirus salmonis* Krøyer and *Caligus rogercresseyi* Boxshall & Bravo) infestations in Atlantic salmon (*Salmo salar* L.). It is also used for the control of a wide range of insect pests (larvae of Lepidoptera and Coleoptera being most sensitive) and some mites on fruits, vegetables, cereals, nuts and seeds. Teflubenzuron acts by inhibition of chitin synthesis and moulting, disrupting chitin deposition in the insect cuticle after ingestion.

An ADI of 0.01 mg/kg bw/day was established by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR, 1995a), based on the dose-related effects in the liver and derived from an 18-month carcinogenicity study in mice described in the toxicological monograph prepared by the meeting (JMPR, 1995b). At the request of the manufacturer, the compound was removed from the review schedule for residues of the JMPR in 1994 and its residue aspects were reviewed for the first time by JMPR in 1996 (JMPR, 1996).

The U.S. Food and Drug Administration has established an import tolerance of 0.5 mg/kg teflubenzuron in muscle with adhering skin of Atlantic salmon (U.S.F.D.A., 2014).

In the European Union, teflubenzuron was included in Annex I of Directive 91/414/EEC by means of Commission Directive 2009/37/EC (EC, 2009) for use as an insecticide only in glasshouses (on artificial substrate or closed hydroponic systems) (EFSA, 2012).

The 22nd Session of the Codex Committee on Residues of Veterinary Drugs in Food (CCRVDF) requested that JECFA conduct an evaluation of teflubenzuron, with establishment of an MRL in finfish (salmon) muscle and skin in natural proportions (FAO/WHO, 2015).

Conditions of use

Teflubenzuron is registered for use in aquaculture in Canada, Norway, UK and Ireland. Medicated feed is prepared by coating commercial fish feed pellets with teflubenzuron (at least 95% chemically pure) as a powder to a concentration of 2 g/kg feed. Spraying the diet with fish oil increases the adherence of the material to the feed pellet. The MRLs and withdrawal periods established in each country are summarized in Table 7.1.

Dosage

The intended oral dose is 10 mg teflubenzuron per kg of fish biomass once daily for seven consecutive days.

Table 7.1. Countries in which teflubenzuron is registered with the MRLs and adopted withdrawal periods.

Country	MRL (µg/kg)	Withdrawal period
United Kingdom	500 (muscle and skin in natural proportion)	7 days
Ireland	500 (muscle and skin in natural proportion)	45 degree days
Canada	300 in muscle and 320 in skin	11 days
Norway	500 (muscle and skin in natural proportion)	96 degree days

Pharmacokinetics and metabolism

Test material used in the radiolabelled pharmacokinetic and metabolism studies in salmon

Pharmacokinetic and metabolism studies were conducted with [^{14}C]-teflubenzuron uniformly labelled within the benzoyl ring (Figure 7.1A) and aniline ring (Figure 7.1B) of the compound.

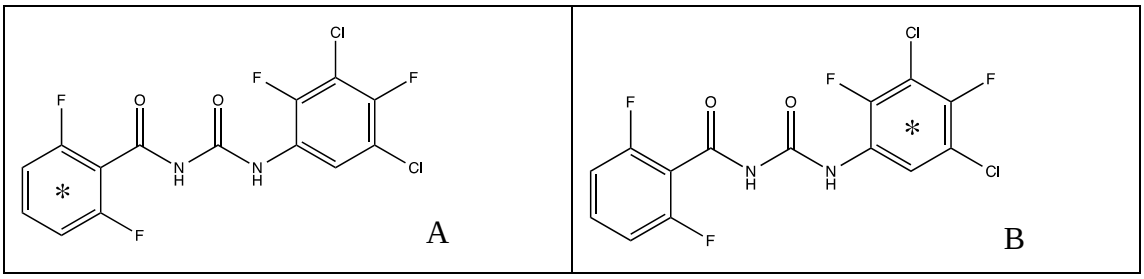


Figure 7.1. Structure of the radiolabelled compounds: A – [^{14}C]-teflubenzuron labelled at the benzoyl ring and B - [^{14}C]-teflubenzuron labelled at the aniline ring.

The purities of the radiolabelled compounds used throughout the studies were greater than 97%, as determined using high performance liquid chromatography (HPLC) coupled to a radiochemical detector.

The radiolabelled compounds were prepared in separate solutions in tetrahydrofuran. For the studies, the solutions were mixed in order to produce an equal mix of radioactivity. Tetrahydrofuran was removed and, the day before dosing, the test material was re-suspended in dimethyl sulphoxide (DMSO). The radiochemical dose was about 1850 kBq/kg (50 µCi/kg) {925 kBq/kg of the labelled compound on the aniline ring and 925 kBq/kg of the labelled compound on the benzoyl ring}.

The radiolabelled teflubenzuron dissolved in DMSO (40 mg/mL, corresponding to a radiochemical concentration of 200 µCi/mL) was added onto control diet at a rate of 100 µL dose formulation per 400 g fish bw. In order to facilitate the detection of regurgitation post-dosing, the formulation containing teflubenzuron was dispensed onto control diet that had been

crushed and mixed with Barbour red food dye. The fortified diet was administered directly into the fish by intra-oesophageal intubation.

Pharmacokinetics in laboratory animals

Rats

Teflubenzuron is only partially absorbed from the gastrointestinal tract in rats (4% to 19%) and the absorption is dose-dependent and saturable (EMEA, 1999). Maximum concentrations in plasma are reached within 8 to 24 h after a single oral dose. The saturable kinetics of teflubenzuron in plasma is essentially constant after repeated administration of teflubenzuron in diet at concentrations greater than or equal to 1000 to 2000 mg/kg feed (77 to 158 mg/kg bw/day). Following repeated administration of radiolabelled [^{14}C]-teflubenzuron, the highest concentrations of the compound are present in fat, liver and kidneys. The distribution is rapid and the maximum concentrations for almost all tissues occur at 6 hours post-dosing. Residues in organs and tissues decline quickly and there is no evidence of accumulation of teflubenzuron. The compound is rapidly and completely excreted, mainly via the faeces (more than 90% of the dose). Absorbed teflubenzuron is largely excreted in the bile (2 to 16% of the dose), while the urinary excretion represents only a minor pathway (0.4% to 1.4% of the dose). The Committee noted that the values provided for the excretion and absorption will exceed 100%. There is no difference in excretion pattern between males and females after single or repeated administrations of teflubenzuron.

Pharmacokinetic in Food-producing Animals

Salmon

In a GLP-compliant study, Atlantic salmon, *Salmo salar* L., (100 fish, 173-395 g) were treated with a single dose of teflubenzuron by intravenous injection to give a nominal concentration of 2 mg/kg bw (Jenkins, 1996a). The fish were maintained in sea water at 13 -14 °C. Plasma samples were taken 15 min, 3 h, 6 h, 9 h, 12 h, 24 h, 48 h, 72 h, 120 h and 168 h post treatment. Teflubenzuron was quantified in the plasma samples (0.5 mL) by a validated HPLC-UV method with the following validation parameters: linear range: 10 to 5000 ng/mL, recovery: 90% and limit of quantification: 10 ng/mL; within-run precision: 1.0 – 5.9% and within-run accuracy: 97.5 to 110%.

In a related experiment, Atlantic salmon, *Salmo salar* L., (90 fish, 104 to 425 g) were treated with a single dose of medicated diet by oral gavage at a nominal concentration of 10 mg/kg bw (Jenkins 1996a). The fish were maintained in sea water at 13 - 14 °C and plasma samples were taken at 3 h, 6 h, 9 h, 12 h, 24 h, 48 h, 72 h, 120 h and 168 h post treatment. Teflubenzuron was quantified in the plasma samples (results shown in Table 2) by the same validated HPLC-UV method described previously with the following validation parameters reported for this study: linear range: 10 to 5000 ng/mL, recovery: 60% and limit of quantification: 25 ng/mL; within-run precision: 3.8 – 19.6% and within-run accuracy: 80.3 – 117%. The change in concentration of teflubenzuron in plasma with time is shown in Table 7.2.

Table 7.2. Mean concentration of teflubenzuron in plasma of Atlantic salmon (n=10 fish) following a single intravenous (IV) administration at a dose of 2 mg/kg bw {single oral (gavage) dose of 10 mg/kg bw}, water temperature: 13-14 °C (Jenkins, 1996a).

AR	Time post-dose (ng/mL)	15 min	3 h	6 h	9 h	12 h	24 h	48 h	72 h	120 h and 168 h
IV	Conc.	5192	883	559	497	518	157	86	29	<LOQ
	±SD	±4934	±297	±301	±217	±128	±103	±29	±14	
Gavage	Conc.	-	226	430	527	521	136	30	13	<LOQ
	±SD		±52	±123	±95	±122	±31	±8	±4	

AR: administration route of the compound; LOQ: 25 ng/mL; SD: standard deviation.

The calculated mean pharmacokinetic parameters for teflubenzuron in Atlantic salmon plasma (intravenous injection and gavage) are shown in Table 7.3.

Table 7.3. Mean pharmacokinetic parameters for teflubenzuron in Atlantic salmon plasma after dosing by intravenous injection or gavage, water temperature: 13-14 °C (Jenkins 1996b).

Route of administration	Dose (mg/kg per day)	T_{max} (h)	C_{max} (µg/mL)	$AUC_{(0-72)}$ (µg.h/mL)	$t_{1/2}$ (h)	CL (mL/kg per minute)
IV	2	0.25	5.2	23.4	15.3	1.4
Gavage	10	9.0	0.57	10.9	14.2	-

AR: administration route of the compound; T_{max}: time to peak plasma concentration; C_{max}: peak plasma concentration; AUC: area under the curve; $t_{1/2}$: elimination half-life and CL: body clearance.

In a multiple dose study (Jenkins, 1995), Atlantic salmon, *Salmo salar* L., weighing from 626 to 918 g, held in sea water at 7 – 8 °C, were treated with medicated feed (dose of 10 mg/kg bw/day) for seven consecutive days. Plasma samples were collected during the feeding period (6 h post-feeding on days 1-7) and 30 h and 48 h after administration of the last medicated feed. Residues of teflubenzuron were quantified with a validated HPLC-UV method. The concentrations of teflubenzuron in plasma found in this study are shown in Table 7.4.

Table 7.4. Mean concentration (n=5) of teflubenzuron in plasma following administration of medicated feed (dose of 10 mg/kg bw/day) to Atlantic salmon for seven consecutive days. Water temperature: 7 - 8 °C (Jenkins 1995).

Time post-dose	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	30 h*	48 h*
Conc. ±SD (ng/mL)	79 ±41	144 ±79	139 ±50	118 ±26	177 ±48	250 ±74	196 ±65	100 ±65	56 ±11

* Time after last dose; SD: standard deviation.

The mean concentration of teflubenzuron in plasma was 144 ng/mL approximately 30 hours (Day 2) after first treatment and a maximum of 250 ng/mL was achieved after six days. The

increased concentration of teflubenzuron in plasma on day 6 may be a result of the variability in the feeding levels of the fish observed during the test. Following the end of the treatment with medicated feed, mean concentrations of teflubenzuron in plasma fell rapidly and on day 9 (48 h after last treatment) at the end of the test, were 29% of the mean concentration determined on day 7. The elimination phase rate constant was estimated to be 0.02949, giving a half-life after oral administration of 23.5 h.

Metabolism in Laboratory Animals

Rats

In an elimination and metabolism study, three test groups of rats, 5 males and 5 females per group, were treated with [^{14}C]-teflubenzuron (purity > 99%) by oral gavage (U.S.F.D.A., 2014). Group A received a single oral dose of 25 mg/kg bw [^{14}C]-teflubenzuron: Group B received a 14-day repeated oral dose of 25 mg/kg bw of non-radiolabelled teflubenzuron followed by a single oral dose of 25 mg/kg bw [^{14}C]-teflubenzuron; and Group C received a single oral dose of 750 mg/kg bw [^{14}C]-teflubenzuron. Urine and faeces were collected at 24, 48, 72, 120, 144, 168 and 192 h post-dosing with the radiolabelled drug.

In group A, more than 85% of the dose was eliminated in the faeces within 24 h after dosing; overall radioactivity recovered in faeces was higher than 90%. Similar results were obtained for rats of group B. For rats that received the higher dose of radiolabelled teflubenzuron (group C), greater than 90% of the administered dose was recovered in faeces within 48 h after dosing. After 8 days, total radioactivity recovered in urine, faeces and carcass was 0.15%, 94.2% and less than 0.1% of the administered dose, respectively. Unextractable and non-extracted radioactive residues in faeces accounted for between 0.7% and 4.5% of the administered dose for all three groups.

The greatest portion of radioactivity in faeces, determined by thin layer chromatography (TLC), was unchanged parent drug (82.2% to 91.4% of the administered dose). One metabolite identified in faeces was 3,5-dichloro-2,4-difluorophenyl-urea (maximum of 0.2% of the administered dose in Group C and 0.5% to 1.0% of the administered dose in Groups A and B).

In another study, the bile ducts of two groups of Wistar rats, 3 males and 3 females per group, were cannulated prior the administration of a single dose of 25 mg/kg bw [^{14}C]-teflubenzuron by oral gavage or a single dose of 750 mg/kg bw [^{14}C]-teflubenzuron by gastric intubation (U.S.F.D.A., 2014; Hawkins & Mayo, 1988). In each test group, bile was collected until 48 h post-dose. Urine and faeces were collected over the periods 0 to 24 h and 24 to 48 h post-dose. At 48 h, all animals were sacrificed and the gastro-intestinal tracts, livers and carcasses were collected. The radioactivity in bile and urine was measured by liquid scintillation counting. For the animals that received the single low dose (25 mg/kg bw), mean quantities of about 16% and 1.4% of the administered dose were excreted in the 0-48 h bile and urine, respectively. A mean of about 46% of the dose was excreted in the faeces collected in the first 48 h and 23% of the administered dose was measured in the gastrointestinal tract at 48 after dosing. About 0.4% and 1.6% of the administered dose were found in the liver and remaining carcass, respectively, at 48 h post-dose.

After the single high-level dose (750 mg/kg bw), means of about 1.9% and 0.4% of the administered dose were excreted in the 0-48 h bile and urine, respectively. A mean of about 65% of the dose was excreted in the 0-48 h faeces and 19% of the administered dose was measured in the gastrointestinal tract at 48 h after dosing. Approximately 0.06% and 1.2% dose were measured in the liver and remaining carcass, respectively, at 48 h. The sum of the radioactivity excreted in urine and bile and the radioactivity in the liver indicated a total absorption of about 18% and 2% of the dose after administration at doses of 25 mg/kg bw and 750 mg/kg bw, respectively. This demonstrates that absorption of teflubenzuron is dose-dependent in rats, with greater absorption at the lower dose.

The majority of radioactivity in the faeces (approximately 43% of the low dose and 56% of the high dose), was identified by TLC as the unchanged parent drug. The concentrations of metabolites found were typically less than 1% of the administered dose. The metabolite, 3,5-dichloro- 2,4-difluorophenyl urea, was found in faeces. A substantial portion of the biotransformation products in bile was unidentified polar material (14.1-15.5% of the administered dose for the low dose rats).

In another study, the nature of the radioactive residues in urine and faeces of male and female rats treated with seven daily doses (25 mg/kg bw) of [^{14}C]-teflubenzuron, labelled in the aniline ring, was investigated (Schlüter, 1984). The radiolabelled compound (purity > 99%, specific radioactivity of 36.7 Bq/µg) was dissolved in dimethyl sulfoxide (0.5 mL) and administered by gavage. Faeces and urine were collected at daily intervals until day 8 post-dose. About 90% of the radioactive material was excreted with the faeces (89.9% male and 92.9% female on day 8 post-dose). Most of this amount (70-75%) passed the gastro-intestinal tract and was excreted as unchanged parent compound. The remainder (11-13%) was composed of various extractable trace compounds (at least 15), none of which exceeded 1% of radioactivity and of a portion of about 5%, which remained unextractable with organic solvents and additional acidic treatment. Only 2-3% of the radioactive material was excreted with the urine. Three metabolites, as hydroxylated compounds, showed that teflubenzuron was metabolized by substitution of a halogen atom and/or hydroxylation once it was resorbed. Differences in biokinetics were not observed between males and females.

In a similar study (JMPR, 1995b), the biotransformation of teflubenzuron was investigated in urine and faeces of Wistar rats treated with a single dose of 25 or 750 mg/kg bw of aniline ring-labelled [^{14}C]-teflubenzuron or single doses of 25 mg/kg bw of non-radiolabelled compound for 14 consecutive days followed by a single dose of 25 mg/kg bw of labelled compound. The main compound identified in faeces was teflubenzuron. Trace amounts of more polar compounds were noted in each treatment group in the faeces. One of these compounds was identified as 3,5-dichloro-2,4-difluorophenyl urea. Thin layer chromatography indicated that the low level of radiolabel found in urine consisted mainly of very polar compounds. A proposed biotransformation pathway is shown in Figure 7.2.

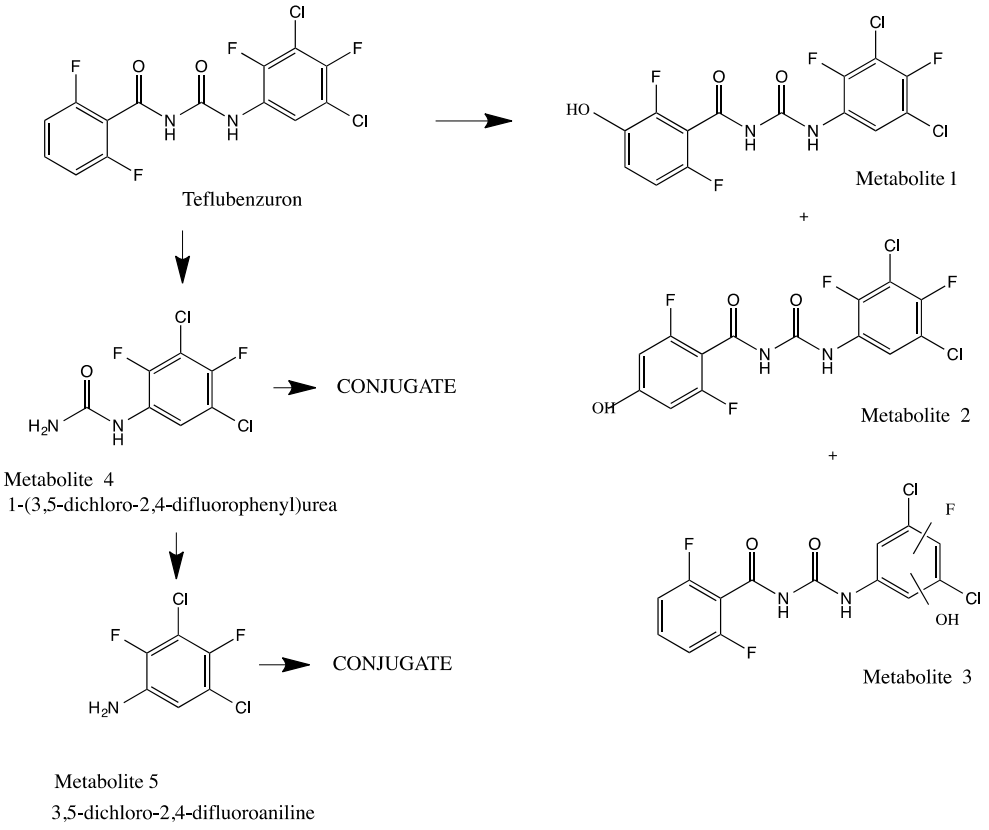


Figure 7.2. Biotransformation pathway of teflubenzuron in rats (JMPR, 1995b).

In an *in vivo* metabolism study (Koerts *et al.*, 1997), male Wistar rats (350 – 400 g) were exposed to 1 to 53 µmol teflubenzuron (in olive oil with 20% DMSO) by oral gavage. After dosing, 0-24 h, 24-48 h urine and 0-48 h faeces samples were collected. Identification of the metabolites was done by ^{19}F-NMR and, for the quantification, 4-fluorobenzoic acid was used as internal standard. Analysis of the faeces revealed the presence of mainly unmodified teflubenzuron. Within 48 h almost the total dose of teflubenzuron was recovered, partly as metabolites in the urine (4-6% of the dose administered) and mainly in unmodified form in the faeces (90% of the dose administered).

The 24-hour urinary metabolic profile of teflubenzuron is shown in Table 7.5.

Table 7.5. Urinary (24-h) metabolic profile of teflubenzuron (Koerts et al. 1997).

Metabolite	Total metabolite (%)
2,6-Difluorobenzoic acid	81.4 ± 3.8
2,6-Difluorobenzoylglycine	4.0 ± 0.5
2,6-difluorobenzamide	2.0 ± 0.3
2-Amino-3,5-difluoro-4,6-dichlorophenylsulfate	2.8 ± 0.5
2-Amino-3,5-difluoro-4,6-dichlorophenylglucoronide	1.2 ± 0.3
4-Amino-2,6-dichloro-3-fluorophenylsulfate	1.2 ± 0.4
4-Acetamido-2,6-dichloro-3-fluorophenylsulfate	0.6 ± 0.5
(3,5-Dichloro-2-fluoro-4-phenylsulfatephenyl)urea	0.3 ± 0.6
(3,5-Dichloro-2-fluoro-4-phenyglucoronidephenyl)urea	5.2 ± 2.4
Unidentified	1.3 ± 0.7

The metabolic profile shows that the benzoate part of teflubenzuron is mainly excreted as 2,6-difluorobenzoic acid and, to a minor extent, as 2,6-difluorobenzoylglycine and 2,6-difluorobenzamide. The aniline ring of teflubenzuron was excreted as the sulphated and glucuronidated conjugates of (4-hydroxy-3,5-dichloro-2-flurophenyl) urea and 2-amino-3,5-difluoro-4,6-dichlorophenol, the sulphated conjugate of 4-amino-2,6-dichloro-3-fluorophenol and the glucuronidated conjugate of 4-acetido-2,6-dichlorofluorophenol. The amount of benzoate-derived metabolites identified in urine was 87% of the total unidentified metabolic pattern whereas the aniline derivative was 13%. It was also shown that the excretion efficiency of the aniline-type compounds from teflubenzuron is at least eight-fold lower than that of benzoates.

Metabolism in Food Producing animals

Goats

In order to study the metabolic profile of teflubenzuron in goats, [^{14}C]-teflubenzuron, uniformly labelled in the aniline ring, was administered orally to two lactating goats, twice daily for 7.5 days, at a daily dose of 7 mg/kg bw (JMPR, 1996). Milk, plasma, urine, faeces, bile, organs and tissues were analysed for the identification and quantification of radioactive metabolites. The main route of elimination of radioactivity was in the faeces, accounting for 99% of the total administered dose, including intestinal contents at post-mortem. The major radioactive component (76.9%) in faeces was attributed to teflubenzuron parent compound using HPLC and TLC analyses. The radioactive residues in all organs, tissues and body fluids examined post-mortem were low in relation to the total dose. The highest mean concentrations of radiolabelled residues were in the liver and lung, with 486 µg eq/kg and 136 µg eq/kg, respectively, corresponding to 0.14 and 0.02% of the total administered dose in the whole organs. Relatively high concentrations were also detected in bile (mean concentrations of 1306 µg eq/L, 0.002% of the total administered dose). The concentrations of radioactivity in the liver and bile indicate biliary excretion as being important in the elimination of the absorbed fraction of an orally administered dose. The absence of similar concentrations in the plasma suggests that much of the absorbed radioactivity is removed by “first-pass metabolism” in the liver. The radioactivity in the bile was mainly in β-glucuronide (or possible sulphate) conjugates. The concentrations of radioactivity in all other organs, tissues and body fluids were generally less than 100 µg eq/kg. Teflubenzuron was, therefore, shown to be poorly absorbed after oral administration; the absorbed fraction appears to be metabolized in the liver and conjugated before elimination, mainly in the bile. Traces of material co-chromatographing with 1-(3,5-dichloro-2,4-difluorphenyl)-3-(2,6-difluoro-3- hydroxybenzoyl) urea (Metabolite 1, Figure 7.2) were detected. None of the extracts contained any radioactive components with similar characteristics to either 3,5-dichloro-2,4-difluoroaniline or 3,5-dichloro-2,4-difluorophenylurea.

Chickens

A total dose of 1.25 mg/kg bw per day was administered to laying hens orally twice daily for 7.5 days (JMPR, 1995b). Teflubenzuron was identified by mass spectrometry as the major component in excreta. Two components were observed in liver and kidney extracts by TLC

and HPLC, one of which was identified as teflubenzuron, while the other could not be identified. A third component found in kidney extracts was shown to be 3,5-dichloro-2,4-difluorophenylurea (Metabolite 4, Figure 7.2). In bile, 22% of the total radioactivity was found and a very polar compound was identified, which on treatment with β-glucuronidase yielded a compound with similar chromatographic characteristics to the meta-hydroxybenzoyl derivative of teflubenzuron (Metabolite 1, Figure 7.2).

Salmon

In a GLP-compliant metabolic profiling study, conducted at a water temperature of 9.9 ± 0.2 °C, a dose equivalent to 10 mg/kg bw (1.85 MBq/kg; 50 µCi/kg) [^{14}C]-teflubenzuron was administered to Atlantic salmon, *Salmo salar* L., (57 fish, 537 to 999 g) by intra-oesophageal intubation (Auger et al. 1995). Two time points after dosing were selected for sampling (days 1 and 8 post-dose). Approximately one quarter of the total liver sample for each fish (5 fish, per time point) was taken and pooled together for extraction. This procedure was also employed for kidney. For muscle, 10 g of sample from each fish per time point was taken and pooled together for extraction. For the skin, half of the total skin for each fish was taken and macerated with solid carbon dioxide and half of the macerated sample was used for extraction. The pooled samples were extracted with three successive portions of acetonitrile (7:3 v/w) at 80 ºC for 3 min. The acetonitrile was partitioned with hexane to remove fatty material, concentrated and analysed by HPLC. Chromatographic separation was achieved using a Lichrosorb RP18 (250 x 4.6 mm; 10 µm) column and a mobile phase of acetonitrile:water:trifluoroacetic acid under gradient elution. Detection was performed using a radiochemical detector and a UV detector (254 nm).

Chromatographic analysis of muscle, skin, liver and kidney tissues collected on days 1 and 8 provided determination of teflubenzuron and some metabolites (Table 7.6). The structures were confirmed by liquid chromatography coupled to mass spectrometry (LC-MS). The analysis of muscle and skin showed the presence of only the parent compound, teflubenzuron. In addition to teflubenzuron, the acetonitrile extracts of the liver showed the presence of three components (one unknown) on day 1. Only parent teflubenzuron and one unknown component were present on day 8. In addition to teflubenzuron, the acetonitrile extracts of the kidney showed the presence of five compounds on day 1 (three unknown); two of the unidentified compounds were more lipophilic in nature than the parent compound. Only parent teflubenzuron and one unknown were present in kidney extracts on day 8 (Table 7.6).

In a similar GLP study using repeated dosing, Atlantic salmon, *Salmo salar* L., (508-1297 g) were maintained at 10 °C in sea water (Auger *et al.*, 1996). Non-radiolabelled teflubenzuron was administered via medicated feed at a dose of 10 mg/kg bw for 6 consecutive days. On the seventh day, a dose of 10 mg/kg bw (1.85 MBq/kg; 50 µCi/kg) [^{14}C]-teflubenzuron was administered directly into the fish by intra-oesophageal intubation. Acetonitrile extracts of tissues samples taken on days 1 and 8 post-dose were analysed by HPLC and LC-MS to study the metabolism. The sample preparation and quantification of the compounds were the same as previously described.

Table 7.6. Compounds identified in the chromatograms (HPLC-radioactivity detector), with their respective concentrations and total radioactive residue in Atlantic salmon tissues sampled

1 and 8 days after single oral administration of [^{14}C]-teflubenzuron at a dose of 10 mg/kg bw (Auger *et al.*, 1995)**.**

Tissue	Compound	Day 1		Day 8	
		Concentration (µk/kg)	TRR (%)	Concentration (µk/kg)	TRR (%)
Muscle	Teflubenzuron	398	97.1	74	78.7
Skin	Teflubenzuron	749	99.5	108	58.1
Liver	Teflubenzuron	888	46.0	212	14.2
	3,5-dichloro-2,4-difluoroaniline	59	3.1		
	3'-hydroxy-teflubenzuron	64	3.3		
	Unknown	124	6.4	181	12.1
Kidney	Teflubenzuron	498	66.3	190	
	2'-hydroxy-teflubenzuron	34	4.55	-	
	3'-hydroxy-teflubenzuron	12	1.6	-	40.2
	Unknown 1	84	11.2	46	9.7
	Unknown 2	16	2.1	-	
	Unknown 3	22	2.9	-	

TRR: total radioactive residue.

Analysis of the acetonitrile extracts of the liver, kidney, muscle and skin from day 1 post-dose revealed that the major component detected was the parent compound; structural confirmation was by LC-MS. In the liver from day 1, three minor components were detected: 3'-hydroxydiflubenzuron and 3,5-dichloro-2,4-difluorophenyl urea and one unknown substance. In samples from day 8, teflubenzuron was detected in muscle and skin. Two minor components were also detected in the muscle but the structures were not elucidated.

Tissue residue depletion studies

Salmon

Several GLP-compliant depletion studies at a water temperature of 6 °C or 10 °C were evaluated to assess total [^{14}C]-teflubenzuron residues (total radiolabelled residues, TRR) or residues of teflubenzuron parent compound (marker residue, MR) from studies using non-radiolabelled drug in Atlantic salmon (*Salmo salar* L.) tissues. These studies included single oral and repeated doses of radiolabelled and/or non-radiolabelled teflubenzuron. A summary of these studies is shown in Table 7.7 and described in more detail in the following paragraph.

Studies 2 and 5 are correlated and were carried out at the same time using the same experimental design with the difference being that, in Study 2, fish received a single oral dose of [^{14}C]-teflubenzuron at Day 7 whereas, in Study 5, the same dose of non-radiolabelled teflubenzuron was administered. The same situation occurs for Studies 3 and 4.

Table 7.7. Summary of the residue depletion studies.

Study	Water temperature (°C)	Dose	Administration	Sampling Time post-dose
1*NTO/007	10 °C	Single oral dose of [^{14}C]-teflubenzuron (10 mg/kg bw; 1.85 MBq/kg).	Intra-oesophageal intubation	9 h, 1, 3, 4, 6, 8, 13 and 18 days
2*NTO/009	10 °C	Daily oral dose of teflubenzuron (10 mg/kg bw) for 6 days + single oral dose [^{14}C]-teflubenzuron (1.85 MBq/kg) on Day 7.	Medicated feed + intra-oesophageal intubation	1, 4, 8, 12 18, 24, 35, 50 and 120 days
3*NTO/013	6 °C	Daily oral dose of teflubenzuron (10 mg/kg bw) for 13 days + single oral dose [^{14}C]-teflubenzuron (1.80 MBq/kg) on Day 14.	Medicated feed + intra-oesophageal intubation	1, 8, 16, 24, 35, 50, 75 and 97 days
4*NTO/014	6 °C	Daily oral dose of teflubenzuron (10 mg/kg bw/day) for 13 days + single oral dose (10 mg/kg bw/day) on Day 14.	Medicated feed + intra-oesophageal intubation	1, 8, 16, 24, 35, 50[a], 75[a] and 97[a] days
5*NTO/010	10 °C	Daily oral dose of teflubenzuron (10 mg/kg bw) for 6 days + single oral dose of teflubenzuron (10 mg/kg bw/day) on Day 7.	Medicated feed + intra-oesophageal intubation	1, 4, 8, 12, 18, 24, 35, 50 and 120 days

*1- (Auger *et al*., 1995); 2 - (Auger *et al.*, 1996); 3 - (Auger & Bounds, 1996); 4 - (McGuire *et al.*, 1996a); 5 - (McGuire *et al.*, 1996b).[a]: samples not analysed.

Radiolabelled residue depletion studies

Salmon

All the following studies used the target species Atlantic salmon (*Salmo salar* L.) with a weight of approximately 1 kg and administration of teflubenzuron with purity higher than 97%. The compound was radiolabelled with carbon-14 to form radiolabelled test materials: [^{14}C]-benzoyl-CME 134 with purity >99% and [^{14}C]-aniline-CME 134 with purity >98%. The purity of both forms was confirmed by HPLC. The test material was prepared with equal amounts of the two radioactive forms of teflubenzuron.

In all studies, the total radioactive residue (TRR) was determined using liquid scintillation counting, either by (i) direct solubilization of tissues using Soluene-350, followed by decolourization using hydrogen peroxide and mixing with scintillation fluid before analysis; or (ii) solubilization of the skin samples using 2 mol/L potassium hydroxide in methanol:water 1:1 v/v; or (iii) combustion of the gastro-intestinal content and tank effluent and mixing with scintillation fluid.

Study 1 – NTO/007

In the first GLP-compliant depletion study (Auger *et al.*, 1995), conducted at a water temperature of 9.9 ± 0.2 °C, [^{14}C]-teflubenzuron at an intended dose equivalent to 10 mg/kg bw (1.85 MBq/kg; 50 µCi/kg) (actual dose 9.45 ± 0.15 mg/kg bw) was administered to Atlantic salmon (537 to 999 g) by intra-oesophageal intubation. In the study, 57 fish, approximately 24 months of age (33 females and 24 males), were housed in two tanks at a stocking density lower than 25 kg/m^3. Six fish were sampled at each of the following intervals: 9 h, and 1, 3, 4, 6, 8, 13 and 18 days post-dosing. Samples (mucus, liver, kidney, muscle, skin and gall bladder) were collected and the TRR was determined using liquid scintillation spectrometry.

Total recovered radioactivity on Day 1 post-dose in the acetonitrile extract was 98.6% (muscle), 103.8% (skin), 59.5% (liver) and 95.5% (kidney). The calculated marker to total residue ratios in the edible tissues are shown in Tables 7.8 and 7.9.

Table 7.8. Concentrations (µg/kg) of total radioactive residues (TRR) and teflubenzuron residues (Marker Residue, MR) in muscle and skin of Atlantic salmon dosed with [^{14}C]-teflubenzuron at 10 mg/kg bw and held in sea water at 10 °C (Auger *et al.*, 1995).

Time	Muscle			Skin		
post-dose (days)	TRR (µg/kg)	MR (µg/kg)	MR/TRR ratio (%)	TRR (µg/kg)	MR (µg/kg)	MR/TRR ratio (%)
1	410	404	98.6	753	782	103.8
8	93.6	79	84.0	185	143	77.1

Table 7.9. Concentrations (µg/kg) of total radioactive residues (TRR) and teflubenzuron residues (Marker Residue, MR) in liver and kidney of Atlantic salmon dosed with [^{14}C]-teflubenzuron at 10 mg/kg bw and held in sea water at 10 °C (Auger *et al.,* 1995).

Time	Liver			Kidney		
post-dose (days)	TRR (µg/kg)	MR (µg/kg)	MR/TRR ratio (%)	TRR (µg/kg)	MR (µg/kg)	MR/TRR ratio (%)
1	1930	1149	59.5	752	718	95.5
8	1490	543	36.4	473	257	54.4

The residues remaining after acetonitrile extraction, for the liver at 1 and 8 days post treatment and the kidney at 8 days post treatment, were further treated by hydrolysis under acidic or basic conditions. Acidic hydrolysis released very little of the remaining radioactivity, whereas basic hydrolysis released 44% and 58% of the activity of the residues in the liver at 1 and 8 days post-dose, respectively, and 97% of the residues in the kidney at 8 days post-dose.

The changes in concentrations of radioactivity in tissues from Atlantic salmon with time are presented in Table 7.10. The highest concentration of radioactivity in muscle (410 ± 89.0 µg eq/kg) and skin (753 ± 224 µg eq/kg) was found 1 day after administration of the drug. The concentrations decreased with elimination half-lives of 4.7 and 6.5 days for muscle and skin, respectively. The highest concentrations of radioactive material were found in the tissues associated with metabolism and excretion. The maximum concentration was determined in gall bladder (119000 ± 31500 µg eq/kg) at 2 days and is assumed to be associated with the bile in this tissue. The concentration in the liver was at a maximum (2500 ± 538 µg eq/kg) at 9 hours after administration and decreased to 1060 ± 319 µg eq/kg at 18 days post-dose. The relatively slow rate of elimination from the liver and gall bladder (half-lives of 16.5 and 7.1 days, respectively) is an indicator of the process of enterohepatic recirculation.

Table 7.10. Mean concentration (six fish at each time) of radioactivity in tissues from Atlantic salmon with time after a single oral dose equivalent to 10 mg/kg bw (1.85 MBq/kg) of [^{14}C]-teflubenzuron (Auger *et al.*, 1995).

Time post-dose	Mean concentration of radioactivity (µg eq/kg) ± SD					
	Liver	Kidney	Muscle	Skin	Mucus	Gall bladder
9 hours	2500 ±538	656 ±103	266 ±61.2	395 ±82.5	93.2 ±85.2	7750 ±8190
1 day	1930 ±451	752 ±156	410 ±89.0	753 ±224	87.8 ±63.3	70100 ±28100
2 days	2100 ±505	670 ±150	215 ±29.0	439 ±75.7	61.6 ±61.6	119000 ±31500
3 days	1830 ±377	558 ±68.9	184 ±29.2	301 ±52.4	62.0 ±59.4	118000 ±20500
4 days	1720 ±524	477 ±125	149 ±35.3	260 ±65.2	40.2 ±29.8	107000 ±57400
6 days	1400 ±344	459 ±127	102 ±17.1	187 ±21.4	5.8 ±8.9	129000 ±67500
8 days	1490 ±230	473 ±93.3	93.6 ±17.3	185 ±31.6	31.6 ±14.6	104000 ±48400
13 days	1050 ±290	314 ±102	37.0 ±9.0	89.3 ±22.6	22.2 ±7.0	41700 ±38200
18 days	1060 ±319	310 ±65.9	20.9 ±4.2	73.0 ±5.7	22.9 ±12.7	28300 ±11500

SD: standard deviation.

Study 2- NTO/009

In a similar depletion study (GLP-compliant), salmon (508 to 1297 g) were maintained at 10 °C and were treated with non-radiolabelled teflubenzuron in medicated feed, at a dose of 10 mg/kg bw, for 6 consecutive days. On the seventh day, a dose of 10 mg/kg bw [14C]-teflubenzuron (1.85 MBq/kg; 50 µCi/kg) was administered directly into the fish by intra-oesophageal intubation (Auger *et al.*, 1996).

The depletion was evaluated (six fish per time) at the sampling days 1, 4, 8, 12 18, 24, 35, 50 and 120 post-dose. The gastro-intestinal contents and the tank environment were also examined to evaluate the excretion of the radiolabelled drug. Only a small amount of radioactive material was distributed into the tissues examined, *i.e.*, the majority of material was excreted from the fish. The highest quantity of radioactive material (1.5 ± 0.6%, corresponding to 310 ± 124 µg eq/kg) was detected in the muscle 1 day after administration, with an initial half-life of elimination of 2.6 days over the period of 1 to 18 days. The drug was slowly eliminated from the muscle with a terminal half-life of 38.5 days over a period of 35 to 120 days, to a level of 1.1 ± 1.3 µg eq/kg by Day 120. The maximum concentration of [^{14}C]-teflubenzuron in skin was determined on day 1 (554 ± 178 µg eq/kg). The initial half-life of elimination from the skin, over the period 1 to 18 days was calculated to be 3.6 days. The terminal half-life of elimination from skin, over the period of 18 to 120 days was calculated to be 49.5 days.

Consistent with the results in *Study 1* (Auger *et al.*, 1995), the highest concentrations of radioactive material were in the tissues associated with metabolism and excretion. The highest concentrations were in the liver, with a maximum of 1880 ± 153 µg eq/kg on day 1. The concentration in liver decreased to 793 ± 153 µg eq/kg on day 8 with an initial half-life of elimination of 5.7 days (over the period of 1 to 8 days). Results are shown in Table 7.11.

Table 7.11. Mean concentration (six fish at each time) of radioactivity in tissues from Atlantic salmon with time after oral daily dose (medicated feed) of teflubenzuron (10 mg/kg bw) for 6 consecutive days followed by a single oral dose of [^{14}C]-teflubenzuron (1.85 MBq/kg) on day 7 via intra-oesophageal intubation. Water temperature of 10 ºC (Auger *et al.*, 1996).

Time post-dose (days)	Mean concentration of radioactivity (µg eq/kg) ± SD				
	Liver	Kidney	Muscle	Skin	Mucus
1	1880± 1110	651 ± 401	310 ± 124	55.4 ± 178	196 ± 165
4	1230 ± 686	239 ± 98.0	57.8± 18.6	122 ± 30.9	8.3 ± 9.3
8	793 ± 153	99.2± 28.2	14.6± 8.0	40.8 ± 13.1	ND
12	864 ± 268	135 ± 60.5	12.0± 5.1	33.7 ± 5.2	5.7 ± 9.4
18	1440 ± 434	217 ± 68.5	<LOD	16.3 ± 2.6	<LOD
24	765 ± 417	103 ± 51.3	<LOD	13.4 ± 3.2	ND
35	573 ± 95.6	64.1± 11.5	6.1± 2.3	8.4 ± 0.7	<LOD
50	522 ± 275	70.2± 36.7	<LOD	9.2 ± 1.5	<LOD
120	127 ± 16.3	25.7± 10.8	<LOD	<LOD	5.8 ± 5.3

SD: standard deviation. ND: not detected. Limit of detection: 5 µg eq/kg.

Total recovered radioactivity on day 1 post-dose in the acetonitrile extract was 95.6% (muscle), 83.4% (skin), 47.7% (liver) and 87.4% (kidney). The calculated marker to total residue ratios in the edible tissues are shown in Tables 7.12 and 7.13.

Table 7.12. Concentrations (µg/kg) of total radioactive residues (TRR) and teflubenzuron residues (Marker Residue, MR) in muscle and skin of Atlantic salmon after oral daily dose (medicated feed) of teflubenzuron (10 mg/kg bw) for 6 consecutive days followed by a single oral dose of [^{14}C]-teflubenzuron (1.85 MBq/kg) on day 7 via intra-oesophageal intubation. Water temperature of 10 ºC (Auger *et al.,* 1996).

Time post-dose (days)	Muscle			Skin		
	TRR (µg/kg)	MR (µg/kg)	MR/TRR ratio (%)	TRR (µg/kg)	MR (µg/kg)	MR/TRR ratio (%)
1	313	300	95.6	568	474	83.4
8	14	18	128.6	41	34	81.9

Table 7.13. Concentrations (µg/kg) of total radioactive residues (TRR) and teflubenzuron residues (Marker Residue, MR) in liver and kidney of Atlantic salmon after oral daily dose (medicated feed) of teflubenzuron (10 mg/kg bw) for 6 consecutive days followed by a single oral dose of [^{14}C]-teflubenzuron (1.85 MBq/kg) on day 7 via intra-oesophageal intubation. Water temperature of 10 ºC (Auger *et al.,* 1996)**.**

Time post-dose (days)	Liver			Kidney		
	TRR (µg/kg)	MR (µg/kg)	MR/TRR ratio (%)	TRR (µg/kg)	MR (µg/kg)	MR/TRR ratio (%)
1	1545	738	47.7	993	868	87.4
8	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

N.A.: not analyzed.

For the combined muscle and skin, the maximum concentration of radioactive material occurred on day 1 and decreased with an initial half-life of elimination of 3.8 days over the period 1 to 24 days. The terminal elimination half-life was 38.4 days over the period 35 to 120 days.

A summary of the pharmacokinetic parameters of radioactive material determined from the depletion data in this study is shown in Table 7.14.

Table 7.14. Pharmacokinetic parameters of radioactivity in tissues from Atlantic salmon with time after oral daily dose (medicated feed) of teflubenzuron (10 mg/kg bw) for 6 consecutive days followed by a single oral dose of [^{14}C]-teflubenzuron (1.85 MBq/kg) on day 7 via intra-oesophageal intubation. Water temperature of 10 ºC (Auger *et al.,* 1996)**.**

	Initial Elimination Phase			Terminal Elimination Phase		
Tissue	**Time, range (d)**	**Initial Half-life (d)**	**Rate constant (d^{-1})**	**Time, range (d)**	**Initial Half-life (d)**	**Rate constant (d^{-1})**
Liver	1-8	5.7	-0.12	24-120	38.5	-0.02
Kidney	1-8	2.6	-0.27	24-120	49.5	-0.01
Muscle	1-18	2.6	-0.26	35-120	38.5	-0.02
Skin	1-18	3.6	-0.19	18-120	49.5	-0.01

It is postulated that there is a biphasic elimination of radioactive material from the tissues. The initial elimination half-lives are between 2.6 and 5.7 days long. The terminal elimination half-lives are between 38.5 and 49.5 days long, which is attributable to the binding of the test material to the tissue matrix

This result is corroborated with the high level of radioactive material (> 95% in liver) that is unextractable from the tissues after 50 days post-dose.

Study 3 – NTO/013

A third depletion study was carried out using a similar experimental design and analytical methods as described in Study 2, using a water temperature of 6 °C instead of 10 °C (Auger & Bounds, 1996). In this study, Atlantic salmon (527 to 1403 g) were fed non-radiolabelled teflubenzuron in the diet for thirteen days at a dose of 10 mg/kg bw. On the fourteenth day the fish were not fed but received a dose equivalent to 10 mg/kg bw of [^{14}C]-teflubenzuron (50.3 ± 0.70 µCi/kg) by oral intubation. Tissues were collected after 1, 8, 16, 24, 35, 50, 75 and 97 days post-treatment. In this study, a background level of teflubenzuron was detected in the closed re-circulating water system, but it was concluded that this did not affect the validity of this study in terms of determining the metabolism and initial rate of depletion (McGuire *et al.,* 1996b). Very little systemic tissue absorption of radioactive material was observed following the final dose. The highest recovery of radioactivity was determined in muscle on Day 1 (0.7% ± 0.2% of the radiochemical dose). All other tissues analysed contained less than 0.1% of the radiochemical dose 8 days following administration. Liver contained the highest concentration of radioactive material, with a maximum of 1170 ± 336 µg eq/kg on day 1, which decreased with an initial elimination half-life of 16.9 days over the period of 1 to 24 days. It was not possible to determine the terminal elimination half-life for residues in liver due to variations in tissue concentrations after day 24. For muscle and skin, the maximum concentrations occurred at 1 day following the final dose (153 ± 40 µg eq/kg for the muscle and 218 ± 83 µg eq/kg for

the skin). The initial elimination half-lives were 3.8 days for muscle and 5.7 days for (skin, respectively, over the period from 1-24 days. The terminal elimination half-life for the skin was 99 days over the period of 27 to 97 days.

The change in concentrations of radioactivity in tissues with time is shown in Table 7.15. The Committee noted that some values provided by the sponsor (marked with * at Table 7.15) are below the limit of detection of the method.

Table 7.15. Mean concentration ± SD (six fish at each time) of radioactivity in tissues from Atlantic salmon with time after oral daily dose (medicated feed) of teflubenzuron (10 mg/kg bw) for 13 consecutive days followed by a single oral dose of [^{14}C]-teflubenzuron (1.85 MBq/kg) on day 14 via intra-oesophageal intubation at a water temperature of 6 ºC (Auger & Bounds, 1996).

Time post-dose (days)	Mean concentration of radioactivity (µg eq/kg) ± SD				
	Liver	Kidney	Muscle	Skin	Mucus
1	1170± 336	328 ± 74.5	153 ± 39.7	218 ± 82.6	8.6 ± 5.5
8	722 ± 181	114 ± 49.1	16.5 ± 6.0	28.5 ± 12.7	0.9* ± 2.3
16	512 ± 204	89.2 ± 31.1	7.5 ± 6.8	16.4 ± 6.9	2.2* ± 3.5
24	455 ± 192	61.3 ± 36.6	1.7* ± 4.2	10.0 ± 1.9	ND
35	655 ± 492	81.9 ± 57.5	ND	10.9 ± 4.9	ND
50	334 ± 122	53.4 ± 9.9	5.9* ± 3.2	8.7 ± 2.7	ND
75	328 ± 100	46.0 ± 22.4	ND	7.2 ± 4.3	ND
97	340 ± 161	31.6 ± 20.4	ND	6.1 ± 1.7	ND

SD: standard deviation. ND: not detected. * Limit of detection: 6 µg eq/kg.

The radiolabelled studies indicated that the main residue in muscle and skin is the parent compound and that the excretion of teflubenzuron is predominantly via faeces.

Residue depletion studies with non-radiolabelled drug

Salmon

Residues of teflubenzuron in salmon tissues in the following studies were quantified by a validated HPLC-UV method with a limit of quantification for the determination of teflubenzuron in muscle and skin of 20 µg/kg (McGuire, 1995). A full evaluation of the method is provided in "Methods of analysis for residues in tissues". Briefly, the samples (approx. 3 g) were extracted with hot acetonitrile and the solvent volume was reduced on a rotary evaporator. The remaining extract was diluted with dichloromethane, reduced by evaporation, then washed with water. The final organic extract was evaporated to dryness at 50 °C and the residue was then re-suspended in 5% diethyl ether in hexane before clean-up on silica and C8 solid phase

extraction cartridges. The quantification of teflubenzuron was carried out by HPLC with a UV detector at 254 nm. The LOQ's for muscle and skin were 20 and 50 µg/kg, respectively.

Study 4 – NTO/014

In a GLP-compliant repeat dose study (McGuire *et al*., 1996a), Atlantic salmon (527 to 1403 g) kept at a water temperature of 6 °C were fed with a diet containing teflubenzuron for thirteen days at an intended dose of 10 mg/kg bw (actual dose of 9.76 mg/kg bw). On the fourteenth day the fish were not fed but were treated with the same dose of teflubenzuron by oral intubation. Tissues were collected 1, 8, 16, 24, 35, 50, 75 and 97 days post treatment, however the samples collected on days 50, 75 and 97 post-dose were not analysed. In this study, a background level of teflubenzuron in the closed re-circulating water system was detected but it was concluded that this did not affect the validity of this study to determine the initial rate of drug depletion.

The results of the analysis of skin and muscle with time are shown in Table 7.16. The recoveries of teflubenzuron in fortified blank skin and muscle samples were in the range of 70 to 129% and 73 to 104%, respectively.

Table 7.16. Mean concentration ± SD (ten fish at each time) of teflubenzuron in tissues from Atlantic salmon with time after oral daily dose (medicated feed) of teflubenzuron (10 mg/kg bw) for 13 consecutive days followed by a single oral dose of teflubenzuron (10 mg/kg bw) on day 14 via intra-oesophageal intubation; water temperature 6 ºC (McGuire *et al.*, 1996a).

Time post-dose (days)	Mean concentration ± SD of Teflubenzuron (µg/kg)		
	Skin	Muscle	Muscle + Skin
1	443 ± 211	405 ± 176	407 ± 155
8	106 ± 32	63 ± 27	67 ± 26
16	54 ± 33	45 ± 7	46 ± 8
24	62 ± 17	41 ± 19	42 ± 17
35	44± 9	23 ± 4	25 ± 4

The initial half-life of elimination calculated from the residue data from days 1 to 16 in the combined tissues muscle and skin was 4.8 days (EMEA, 1999).

Study 5 – NTO/010

In a GLP-compliant repeated oral dose residue study (McGuire *et al.*, 1996b) conducted at a water temperature of 10 ± 1 °C, Atlantic salmon (508 to 1297 g; 25 male and 29 female) were treated with teflubenzuron medicated feed (5.7 g of medicated diet/kg bw, corresponding to 9.46 mg of teflubenzuron per kg bw; intended dose 10 mg/kg bw) over a seven day period. One fish per group received a single oral dose of 10 mg/kg bw teflubenzuron by intra-oesophageal intubation on feeding Day 7. Samples of muscle and skin (3 g) were collected on days 1, 4, 8, 12, 18, 24, 35, 50 and 120 post-dose. The concentrations of teflubenzuron in muscle and skin

were determined using a validated HPLC-UV method (McGuire, 1995). Recoveries for muscle and skin ranged from 75-391% and 70-187% of the administered dose, respectively. The depletion results are presented in Table 7.17. Values lower than LOQ of 20 µg/kg in muscle and skin were considered in the reported values as 20 µg/kg for calculations of the average values with their respective standard deviations.

Table 7.17. Mean concentration ± SD (ten fish at each time) of teflubenzuron in tissues from Atlantic salmon with time after oral daily dose (medicated feed) of teflubenzuron (10 mg/kg bw) for 6 consecutive days followed by a single oral dose of teflubenzuron (10 mg/kg bw) on day 7 via intra-oesophageal intubation; water temperature 10 ºC (McGuire *et al.*, 1996b)**.**

Time after treatment (days)	Mean concentration ± SD of Teflubenzuron (µg/kg)		
	Skin	Muscle	Muscle and skin*
1	1310 ± 436	894 ± 501	932 ± 475
4	353 ± 316	329 ± 206	331 ± 213
8	221 ± 229	103 ± 52	116 ± 64
12	86 ± 42	52 ± 23	56 ± 22
18	50 ± 12	26 ± 9	29 ± 8
24	39 ± 20	28 ± 16	29 ± 16
35	43 ± 13	37 ± 17	38 ± 16

* The combined concentrations of teflubenzuron in muscle and skin were obtained using the following equation:

$$\text{Muscle+skin} = \frac{\left(\text{Conc. Teflubenzuron}_{\text{muscle}} \times \text{weight}_{\text{muscle}}\right) + \left(\text{Conc. Teflubenzuron}_{\text{skin}} \times \text{weight}_{\text{skin}}\right)}{\text{Total weight}_{\text{muscle+skin}}}$$

The initial half-life of elimination calculated from the residue data from days 1 to 18 in the combined muscle and skin was 3.4 days. The tissue concentrations appeared to reach a plateau of 30 to 40 µg/kg at 24 to 35 days post treatment. This has been attributed to the background level of teflubenzuron which was found in the test system (EMEA, 1999).

A summary of the pharmacokinetic parameters obtained in the depletion studies (*Study 1, Study 2, Study 3, Study 4 and Study 5*) is presented in Table 7.18.

Table 7.18. Elimination rate constants and half-lives of elimination obtained from the total radioactive depletion and residue Studies 1 to 5 (O'Connor, 1996).

		Initial Elimination Phase			Terminal Elimination Phase		
Study	**Tissue**	**Time range (d)**	**Initial half-life(d)**	**Rate constant (d^{-1})**	**Time range (d)**	**Initial half-life(d)**	**Rate constant(d^{-1})**
1	Liver	1-18	16.5	-0.04	--	--	--
	Kidney	3-18	17.4	-0.04	--	--	--
	Muscle	2-18	4.7	-0.15	--	--	--
	Skin	2-18	6.5	-0.11	--	--	--
	Muscle and skin	1-18	4.6	-0.15	--	--	--
	Mucus[a]	0.375-18	8.4	-0.08	--	-	--
	Gall bladder	2-18	7.1	-0.10	--	--	--
2	Liver	1-18	5.7	-0.12	24-120	38.5	-0.02
	Kidney	1-18	2.6	-0.27	24-120	49.5	-0.01
	Muscle	1-18	2.6	-0.26	35-120	38.5	-0.02
	Skin	1-18	3.6	-0.19	18-120	49.5	-0.01
	Muscle and skin	1-24	3.8	-0.18	35-120	38.4	-0.02
3	Liver	1-18	16.9	-0.04	*	*	*
	Kidney	1-24	10.0	-0.07	35-97	49.5	-0.01
	Muscle	1-24	3.8	-0.18	**	**	**
**	Skin	1-24	5.5	-0.13	24-97	99.0	-0.01
	Muscle and skin	1-35	4.9	-0.14	**	**	**
4	Muscle	1-16	4.8	-0.14	--	--	--
	Skin	1-16	5.0	-0.14	--	--	--
	Muscle and skin	1-16	4.8	-0.14	--	--	--
5	Muscle	1-18	3.4	-0.21	--	--	--
	Skin	1-18	3.8	-0.18	--	--	--
	Muscle and skin	1-18	3.4	-0.20	--	--	--

-- Insufficient data to determine terminal elimination phase.

[a] Day 6 was not included for mucus as data were considered as outliers.

* Data variation too high to determine the terminal elimination phase.

**Terminal elimination phase not observed.

Teflubenzuron residues depleted in muscle and skin with different half-lives depending on the water temperature. Peak residue concentrations were higher in the experiment performed at 10 ºC than in the experiment at 6 ºC, however the initial rates of depletion of tissue residues were similar. The slow terminal phase of elimination was attributed to background levels of teflubenzuron in the recirculated sea water in the tanks were the fish were maintained.

Methods of analysis for residues in tissues

Due to the physical and chemical properties of teflubenzuron (*i.e.*, its polarity and low volatility), liquid chromatography is the method of choice for the determination of drug residues in food, feed and biological matrices.

Most protocols use solvent extraction of teflubenzuron from the sample followed by clean-up steps, including solid phase extraction procedures and more recently the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approach (Anastassiades *et al.*, 2003). Pressurized liquid extraction (PLE) has also been employed (Brutti *et al.*, 2010). Chromatographic separation is commonly performed using reverse-phase chromatography. For the quantification of teflubenzuron, UV and, more recently, tandem mass spectrometry detectors have been employed. In the latter case, electrospray ionization is usually employed in the positive ion mode using acquisition of ions in the selected reaction-monitoring mode (SRM). The Committee assessed the validation data against the analytical requirements as published in the Codex guidelines for analytical methods for residue control, CAC/GL 71-2009 (FAO/WHO, 2014).

Quantitative methods

Liquid chromatography

A validated single-residue method, using high performance liquid chromatography (HPLC) coupled to ultraviolet (UV) detection was used in depletion studies carried out twenty years ago for the determination of teflubenzuron in salmon tissues (McGuire, 1995). Teflubenzuron was extracted from the tissues (3 g) using acetonitrile (3 x 7 mL) at 80 °C. The combined acetonitrile extracts were reduced to approximately 2 mL on a rotary evaporator at 50 °C. The extract was diluted in dichloromethane (approximately 10 mL) and the volume was reduced again to 2 mL. The remaining dichloromethane extract was quantitatively diluted to 10 mL with dichloromethane. An aliquot of 5 mL of this extract was diluted with dichloromethane (20 mL) and the organic phase partitioned with 3 x 50 mL of water. The aqueous phase was back-partitioned with 25 mL dichloromethane. The organic extract was reduced to dryness and the residue re-dissolved in 5 mL of diethyl ether:hexane, 5:95 v/v. Finally, the extract was cleaned-up by solid phase extraction, first using a silica cartridge (500 mg), followed by a C8 cartridge (500 mg). The eluate (1 mL) was transferred to a vial and analysed by HPLC-UV at 250 nm or 254 nm. For the chromatographic separation a Supelcosil LC-ABZ column (25 cm x 4.6 mm) column at 28 °C and a mobile phase containing methanol:acetonitrile:water 60:20:20 v/v/v was employed. The external calibration curve covered the concentration range of 0.02 to 1.0 µg/mL (corresponding to 20 µg/kg to 1000 µg/kg) with a linearity of 0.9950. The recoveries of teflubenzuron were >70% and the limit of quantification was 20 µg/kg in salmon skin and muscle.

Confirmatory methods

Liquid chromatography – tandem mass spectrometry (LC-MS/MS)

Liquid chromatography coupled to tandem mass spectrometry, using electrospray ionization (ESI), has been widely used as technique for the determination of residues of teflubenzuron in food and biological matrices due to its selectivity. However, in order to overcome matrix suppression, matrix-matched standards are mandatory.

The FDA monitors teflubenzuron in salmon tissues using a multi-residue pesticide monitoring procedure, described in the FDA Laboratory Information Bulletin 4463 (Chamkasem *et al.*,

2010). The sample preparation is a modification of the QuEChERS approach developed by Anastassiades and co-workers (Anastassiades *et al.*, 2003) without using the dispersive sample clean-up step. Briefly, salmon tissue (10 g) is shaken with a mixture of 5 mL water and 15 mL acetonitrile in the presence of 1.5 g NaCl and 6 g $MgSO_4$. The mixture is centrifuged, and a volume of 600 µL of the extract is added of the same volume of a solution of 4 mmol/L ammonium formate and 0.1% formic acid. The solution is mixed and filtered through a 0.2 µm PVDF syringe filter. The filtrate is injected into the LC-MS/MS system, using the electrospray ionization source in the negative mode. The chromatographic separation is carried out on a reversed phase C18 column (Restek Ultra Aqueous, 100 x 2.1 mm; 3 µm), at 50 °C, and a mobile phase of ammonium formate and formic acid under gradient elution. Teflubenzuron has a precursor ion at 379 *m/z* and two product ions at 339.1 *m/z* and 358.9 *m/z*. The two product ions were used for quantification and confirmation purposes. Concentrations in salmon muscle and skin were determined by external calibration curves (standards in acetonitrile); i.e., without using an internal standard. The linear range of 0.1 to 100 ng/mL corresponds to 0.3 to 300 µg/kg of teflubenzuron in tissues. The limit of quantification was estimated at a signal to noise ratio ≥ 10.

The method validation parameters are presented in Table 7.19. Analysis of incurred residues in salmon was also carried out. Salmon were fed by oral gavage with a single dose of teflubenzuron at 20 mg/kg bw. Four fish were sacrificed at 24 h and 48 h after feeding and average concentrations of teflubenzuron in muscle tissue with skin of 4.4 µg/kg and 16.4 µg/kg were determined, respectively.

Table 7.19. Validation parameters of the LC-MS/MS method for the determination of teflubenzuron in salmon tissues (Chamkasem *et al.,* 2010)**.**

Parameter Assessed	Muscle with skin Fortified samples	 Incurred samples
Intraday accuracy (% bias)		
Intraday precision (% CV)	6.02% (1 µg /kg) 1.64% (10 µg /kg) 3.31% (100 µg /kg)	 3.2% (4.4 µg/kg)[a] 2.2% (16.4 µg/kg)[b]
Accuracy	65.9% (1 µg /kg) 88.4% (10 µg /kg) 101.4% (100 µg /kg)	
Estimated LOQ (µg/kg)	0.3	
Analytical range (ng/mL)	0.1 -100	
Linearity (r)	0.9995	
Selectivity	No interference observed	
Extraction recovery (n=6)	65.9 (1 µg/kg) 88.5 (10 µg/kg) 101.37 (100 µg/kg)	

a: 24 h post feeding (n=4); b: 48 post feeding (n=4)

A method using pressurized liquid extraction (PLE) for the extraction of benzoylureas, including teflubenzuron, in animal products (milk, eggs and meat) has been developed and validated (Brutti *et al.,* 2010). Quantification was carried out by LC-MS/MS using an ion trap (IT) mass analyser and an APCI source. Sample test portions (5 g) were homogenized with diatomaceous earth (4-5 g) and extracted with 22 mL ethyl acetate at 80 °C and 1500 psi. The extract was concentrated to 1 mL at 40 °C, transferred quantitatively to another flask using 2 x 2.5 mL methanol and evaporated to dryness. The residue was reconstituted in methanol. The method presented linearity (r) higher than 0.99. Recoveries at a fortification level of 10 µg/kg were 78% (RSD, 16%), 84% (RSD, 8%) and 90% (RSD 10%) for milk, eggs and beef meat, respectively. The limit of quantification was in the range of 2 to 10 µg/kg.

A simple and fast method for the determination and monitoring of occurrence of eight pesticides, including teflubenzuron, in fish and shellfish by matrix solid-phase dispersion (MSPD) with anhydrous sodium sulphate and C18 as dispersants, silica as an adsorbent and LC-MS/MS quantification has been reported (Carro *et al.*, 2012). Sample test portions (0.2 g) were blended with 0.5 g sodium sulphate anhydrous and 2 g of C18. The mixture was transferred to a 6 mL SPE empty cartridge and 2 g of silica was added at the top. Acetic acid:acetonitrile, 5:95 v/v, was used to elute the analytes. The quantification was performed by LC-MS/MS with an ESI interface, which was operated simultaneously in the positive and negative mode. For the chromatographic separation, a Hypersil ODS (100 mm x 3.2 mm, 3 µm particle size) column at 30 °C and a mobile phase 5 mM ammonium acetate in acetonitrile were used. Linearity was evaluated using matrix-matched standards in the range of 5 to 500 µg/kg. Linearity was demonstrated, with a correlation coefficient > 0.996 and intra-day precision

(n=6) was 5.8%. A matrix effect of 18.1% was verified. The limits of detection and quantification for teflubenzuron were 1.5 µg/kg and 4.7 µg/kg, respectively.

Appraisal

Teflubenzuron has not been previously reviewed by the Committee, but was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), in 1994, when an ADI of 0-0.01 mg/kg bw per day was established based on the dose-related effects in the liver and derived from the 18-month carcinogenicity study in mice.

Teflubenzuron is an acyl urea insecticide registered for aquaculture use in the treatment of Atlantic salmon at a maximum dose of 10 mg per kg fish for seven days, administered through feed (pelleted diet at a level of 2 g/kg), for control of infestation of sea lice. It is also used in agriculture to control a wide range of insect pests.

Teflubenzuron residues depleted in muscle and skin with different half-lives, depending on the water temperature. Peak residue concentrations were higher in the experiment performed at 10 ºC than in the experiment at 6 ºC, however the initial rates of depletion of tissue residues were similar.

Metabolism data are available for a variety of animal species, including rats, goats, chicken and salmon. Teflubenzuron is predominantly unmetabolized and biliary excretion is the main path for elimination. The metabolic profiles are similar throughout animal species.

Metabolic profiling in salmon was available. Two studies were carried out following single or repeated dose administration of radiolabelled teflubenzuron to salmon. Unchanged teflubenzuron was the major component in liver and kidney. Minor metabolites (less than 5%) were identified in salmon liver and kidney: 3,5-dichloro-2,4-difluoroaniline, 2'-hydroxy-teflubenzuron, 3'-hydroxy-teflubenzuron and 3,5-dichloro-2,4-difluorophenyl urea. Some metabolites remained unknown. In salmon muscle and skin only teflubenzuron was identified.

Radiolabelled data are available for the depletion of teflubenzuron residues in salmon at a water temperature of 10 ºC, following single or repeated dose. Teflubenzuron was identified as the marker residue in salmon muscle and skin. Based on the results of these two studies, the Committee identified teflubenzuron as the marker residue in salmon muscle and skin and determined that a value of 0.8 was appropriate for the MR:TRR. This value was the mean value of the MR:TRR of muscle and skin determined 8 days post last dose of teflubenzuron at a water temperature of 10 ºC, excluding the value of 1.28 that was considered an outlier.

The highest concentration (less than 1000 µg/kg) of teflubenzuron in salmon muscle and skin occurs 1 day after administration of the drug.

The residue depletion studies in salmon were conducted in the mid 90's using HPLC-UV methods, which required complex sample preparation procedures for extraction and clean-up. The limit of quantification (LOQ) was 20 µg/kg in salmon muscle and skin. The state-of-the-art methods (LC-MS/MS) use simpler sample preparation procedures, based on the QuEChERS approach, and have a LOQ about of 0.3 µg/kg.

The recommended MRL of 400 µg/kg of teflubenzuron in fillet (muscle with skin in natural proportions) and salmon muscle was based on a withdrawal period of 96 degree days and

calculated on the basis of the upper limit of the one-sided 95% confidence interval over the 95th percentile (UTL 95/95) of residue concentrations in salmon muscle and skin derived from the pivotal residue depletion study, conducted at a water temperature of 10 ºC, from 1 to 18 days after treatment. The tolerance limits for teflubenzuron residues in salmon muscle and skin are shown in Figure 7.3.

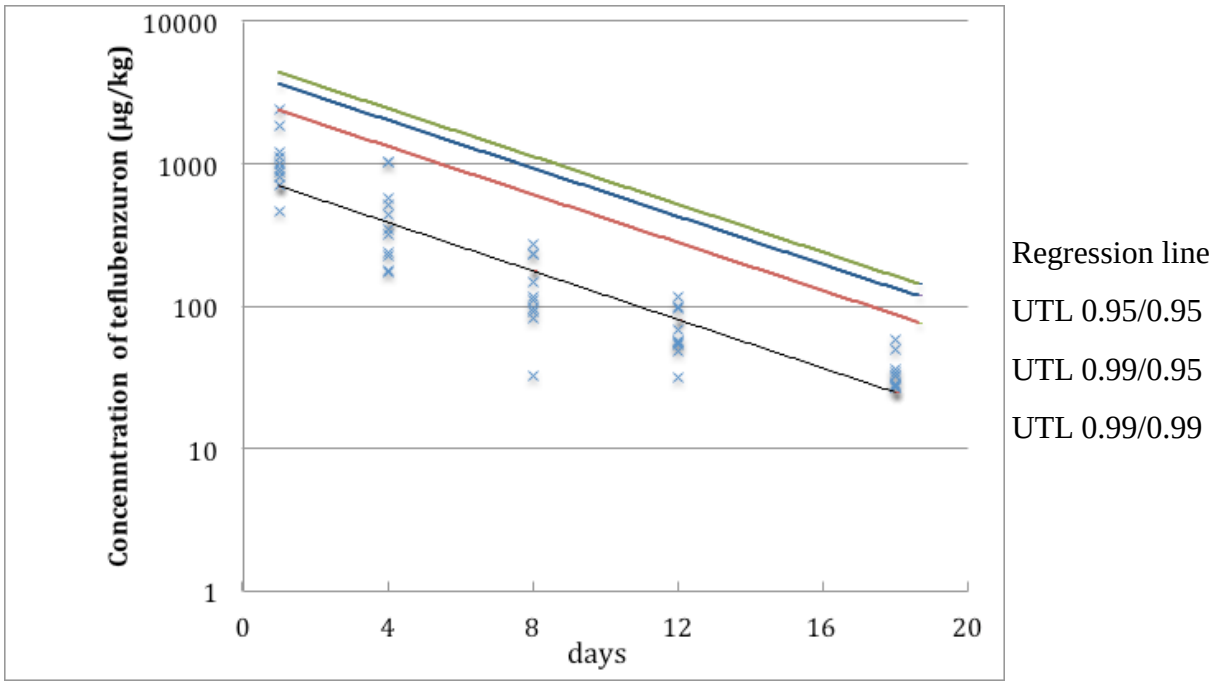


Figure 7.3. Tolerance limit considerations for teflubenzuron in salmon muscle with skin.

Dietary exposure assessment

The ADI of 0-5 µg/kg bw for teflubenzuron established by the Committee was based on a chronic effect, so a GEADE was not determined. In this dietary exposure assessment, fish was the only contributor to dietary exposure.

The EDI for teflubenzuron was calculated based on median residues found in salmon muscle and skin (water temperature 10 ºC) at 8 days post-dosing, with an associated ratio of the concentration of marker residue to the concentration of total residue of 80%. The median (n = 10) calculated 8 days after treatment was used as input into the chronic dietary exposure estimates (Table 7.20).

Table 7.20. Median concentrations of teflubenzuron residues in salmon skin and muscle at a water temperature of 10 ºC.

Time post dosing (days)	Concentration of marker residue in salmon muscle and skin µg/kg										
	animal 1	animal 2	animal 3	animal 4	animal 5	animal 6	animal 7	animal 8	animal 9	animal 10	Median
1	813	882	465	1123	1021	712	946	1862	1226	2448	984
4	352	177	180	577	450	321	243	519	226	1045	337
8	237	83	32	92	99	236	116	148	112	273	114
12	101	55	117	57	56	49	69	57	32	98	57
18	29	58	50	35	37	33	27	28	27	33	33

The estimated dietary exposure expressed as the EDI was 42.9 µg/person/day, which represents 14% of the upper bound of the ADI of 0-5 µg/kg bw/day (or 300 µg/person/day) (Table 7.21).

Table 7.21. The Estimated Dietary Intake of teflubenzuron residues from salmon muscle.

Tissue	Median concentration*(µg/kg)	Standard Food Basket (kg)	MR:TR ratio[1]	Daily intake (µg/person/day)
Muscle	114	0.3	0.8	42.9
TOTAL				42.9

Using the median residue in salmon muscle and skin and fish consumption as inputs, the GECDE for the general population was 1.6 µg/kg bw/day, which is equivalent to 31% of the upper bound of the ADI. The higher exposure estimate compared to the EDI was due to the higher consumption of fish used in the GECDE, 655 g/person compared 300 g of muscle (fish) used in the EDI model diet (Table 7.22).

In children, the GECDE was 2.1 µg/kg bw/day which represents 43% of the upper bound of the ADI. This estimate was higher than the whole population estimate. While the fish consumption amount is lower than for adults and the model diet, the lower bodyweight of children leads to comparatively higher exposure on a bodyweight basis. Exposure of infants was estimated to be lower at 0.9 µg/kg bw/day (18% of the upper bound of the ADI) because fish consumption of infants is only 10% of the consumption amount used in the model diet.

Table 7.22. The Global Estimate of Chronic Dietary Exposure (GECDE) to teflubenzuron residues in salmon muscle for the general population, children and infants.

Category	Type	Mean consumption[1] whole population, g/d	97.5th consumption[2] consumers only, g/d	Exposure µg/kg bw/day		GECDE[3] µg/kg bw/day	ADI %
				Mean	97.5th		
General Population							
Fish and seafood	Fish	27	655	0.06	1.56	1.56	31.1
TOTAL				0.0	1.6	1.6	31
Children							
Fish and seafood	Fish	24	226	0.22	2.15	2.15	42.93
TOTAL				0.0	2.0	2.0	43
Infants							
Fish and seafood	Fish	1	33	0.04	0.92	0.92	8.8
TOTAL				0.0	0.9	0.9	18

[1]highest mean consumption figures based on whole population considered from the available dataset
[2]highest 97.5th food consumption figures based on consumers only considered from the available dataset
[3]GECDE is the sum of the highest exposure at the 97.5th percentile of consumption for a food and the mean dietary exposures of the other foods.

Maximum Residue Limits

In recommending MRLs for teflubenzuron in salmon, the Committee considered the following factors:

- Teflubenzuron is authorized for use in salmon in several countries. The maximum recommended dose is 10 mg/kg fish per day for 7 consecutive days, administered through medicated feed. The withdrawal periods range from 7 to 11 days and from 45 to 96 degree-days.
- An ADI of 0-5 µg/kg bw for teflubenzuron was established by the Committee.
- Teflubenzuron is the marker residue in tissues.
- The ratio of the concentration of marker residue to the concentration of total residue of 0.8 was calculated in muscle and skin in natural proportion of salmon. Residue data were provided using a validated analytical method to quantify teflubenzuron in salmon tissues.
- A validated analytical method for the determination of teflubenzuron in edible salmon tissues is available in the literature and may be used for monitoring purposes.

The MRLs were calculated on the basis of the upper limit of the one-sided 95% confidence interval over the 95th percentile of total residue concentrations (95/95 UTL) in salmon muscle and skin derived from the pivotal study used for this assessment, conducted at a water temperature of 10 °C and a withdrawal period of 96 degree-days (10 days).

The Committee recommended MRLs for teflubenzuron in salmon of 400 µg/kg in fillet (muscle plus skin in natural proportion) and in muscle.

The EDI is 42.9 µg/person per day, on the basis of a 60 kg individual, which represents approximately 14% of the upper bound of the ADI.

The GECDE for the general population is 1.6 µg/kg bw per day, which represents 31% of the upper bound of the ADI; for children, 2.1 µg/kg bw per day, which represents 43% of the upper bound of the ADI; and for infants, 0.9 µg/kg bw per day, which represents 18% of the upper bound of the ADI.

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8. Zilpaterol hydrochloride

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Addendum to the monograph prepared by the seventy-eighth meeting of the Committee and published in the series FAO JECFA Monograph 15.

Background

The 78th meeting of the Committee, at the request of the 21st Session of CCRVDF (FAO/WHO, 2014a), evaluated zilpaterol HCl and established an ADI of 0–0.04 µg/kg bw on the basis of a LOAEL for a slight increase of tremor in humans in a single dose study (FAO/WHO, 2014b).

The 78th meeting of the Committee also agreed that parent zilpaterol was an appropriate marker residue in muscle. Only limited data were available for tissues other than muscle, and the Committee was unable to determine a suitable marker residue in other edible tissues. Liver and kidney contained the highest concentration of zilpaterol at all sampling times, followed by muscle. The ratios of the concentration of zilpaterol to the concentration of the total residues for liver and for kidney over the 96-hour withdrawal period after the last drug administration could not be determined with any confidence due to the very limited data available and lack of sensitivity of the methods used. The data provided were not sufficient to determine the total residue half-life in the liver after 96 hours. There are no measurable residues in adipose fat.

The 78th meeting of the Committee therefore concluded that it was not possible to recommend MRLs for zilpaterol and that the following data were needed to establish MRLs:

- Results from studies investigating marker residue in liver and kidney;
- Results from studies determining marker residue to total residue ratios in liver and kidney;
- Results from depletion studies to enable the derivation of MLRs compatible with the ADI.

The Committee also stated that "All such studies should use sufficiently sensitive, validated analytical methods capable of measuring zilpaterol and its major metabolites in edible tissues of cattle".

The 22nd session of the CCRVDF, requested JECFA to consider the new data submitted to recommend MRLs for bovine tissues and to also consider potential risks of zilpaterol residues in animal lungs and other edible offal (FAO/WHO, 2015).

The Sponsor submitted data that included results from two residue depletion studies in cattle (Crouch, 2014; Crouch, 2015) and a new validated analytical method for zilpaterol free base residues in bovine tissues (Wrzesinski, 2015). Additional data from two earlier non-pivotal residue studies (Wray, 2008a, Wray, 2008b) that were not provided to the 78th JECFA were also provided by the sponsor for the 81st JECFA. In addition, the Sponsor submitted a new structure–activity relationship assessment of *N*-acetylated deisopropyl zilpaterol, provided an assessment of zilpaterol pharmacokinetics, pharmacology and the impact on exposure and submitted a number of comments on the evaluation of zilpaterol HCl conducted by the 78th meeting of JECFA.

Overview of previous assessment

Zilpaterol hydrochloride, (±)-*trans*-4,5,6,7-tetrahydro-7-hydroxy-6-(isopropylamino) imidazo[4,5,1-jk]-[1]benzazepin-2(1*H*)-one hydrochloride; (zilpaterol HCl; CAS No. 119520-06-8), is a β_2-adrenoreceptor agonist repartitioning agent (FAO, 2013). It is used to increase rate of body weight gain, improve feed efficiency and increase carcass muscle ratio in cattle fed in confinement before slaughter . There are four enantiomers of zilpaterol HCl. The product in use is racemic *trans*-zilpaterol HCl, a mixture of the (6*R*,7*R*) and (6*S*,7*S*) enantiomers; it will be referred to as zilpaterol HCl in this report.

Zilpaterol HCl is to be mixed into the feed at a concentration of 7.5 mg/kg on a 90% dry matter basis. This will result in a dose of approximately 0.15 mg/kg bw, or 60–90 mg zilpaterol HCl per animal per day. It is administered for a period of 20–40 consecutive days before withdrawal from the feed. Zilpaterol HCl is not approved for use in lactating dairy cattle. Where information on authorized uses was provided, withdrawal periods ranged from 2 to 4 days.

Zilpaterol is readily absorbed after oral administration, though the degree of absorption may vary depending on the specific method of oral dosing. Studies conducted in rats, swine and cattle demonstrated the metabolism of zilpaterol as qualitatively and quantitatively comparable in these three species following oral administration. Two major metabolites, deisopropyl-zilpaterol and hydroxy-zilpaterol, together with the parent zilpaterol free base, were observed. Parent compound and metabolites are readily eliminated, primarily in the urine (80% in cattle, 85% in swine and 50% in rats) with the remainder in the faeces. Unchanged parent zilpaterol is the main compound excreted in the urine of these three species. Zilpaterol residue concentrations were approximately 4 - 10 times higher than those of the only significant metabolite, deisopropyl-zilpaterol, in tissues and urine. In rat faeces, the major metabolite is hydroxy-zilpaterol. A metabolism study conducted in cattle with [^{14}C]zilpaterol shows that radioactive residues are detectable in liver at 192 hours (8 days) following a single oral dose of 0.2 mg/kg bw.

Radiolabelled residue depletion studies conducted in cattle after treatment at the recommended dose of 0.15 mg/kg bw/day demonstrated that a steady state is achieved by 12 days on treatment. Residues were detected in liver and kidney until 96 h post-dose. No residues were detected in fat after 12 hours, and no residues were detected in muscle after 48 hours. Extractable residues from liver decreased from 52% to 24 % between 12 h to 96h, and from 89 % to 38 % for kidney over the same time period. Residues in muscle are approximately 100% extractable between 12 and 48 h.

Parent zilpaterol hydrochloride represents a significant part of the extractable residue in liver, kidney and muscle. The ratio of zilpaterol hydrochloride to extracted residue decreased with time for liver, kidney and muscle. Deisopropyl zilpaterol was identified in the extractable fraction and represent a minor fraction of the extractable residue. Other metabolites detected in small quantities in cattle include N-acetylated deisopropyl zilpaterol (urine only) and one unidentified metabolite (3.3% of liver and 5.7% of kidney residue).

To facilitate an understanding of the evaluation of zilpaterol hydrochloride by the present meeting of the Committee, the summaries of key studies reported in the residue monograph prepared by the 78th meeting of the Committee (FAO, 2013) have been included in the current monograph. These studies are identified where they appear and include corrections to some transcription errors contained in the tables published in the previous monograph.

Residues in food and their evaluation

Pharmacokinetics and metabolism

No new data or studies were provided for the current evaluation. The following studies were summarized in the monograph prepared by the 78th meeting of the Committee (FAO, 2013) and are included here due to their relevance to the current evaluation.

Pharmacokinetics and metabolism in laboratory animals

Rats

In a non-GLP-compliant study reviewed by the 78th meeting of the Committee (FAO, 2013), [^{14}C]zilpaterol hydrochloride was administered as a single oral dose of 1 mg/kg by gastric intubation to 10 male and 10 female Sprague-Dawley rats with a mean weight 203 g (Tremblay *et al.*, 1989, V-0238-0211). The 10 rats were divided into two groups of 5 each. The first group was anaesthetized and killed 0.5 h after drug administration and the second group 24 h after drug administration. The total radioactivity in the different tissues and plasma collected was determined by liquid scintillation counting (LSC). The ratio of tissue radioactivity concentration to that of plasma ($R_{t/p}$) was calculated for each tissue collected (Table 8.1).

Note that the same tissues were not always collected between male and female rats. The $R_{t/p}$ results of the study conducted to determine the tissue distribution of zilpaterol as a function of time in the rat after a single oral dose administration showed that the radioactivity concentration of the drug depletes between 0.5 h and 24 h for all tissue matrices and organs of the males or females tested. Kidneys and liver involved in the metabolism and elimination of zilpaterol hydrochloride and its metabolite displayed the highest $R_{t/p}$. At 24 h, the residual radioactivity

was low and there was no retention in the organ samples, with no marked difference between male and female rats.

Table 8.1.[a] Tissue distribution of zilpaterol at 0.5 and 24 h following a single administration of 1 mg/kg [^{14}C]zilpaterol hydrochloride by gastric intubation to male and female Sprague-Dawley rats (Tremblay et al., 1989).

	$R_{t/p}$* >>>1	**$R_{t/p}$ <<<<1**
0.5 h Male rats	Vascular system (heart, spleen, bone marrow) Respiratory system (diaphragm, lung) Endocrine system (thyroid, adrenals, pituitary) Digestive system (pancreas, duodenum, stomach) Liver – 5.96, Kidney – 34.4	CNS (cortex, cerebellum, medulla) Eyes Fat (subcutaneous, perirenal) Testis and thymus
0.5 h Female rats**	Reproductive system (vagina, oviducts, uterus, & ovaries) Skeletal muscle, adrenals, liver -7.24, kidneys – 37.4	Subcutaneous fat
24 h Male rats	Respiratory system (lung, diaphragm) Vascular system (blood, erythrocytes) Relational system (skin, skeletal muscle) Adrenals – 7.0, stomach – 13.3, kidney – 16.6, urinary bladder – 24.2, liver – 75	CNS (cortex, cerebellum, medulla) Endocrine system (thyroid, pituitary) Vascular system (heart, bone marrow) Thymus, pancreas, eyes, perirenal fat
24 h Female rats	Ovaries, liver – 71, kidneys – 11.4	

[a] Reprinted without modification Table 10.1 from the 78th monograph (FAO, 2014).
* $R_{t/p}$ = ratio of tissue radioactivity concentration to that of plasma.
** Different tissues were collected from female rats than male rats (e.g., lung was only collected from male rats).

The $R_{t/p}$'s measured for plasma, liver, kidneys, skeletal muscle and lung tissues are given in Table 8.2 for the male rats used in the above study and sacrificed at 0.5 and 24 h after the oral

dose. These results show that the concentration of residues likely to be found in muscle are lower than would be found in kidney, liver and lung tissue.

Table 8.2.[a] Ratio ($R_{t/p}$) of concentrations of [^{14}C]zilpaterol in male Sprague-Dawley rats killed 0.5 and 24 h after a single oral dose (Tremblay et al., 1989).

Tissue	0.5 hours withdrawal		24 hours withdrawal	
	n	Mean ± S.D.	n	Mean ± S.D.
Plasma	5	1	5	
Liver	5	5.96 ± 0.24	5	75 ± 14
Kidneys	5	34.4 ± 3.7	5	16.6 ± 3.7
Skeletal muscle	5	1.24 ± 0.08	5	2.46 ± 0.44
Lung	5	1.65 ± 0.27	5	1.43 ± 0.19

[a] Reprinted without modification Table 10.2 from the 78th monograph (FAO, 2014).

A GLP compliant study (reviewed by the 78th meeting of the Committee) was undertaken in which 70 male (mean bodyweight 272 g) and 70 female (mean bodyweight 213 g) Sprague-Dawley rats (about 8 weeks old) were allocated to two groups of 15 animals/sex/group, which received a dietary admixture, and two groups of 20 animals/sex/group dosed by gavage (Sauvez, 1995). Unlabelled zilpaterol doses used were 0.05 or 1.10 mg/kg/day (gavage and dietary admixture) for 13 days. All the animals were fasted for gavage purposes. Blood samples were collected Days 2–3 and Days 13–14, and harvested plasmas were analysed for unchanged zilpaterol using a validated radioimmunoassay method with a LOQ of 0.025 ng/ml. After a 2-week repeated administration by oral route (dietary or gavage) at a dose of 0.055 mg/kg or 1.1 mg/kg bw in male and female rats, the mean plasma AUC (24h period)/dose was roughly 2 – 6 times higher in females than in males. The mean plasma AUC (24h period) after dietary admixture administration was 38.8 – 105.7% of that obtained after oral gavage (high and low dose, respectively). The mean plasma C_{max} after dietary admixture administration was 8.5 – 15.7% of that obtained after oral gavage (high and low dose, respectively; Table 8.3).

Table 8.3. Mean pharmacokinetic parameters for zilpaterol in Sprague-Dawley rat plasma after dosing by dietary admixture or gavage (Sauvez, 1995).*

ROUTE	Dose	Sex	Study Days	AUC 0-24 (ng*h mL)	AUC_{avg} (bothdays)	AUC_{avg} (M+F)	F = AUC_{FED}/ AUC_{gavage}	C_{max} (ng/mL)	C_{max} AVG (both days)	C_{max} AVGM+F	F =C_{maxFED}/ $C_{max\ gavage}$
Dietary admixture	0.055	M	D1-2 D13-14	3.64 2.92	3.3	11.5	105.7%	0.24 0.18	0.2	0.8	15.8%
		F	D1-2 D13-14	29.5 10	19.8			1.9 0.68	1.3		
	1.1	M	D1-2 D13-14	56.1 47.2	51.7	104.9	38.8%	3.65 3.78	3.7	6.4	8.5%
		F	D1-2 D13-14	159 157.2	158.1			8.71 9.46	9.1		
Gavage	0.055	M	D1-2 D13-14	5.44 6.35	5.9	10.9		2.5 2.35	2.4	4.8	
		F	D1-2 D13-14	13.4 18.4	15.9			5.99 8.29	7.1		
	1.1	M	D1-2 D13-14	139 188	163.5	270.0		44.47 46.03	45.3	75.3	
		F	D1-2 D13-14	356 397	376.5			115.31 95.49	105.4		

*This table has been modified from the version (Table 10.3) published in the 78th JECFA monograph (FAO, 2014).

The bioavailability of non-extractable (bound) zilpaterol residues from cattle liver fed to rats was assessed in a GLP-compliant study as per the Gallo-Torres model (Girkin, 1999), reviewed by the 78th meeting of the Committee. Non-extractable residues remaining in the liver from cattle administered radiolabelled zilpaterol were fed to Sprague-Dawley rats (16 male, 16 female) ranging in age from 6 to 10 weeks. Liver was obtained from cattle killed at 12, 24, 48 and 96 h; following either 12 repeated daily doses of zilpaterol or 12 h after the last of 15 repeated daily doses. Pooled liver samples from each dosage × withdrawal time were extracted, lyophilized, finely powdered and pelleted.

Groups of 4 rats (2 males and 2 females per group) were surgically altered. After a 24-hour recovery, lyophilized pelleted cattle liver (either non-zilpaterol-containing control liver, or liver containing non-extracted residues), or rat diet was administered by gastric cannulae to bile-duct cannulated rats. An intragastric dose of radiolabelled zilpaterol was administered to the control liver and rat diet groups; mean absorption was > 88%. All rat groups fed non-extractable zilpaterol residues had > 90% of the radioactivity in faeces or GI contents. The results show that the non-extractable residues from livers of cattle at all sacrifice points were only poorly absorbed by the rats. Group III had the highest proportion of the zilpaterol dose being absorbed (and therefore bioavailable), with a mean of 3.3% total absorption (see Table 8.4). The bioavailability of the non-extractable portion of incurred non-extractable (bound) residues is considered to be no more than 5%.

Table 8.4. Recovery of [^{14}C]zilpaterol radioactivity concentration expressed as % of administered dose following intra-gastric administration to Sprague-Dawley rats (Girkin, 1999).*

	% Radioactivity (n=4 per group)				
	Group III	**Group IV**	**Group V**	**Group VI**	**Group VII**
Days of administration(d)	12	15	12	12	12
Withdrawal period (h)	12	12	24	48	96
Absorbed					
Urine	2.4	2.2	2.0	0.8	1.1
Bile	0.0	0.2	0.0	0.0	0.1
Carcass & Tissues	0.9	0.1	0	0	0
Total absorbed	3.3	2.5	2.0	0.8	1.2
Non-absorbed					
Faeces	88.0	97.2	101.9	96.1	99.3
GIT contents	2.4	0.5	0.1	0.0	0.1
Cage washes	0.0	0.2	0.0	0.0	0.0
Total non-absorbed	90.4	97.8	102.0	96.1	99.4
Total Recovery	93.7	100.3	104.0	96.8	100.6

*Reprinted and corrected from Table 10.24 in the 78th JECFA monograph (FAO, 2014).

Dogs

An open, randomized cross-over study (reviewed by the 78th meeting of the Committee) using 4 fasted male beagle dogs (mean weight of 10 kg) in a non-GLP-compliant study was undertaken to measure the absolute bioavailability of [^{14}C]zilpaterol hydrochloride in the dog after a single dose administration of 1 mg/kg bw intravenously or orally (Tremblay *et al*, 1990). The dogs were fasted for 24 h before and 8 h after drug administration. Urine samples were collected over a 48 h period and analysed for zilpaterol by LSC. The amount of radioactivity excreted in urine was 22.8 ± 2.1% of the intravenous dose, and 23.9 ± 2.4% of the oral gavage dose. The absolute bioavailability of zilpaterol after oral gavage administration was calculated as 100%.

Humans

A study (non-GLP-compliant) reviewed by the 78th meeting of the Committee was conducted with 9 healthy male fasted volunteers aged between 28 and 55 years weighing between 56 and 76 kg, using a single-blind protocol versus a placebo to measure the clinical tolerance of humans to zilpaterol (Sutton and Budhram, 1987; Tremblay and Mouren, 1988). Zilpaterol was administered as a solution at single doses of 0.25, 0.50, 1.0 and 2.0 mg to the healthy volunteers and blood was collected from each volunteer at 15 minutes following drug administration, then 1, 2, 3, 4, 5, 6, 8 and 24 h after dosing. Zilpaterol concentrations in plasma were analysed by radioimmunoassay (LOQ = 0.1 ng/ml). Time (T_{max}) to reach the maximal concentration (C_{max}) was observed 1 hour after dosing whatever the dose, and there was a linear relationship between both the C_{max} or AUC, and the dose. The plasma concentrations were proportional to the dose administered and the $t_{1/2}$ was independent of the administered dose. In this study, it was observed that the 1.0 mg dose was badly tolerated by volunteers and as result, none of the volunteers was given a dose greater than 2.0 mg.

Pharmacokinetics and metabolism in food producing animals

Cattle

A GLP-compliant study reviewed by the 78th meeting of the Committee was conducted with four Salers steers and four Charolais × Salers heifers averaging 295 kg bw and allotted to four groups of two animals, each group comprising one steer and one heifer (Tulliez, 1992). The first group was kept on feed supplemented with unlabelled zilpaterol and was used as control. Animals in the three other groups were given a single dose of 0.2 mg/kg bw of [^{14}C]zilpaterol hydrochloride by gavage of the pellet and were killed at 12 h, 48 h and 8 days, respectively. Plasma was collected from each animal during the first 10 h and then at the 14th, 21st and 24th hours, and then every day until they were killed. Urine and faeces were collected daily from the individual animals for the 8-day surviving animals. At kill point, liver, kidneys and samples of muscle (*longissimus dorsi*), perirenal and visceral fat and of the four stomachs were excised and frozen until analysis. There was a rapid increase in radioactivity in plasma, which reached a maximal value 12 h and 10 h following drug administration in the male and female, respectively. The corresponding highest concentrations in plasma were 16.8 ng/ml and 22.4 ng/ml zilpaterol equivalents. Depletion of radioactivity in plasma occurred on a biphasic basis. The $T_{1/2}$ for the first phase was observed at 11.9 and 13.2 h for male and female, respectively.

The second phase corresponded to a very slow decrease of radioactivity, but could not be described accurately because the signal was not significantly different from the background. Over 90% of the dose (97% in steers and 93% in heifers) was excreted over the 8 days (Table 8.5).

Table 8.5.[a] Excretion of [^{14}C]zilpaterol in steers (Salers) and heifers (Charolais × Salers) over the 8 days following a single administration of [^{14}C]zilpaterol by gavage (Tulliez, 1992).

	Radioactivity excreted as % of administered dose	
Sample material	Steer	Heifer
Urine	88.2	84.3
Faeces	8.7	8.6
Total	96.9	92.9

[a] Reprinted without modification from Table 10.14 in the 78th Monograph (FAO, 2014).

In males, 88% of the excreted material was in the urine and 8.7% was in the faeces, while in females 84% was in the urine and 8.6% was in the faeces. At 12 h post-dose, the radioactive concentrations were observed in the following order: liver=kidney>reticulum> omasum>abomasum>rumen >muscle >fat. Radioactivity was not detectable in any tissues except liver at 192 h post-dose (Table 8.6).

Table 8.6.[a] [^{14}C]zilpaterol hydrochloride-equivalents (µg/kg of fresh sample) in tissues and stomachs of steers and heifers (n=1 animal per sex at each withdrawal period) following a single administration of [^{14}C]zilpaterol hydrochloride by gavage (Tulliez, 1992).

Tissue	**Sex**	**[^{14}C]zilpaterol hydrochloride equivalents (µg/kg) Withdrawal period (h)**		
	(Avg)	**12**	**48**	**192**
Liver	M/F	112/116	42/39	15/11
	Avg	(114)	(41)	(13)
Kidney	M/F	110/118	25/23	NS/NS*
	Avg	(114)	(24)	NS
Perirenal fat	M/F	2/2	1/NS	NS/NS
	Avg	(2)	NS	NS
Visceral fat	M/F	7/3	4/2	NS/NS
	Avg	(5)	(3)	NS
Muscle	M/F	17/15	4/3	NS/NS
	Avg	(16)	(4)	NS
Rumen	M/F	61/43	20/20	NS/NS
	Avg	(52)	(20)	NS
Reticulum	M/F	83/147	14/16	NS/NS
	Avg	(115)	(15)	NS
Omasum	M/F	82/79	60/34	NS/NS
	Avg	(81)	(47)	NS
Abomasum	M/F	50/63	12/16	NS/NS
	Avg	(57)	(14)	NS

[a] Reprinted without modification Table 10.15 in the 78th JECFA Monograph (FAO, 2014).
* NS = not significant; the result in brackets represents the average of the readings from the 2 animals.

In a GLP-compliant pilot steady state study reviewed by the 78th meeting of the Committee, four groups of two animals each (one Charolais steer and one Charolais heifer, 200–330 kg bw) were used in 4 consecutive trials (multi-dose administration) (Tulliez, 2000). The animals were administered daily an oral dose of [^{14}C]zilpaterol at 0.15 mg/kg bw for 10, 12, 15 and 21 days, and killed 20–24 h after the last dose administration. Another group of 2 non-medicated animals served as controls. Radio analysis of the extractable radioactivity from liver, muscle, kidneys showed that, other than parent drug, the only other major metabolite was deisopropyl zilpaterol (10–15%). Blood samples were collected daily before the daily dosing, and at kill time, liver, kidneys and muscle (*longissimus dorsi*) and fat (perirenal) were collected. Total radioactivity in the tissues was determined by LSC, and zilpaterol-related metabolites were isolated, purified by HPLC and identified by mass spectrometry (ESI-MS, GC-MS, and thermospray mass spectrometry–TSP/MS). Radioactivity levels reached a steady state concentration of 20 µg/kg in plasma after 4–6 days of dosing. No significant radioactivity could be detected in fat samples. The proportion each component comprised of the extractable radioactivity in liver, muscle and kidney are presented in Table 8.7.

Table 8.7.[a] Percentage distribution of extractable [^{14}C]zilpaterol-related metabolites in tissues of cattle killed 20–24 h after the last dose of [^{14}C]zilpaterol (Tulliez, 2000).

	Proportions of zilpaterol and deisopropyl-zilpaterol in extractable [^{14}C]zilpaterol hydrochloride equivalents (% of total radioactivity)					
Treatment	**Liver**		**Kidney**		**Muscle**	
Days	**Zilpaterol**	**Deisopropyl-zilpaterol**	**Zilpaterol**	**Deisopropyl-zilpaterol**	**Zilpaterol**	**Deisopropyl-zilpaterol**
10	68	16	62	13(1)	73	13(1)
12	76	8	87	5	85	10
15	67	12	79	6	86	15(1)
21	69	13	72	7(1)	94	13(1)

[a] The caption and heading of this table has been corrected from Table 10.18 published in the 78th JECFA monograph (FAO, 2014).
(1) Values are the average of percent distribution of one male and one female except for footnoted values which represent only one animal.

Tissue residue depletion studies

Radiolabelled residue depletion studies

No new data or studies were provided for the current evaluation.

Cattle

A GLP-compliant study reviewed by the 78th meeting of the Committee was conducted in which 17 healthy Hereford cattle (9 steers, 6 heifers) weighing between 200 and 230 kg were allocated into six groups (Tulliez, 1999, V-0238-0158). Group I (1 male and 1 female) was a non-medicated group designed to provide control samples. Each of the remaining Groups (II–VI) comprised 3 animals (2 males and 1 female, or the opposite). During the experimental period, each animal received the radiolabelled [**14**C]-zilpaterol and unlabelled zilpaterol at 0.15 mg/kg bw/day for 12 days. The Group II animals were killed 12 h after the last dose on the 12th day, Group IV 24 h, Group V 48 h and Group VI 96 h after the last dose. Group III animals were fed for 15 days and killed 12 h after the last dose. Liver samples were collected as follows: LL – left lobe; RL – right lobe; CL – caudate lobe; SL – square lobe. Adipose tissue was either PR – perirenal; or SC – subcutaneous. A validated liquid chromatographic/fluorescence method was used for the analysis of zilpaterol and zilpaterol metabolites in edible tissues and fat of cattle. The tissue samples were analysed for total radioactivity (Table 8.8), percentage of extractable radioactivity (Table 8.9), as well as for unchanged zilpaterol and deisopropyl-zilpaterol metabolite by HPLC with radiometric detection (Table 8.10).

Table 8.8.[a] Total residues (eq. Zilpaterol HCl) in tissues of cattle fed 0.15 mg /kg bw/day of [^{14}C]zilpaterol hydrochloride for 12 days (Tulliez, 1999)

Withdrawal time (hours)	Liver (µg/kg ± S.D.)	Kidney (µg/kg ± S.D.)	Muscle (µg/kg ± S.D.)	Fat (µg/kg)
12 [1]	291 ± 56	184 ± 31	22 ± 2.4	10.5
24	205 ± 14	100 ± 5	12 ± 2.6	ND
48	157 ± 23	37 ± 25	6, ND,ND[2]	ND
96	113 ± 17	9 ± 4	ND	ND

[a] This table contains data previously reported in Table 10.23 published in the 78th JECFA monograph (FAO, 2014).

[1] Data from the 12 and 15-day feeding period were combined. [2] ND = not detected.

A mass balance for unchanged zilpaterol and its metabolites in tissues was calculated from the recovery of the radioactivity after different extraction steps. Labelled zilpaterol and labelled metabolites were extracted from liver, kidney and muscle using an ammonia-acetonitrile-methanol mixture and then purified by solid phase extraction. Liver was again the tissue containing the highest total residue concentrations, expressed as zilpaterol HCl-equivalents, with concentrations of 291 ± 56, 205 ± 14, 157 ± 23, and 113 ± 17 µg/kg at 12, 24, 48 and 96 h, respectively, after the last dose for the animals administered 12 daily doses of the drug (Table 8.8). The next highest total residue concentrations were observed in kidney, with concentrations of 184 ± 31, 100 ± 5, 37 ± 25 and 9 ± 4 µg/kg at 12, 24, 48 and 96 h, respectively,

after administration of the final dose. The total residue concentration in muscle was already very low 12 h after the last dose, at 22 ± 2 µg/kg, 12 ± 3 µg/kg at 24 h, and depleted to non-detectable concentrations by 96 h after the last dose.

Residues in tissues were similar in animals administered zilpaterol when slaughtered at zero withdrawal time (12 h after the last dose) whether the drug was administered for 12 or 15 days. The residue levels reached a steady state by 12 days after dosing. Analysis of the total [^{14}C]zilpaterol-related residues showed that percentage of extractability decreased from about 50% in liver at 12 h to 24% at 96 h. In kidney, percentage of extractability also decreased with time. Essentially all of the residues in muscle were extractable at the 12 and 24 h withdrawal periods (Table 8.9).

Table 8.9.[a] Percentage extractability of [^{14}C]zilpaterol HCl-related residues and distribution of residues in kidney, muscle and liver in cattle over four-day (96 h) tissue withdrawal period (Tulliez, 1999).

Tissue	Withdrawal Time (hours)	Total Radioactive Residue (TRR)[1] Eq µg/kg	Extracted Radioactive Residue (ERR)[1] Eq µg/kg	% Extract-ability	LC-R Zilpaterol HCl (MR)[2] µg/kg	LC-F Zilpaterol HCl (MR)[3] µg/kg
Liver	12	291 ± 56	149± 29	52 ± 7	95 ± 27	82 ± 22
	24	205 ± 14	82 ± 4	40 ± 1	48 ± 5	40 ± 1
	48	157 ± 23	49± 19	31 ± 9	23 ± 13	15 ± 12
	96	113 ± 17	27 ± 3	24 ± 2	7.5 ± 3.4	1.4 ± 0.2
Kidney	12	184 ± 31	162 ± 23	89 ± 8	110 ± 30	106 ± 25
	24	100 ± 5	85 ± 2	85 ± 3	58 ± 5	58 ± 5
	48	37 ± 25	30 ± 25	74 ± 14	19 ± 23	21 ± 23
	96	9 ± 4	4 ± 2	38 ± 4	0.3 ± 0.3	NQ
Muscle	12	22 ± 2.4	22 ± 3.8	102 ± 9	13 ± 3	15 ± 2
	24	12 ± 2.6	12 ± 2.0	99 ± 6	5 ± 2	5 ± 2
	48	NQ	ND	NA	ND	NQ
	96	ND	ND	NA	ND	NQ

[a] This table contains data previously reported in Table 10.23 published in the 78th JECFA monograph (FAO, 2014).

[1]TRR = Total radioactive residue (as Zilpaterol HCL equivalents). ERR = Extracted radioactive residue (as Zilpaterol HCL equivalents).
[2]MR = Parent zilpaterol (Marker residue) determined by radio-HPLC.
[3]MR = Parent zilpaterol (Marker residue) measured by HPLC/fluorescence. ND = not detected. NQ = Not quantifiable. NA = applicable.

The radioactivity extracted from tissues was analysed using radio-HPLC. Radioactivity extracted from liver and kidney is mainly associated with unchanged zilpaterol and deisopropyl-zilpaterol. Very minor metabolites are also present. No difference is observed between sexes, and the distribution between zilpaterol and deisopropyl zilpaterol does not vary significantly with the withdrawal time. In muscle, the same pattern is generally observed, although in some samples, deisopropyl-zilpaterol is not detectable. The results are shown in Table 8.10. Parent zilpaterol together with smaller amounts of deisopropyl-zilpaterol were the predominant compounds found in the extractable residues from tissues. Parent zilpaterol was approximately 4–8 times more abundant than the deisopropyl-zilpaterol.

Table 8.10.[a] Measurement of [^{14}C]zilpaterol and [14C]deisopropyl-zilpaterol residues by radio-HPLC in cattle tissues, Mean±S.D. expressed as zilpaterol HCl equivalents in µg/kg (Tulliez, 1999).*

Withdrawal Time (hours)	Residues of [^{14}C]zilpaterol and [^{14}C]deisopropyl-zilpaterol (µg/kg)					
	Liver		Kidney		Muscle	
	Zilpaterol	Deisopropyl-zilpaterol	Zilpaterol	Deisopropyl-Zilpaterol	Zilpaterol	Deisopropyl-Zilpaterol
12[1]	104.7 ± 33.3	11.2 ± 1.7	127.1 ± 22.3	14.9 ± 1.9	13.3 ± 1.8	1.6 ± 0.1
12[2]	84.4 ± 19.8	15.7± 2.3	92.6 ± 28.5	16.3 ± 3.4	12.7 ± 3.8	3.7 ± 0.4
24[1]	48.4 ± 5.3	6.5 ± 1.4	57.9 ± 5.0	7.8 ± 1.7	4.8 ± 2.0	ND[3]
48[1]	22.9 ± 13.3	2.5 ± 0.3	18.9 ± 22.8	1.4 ± 0.8	2.3[4],	0.3[4]
96[1]	7.5 ± 3.4	1.1 ± 0.2	0.3 ± 0.3	0.1[4]	ND	ND

[a] This table has been modified from the Table 10.22 published in the 78th JECFA monograph (FAO, 2014).
[1] Group was fed medicated feed for 12 days. [2] Group was fed medicated feed for 15 days. [3] ND = Not detectable. [4] Only one value available.

Parent zilpaterol was also measured by a validated HPLC/FL method (Table 8.9). At 12 h, it represented 28 ±7% of the total radioactivity residue (TRR) and 54 ± 8% of extracted radioactive residue (ERR) in liver. The MR:TRR and MR:ERR ratios decreased with time to reach, respectively, 1.2 ± 0.1 and 5.2 ± 0.3% at 96 h. For kidney, a similar trend was observed.

Zilpaterol residues in liver show a biphasic curve of depletion for total radioactive residue related to a slow decrease of non-extractable radioactive residue. It should also be noted that there was a difference in the sensitivities of the radiometric versus the fluorescence detection methods used for the quantification of zilpaterol hydrochloride.

Residue depletion studies with non-radiolabelled drug

Cattle

In the first of three GLP-compliant tissue residue depletion studies reviewed by the 78th meeting of the Committee (Table 8.11) measuring the concentration of zilpaterol in liver, muscle and kidney of cattle (Hughes, McDonald and Bomkamp, 1999), 18 crossbred beef cattle (9 steers weighing 455 to 595 kg and 9 heifers weighing 480 kg to 573 kg at the initiation of treatment) were randomly assigned to four groups (2 of each sex per group). The cattle were treated for 12 consecutive days with the commercial pre-mix medicated feed at the recommended dosage of 0.15 mg/kg bw per day or 7.5 mg/kg in feed. After receiving the final dose via medicated feed, one group of animals was killed at each of 12, 24, 48 or 96 h post-dose. Two animals were non-medicated control animals. These animals were considered representative of standard feedlot cattle.

Table 8.11.[a] Mean zilpaterol hydrochloride concentrations in cattle liver, muscle and kidney tissues in the four day (96-h) withdrawal period pivotal study (Hughes, McDonald and Bomkamp, 1999).

Withdrawal Period (hours)	Mean zilpaterol hydrochloride equivalents (µg/kg) (n=4)		
	Liver	Muscle	Kidney
Group II (12)	28.3 ± 9.1	5.0 ± 1.9	50.8 ± 33.1
Group III (24)	11.4 ± 2.8	2.1 ± 0.5	12.9 ± 1.5
Group IV (48)	4.5 ± 4.0	<LOQ[1]	5.7 ± 5.2
Group V (96)	<LOD[2]	<LOD[3]	<LOD[4]
LOD (µg/kg)	1	0.1	0.5
LOQ (µg/kg)	3	1	1

[a] Reprinted without modification Table 10.25 in the 78th JECFA Monograph (FAO, 2014).
[1] LOQ = 1 µg/kg. [2] LOD = 1 µg/kg. [3] LOD = 0.1 µg/kg. [4] LOD = 0.5 µg/kg.

Samples of liver, muscle and kidney from the four-day withdrawal study were assayed by the validated HPLC/FL method (Table 8.11). Recoveries of marker residue were 91.8 ±3.72%, 86.1 ±13.9% and 98.4 ±4.57%, respectively, for the liver, muscle and kidney. The LOQs for the method were 3 µg/kg, 1 µg/kg and 1 µg/kg, respectively, for liver, muscle and kidney, while LODs were 1 µg/kg, 0.1 µg/kg and 0.5 µg/kg, respectively, for the liver, muscle and kidney. The mean concentrations of zilpaterol in liver depleted from 28.3 µg/kg 12 h after the last 12th-day dose to 11.4 µg/kg 24 h after the last dose and to 4.5 µg/kg 48 h after the last dose. At 12,

24 and 48 h after the last dose, the concentrations of residues in kidney were 51, 13 and 6 µg/kg, respectively. Notable in this particular study, the residue concentrations in kidney were slightly higher than the residue concentrations in liver. This is contrary to all other zilpaterol residue depletion studies in cattle.

In the remaining two GLP-compliant studies reviewed by the 78th meeting of the Committee, a total of 25 steers and 25 heifers, including 48 treated animals and 2 controls, forming 9 groups, were used in each of the studies (Crouch, 2011a, 2011b). The group assignments, treatments, and withdrawal periods are shown in Table 8.12.

For the purpose of these two studies, cattle were administered Zilmax® either via component feeding (Crouch, 2011a) or via a pelleted type C top dress supplement (Crouch, 2011b) at the recommended dosage regimen of 90 mg zilpaterol hydrochloride per head, and for 20 consecutive days. The males were castrated and no female was pregnant. The bodyweights ranged from 433 kg to 574 kg for heifers, and from 480 kg to 584 kg for steers. Samples (muscle and liver only, no kidney) were assayed by the validated HPLC/FL method.

Table 8.12.[a] Experimental design used in the two 10-days withdrawal period pivotal studies for zilpaterol hydrochloride (Zilmax) residue depletion study in cattle (Crouch, 2011a, b)**.**

Group	Withdrawal time (days)	Zilmax dose (mg/head/day)	Dosing period (consecutive days)	Steers	Heifers
I	0.5	90	20	3	3
II	1	90	20	3	3
III	2	90	20	3	3
IV	3	90	20	3	3
V	4	90	20	3	3
VI	6	90	20	3	3
VII	8	90	20	3	3
VIII	10	90	20	3	3
Control	NA[1]	NA	NA	1	1

[a] Reprinted without modification Table 10.25 in the 78th JECFA Monograph (FAO, 2014).

The method LOD for liver was 0.90 µg/kg with an LOQ of 2.0 µg/kg, and the muscle LOD and LOQ were 0.53 µg/kg and 2.0 µg/kg, respectively. The concentrations of residues in liver were significantly lower than the residue levels observed in the earlier GLP-compliant study (Hughes, McDonald and Bomkamp, 1999). Residues in muscle tissue were too low to permit a depletion curve plot (Table 8.13).

Table 8.13. Mean zilpaterol free base residue concentrations (ug/kg) in liver and muscle at 12 – 240 hour withdrawal times in cattle fed 90 mg zilpaterol / head / day for 20 days. [Crouch, 2011a, 2011b].[a]

Slaughter time(hours)	Top dress supplement (Crouch, 2011b)		Component feeding (Crouch, 2011a)	
	Liver (µg/kg)	Muscle (µg/kg)	Liver (µg/kg)	Muscle (µg/kg)
12	12.9 ± 5.3	3.0 ± 0.7[1]	13.9 ± 7.3	3.8 ± 0.5 [2]
24	All values but one (3.6) <LOQ [4]	All values <LOQ	5.7 ± 2.4	All values <LOQ
48	All values <LOQ	All values <LOQ	3.8 ± 1.0 [3]	All values <LOQ
72	All values but one (2.9) <LOQ	All values <LOD[5]	2.3 ± 0.4 [3]	All values <LOD
96	All values <LOD[6]	All values <LOD	All values <LOQ	All values <LOD
144	All values <LOD	All values <LOD	All values but one (2.01) <LOQ	All values <LOD
192	All values <LOQ	All values <LOD	All values <LOQ	All values <LOD
240	All values <LOD	All values <LOD	All values <LOD	All values <LOD

[a] This table has been modified from the Table 10.27 published in the 78th JECFA monograph (FAO, 2014).
[1] 4 out of 6 values >LOQ. [2] 2 out of 6 values >LOQ. [3] 3 out of 6 values >LOQ. [4] LOQ = 2 µg/kg.
[5] LOD = 0.527 µg/kg. [6]LOD = 0.985 µg/kg.

Data from two new depletion studies using non-radiolabelled drug were submitted by the Sponsor. These studies had not been submitted for evaluation by the 78th meeting of the Committee.

In a GLP-compliant study conducted to determine the concentration of the marker residue, zilpaterol free base, at "zero withdrawal" (12 ± 2 hours) following administration of Zilmax® to male and female finishing cattle (Crouch et al, 2014), zilpaterol was administered for 30 days as a Type B pelleted supplement via component feeding dose rates of 30, 45, 60, and 75 mg zilpaterol hydrochloride / head / day for 30 days. The study animals were commercial breed steers and heifers (Black Angus, Black Angus Cross), approximately 12 months of age. The body weights of the animals at the beginning of the study ranged from 359 to 458 kg. Ten steers and 10 heifers were randomized by body weight within sex to each of 4 Zilmax® dose level groups (Group 2 = 30, Group 3 = 45, Group 4 = 60, and Group 5 = 75 mg zilpaterol hydrochloride per head per day) for a total of 80 medicated animals.

Liver and muscle tissues were collected after a withdrawal period of 12 + 2 hours for all treated groups. At least 1 kg of liver was retrieved and trimmed of large blood vessels, carefully avoiding puncturing the gall bladder. All three lobes of the liver were sampled in cross section for homogenization of the liver specimens. At least 1 kg of the longissimus dorsi muscle was retrieved and trimmed of extraneous fat for the muscle specimens. After collection, each tissue specimen was rinsed to remove contamination such as blood or intestinal contents, weighed, and cut into smaller pieces. The pieces were well mixed and aliquots of approximately ¼ of the total (250 g) were placed into each of four resealable plastic bags and flattened. The bags were labelled aliquot number as 1,2,3 or 4 of 4 and immediately placed in an insulated ice chest containing dry ice for rapid freezing and stored in a freezer set at -70ºC for storage until they were homogenized at the Testing Facility within 15 days of collection. The samples were analyzed in duplicate. The averaged results of the analysis of liver and muscle samples are shown in Table 8.14. No kidney samples were analysed in this study.

Table 8.14. Mean ± S.D. concentrations of zilpaterol free base in tissues of finishing cattle administered oral zilpaterol hydrochloride via Type B pelleted supplement at 30, 45, 60 and 75 mg/head/day for 30 days, and killed at 12 hours post-feeding. (Crouch *et al*, 2014).

	N	30 mg/head/ day	N	45 mg/head/ day	N	60 mg/head/ day	N	90 mg/head/ Day
Liver	20	11.2±5.9	20	14.7±6.1	20	18.1±7.6	20	19.8±6.1
Muscle	5[a]	2.68±0.50	11[a]	2.57±0.57	14[a]	2.88±0.90	11[a]	2.52±0.53

Calibration curves = 2.0 - 30.0 µg/kg for liver, 2.0 - 20 µg/kg for muscle.

LOQ = 2.0 µg/kg

[a] below LOQ results not included in mean calculations.

Another GLP-compliant study was conducted to determine the depletion over time of the marker residue, zilpaterol free base, following administration of Zilmax® to male and female finishing cattle (Crouch et al., 2015). Zilpaterol was administered for 30 days as a medicated complete Type C feed at dietary concentrations required to provide 60 or 90 mg zilpaterol hydrochloride/head/day. The 84 pool animals from which the study animals were selected were commercial breed steers and heifers (Angus and Angus cross). They received no treatment upon arrival at the testing facility and no treatment was administered at any subsequent time. Animals at arrival to the feedlot ranged from 388-523 kg and were approximately 12 months old.

Thirty eight steers and 38 heifers selected from the pool were randomized by body weight within sex to three Zilmax dose level groups: Group 1 = control (1 steer and 1 heifer plus one spare steer and heifer), Group 2 = 60 mg zilpaterol HCl per head per day (18 steers and 18 heifers), Group 3 = 90 mg zilpaterol HCl per head per day (18 steers and 18 heifers). The medicated groups were further subdivided by post-medicated feed withdrawal time in sub-groups of 3 steers and heifers each. Groups A, B, C, D, E and F corresponded to 12, 24, 48, 72,

120 and 240 hours withdrawal period, respectively (Table 8.15). Treated animals were kept in pens of 18 per sex, and controls in pens of 2 per sex. Animals when assigned to the study groups weighed from 403 - 535 kg. They were fed a complete non-medicated Type C feed *ad libitum* except during the 30 day treatment period for the medicated animals. The complete medicated Type C feed zilpaterol HCL concentration in the daily batches was adjusted as needed to ensure that the average daily zilpaterol consumption per head per pen remained at least at the targeted 60 (Group 2) or 90 mg (Group 3).

The body weights of the medicated animals in the study ranged from 451-619 kg at tissue collection. Tissues (liver lobe subsamples ~ 1 kg total, longissimus dorsi muscle ~ 1 kg total, both kidneys) were collected from the medicated animals at withdrawal intervals of 12, 24, 48, 72, 120 and 240 hours and control animal tissues were collected prior to the medicated ones. The tissues were chopped, thoroughly mixed, divided into 4 approximately equal portions per tissue type, bagged, labeled and quick frozen on dry ice immediately after collection. These aliquots, labeled as 1-4, were transferred to a freezer for storage at < - 20 ºC until processing. The frozen tissue pieces were processed at the testing facility by homogenization with dry ice for subsequent residue analysis. The 4 portions of kidney were combined for homogenization. The 4 portions of liver and muscle were each homogenized individually. Four aliquots of each tissue homogenate prepared were sampled by placing the dry ice/homogenate mixture into 50 mL tubes for sublimation of residual dry ice at -20 ºC and subsequent storage at < - 20 ºC until transfer for residue analysis. The remainder of each dry ice/homogenate mixture was discarded after sampling the 4 homogenate aliquots. The zilpaterol free base concentrations measured for all groups are summarized in Table 8.15.

Table 8.15. Mean ± S.D. Zilpaterol Free Base Concentrations after administration of Zilmax® Type C medicated feed at 60 or 90 mg/head/day (Crouch 2015).

Group ID	Withdrawal Period (h)	Number of animals (Steers / Heifers)	Mean zilpaterol free base ± S.D. (µg/kg)		
			Liver	Muscle	Kidney
Control (0 mg zilpaterol HCl)					
1	NA	2 (1/1)	< LOD	< LOD	< LOD
60 mg zilpaterol HCl/animal/day for 30 days					
2A	12	6 (3/3)	42.0±16.3	5.66±1.97	37.7±4.37
2B	24	6 (3/3)	10.1±5.81	1.23±0.53	10.2±1.46
2C	48	6 (3/3)	1.58±0.97	0.82±0.79	1.93±0.79
2D	72	6 (3/3)	0.48±0.25	0.32±NA	0.46±0.041
2E	120	6 (3/3)	0.38±NA	BLQ±NA	BLQ±NA
2F	240	6 (3/3)	0.29±NA	0.251±NA	BLQ±NA

90 mg zilpaterol HCl/animal/day for 30 days					
3A	12	6 (3/3)	35.9±12.28	4.92±1.42	27.9±0.89
3B	24	6 (3/3)	15.6±5.22	1.84±0.55	9.8±0.21
3C	48	6 (3/3)	4.49±1.54	0.74±0.16	5.47±0.08
3D	72	6 (3/3)	1.22±0.34	0.306±0.04	1.02±0.02
3E	120	6 (3/3)	0.27±0.02	BLQ±NA	0.36±0.03
3F	240	6 (3/3)	0.59±0.14	BLQ±NA	0.47±0.03

LOQ = 0.25 μg/kg (ppb) for all tissues.

In addition, two other residue depletion studies were evaluated which had not been previously submitted by the Sponsor. These two studies (Wray, 2008a; Wray, 2008b) were conducted to estimate a withdrawal period for ZILMAX used as top dress supplement. After review of the study designs in these two residue depletion studies by the Committee, the data were not considered suitable for use in the development of MRL recommendations. There was marked matrix interference in the LC-mass chromatograms of the liver samples analyzed using the method described in the first study (Wray, 2008a) and no efforts were made to minimize or eliminate them. In the second study (Wray, 2008b) there was only one slaughter time point (2 days) at which residue concentrations were greater than or equal to the limit of quantification of the method (see also Appraisal).

From the evaluation of the residue depletion data considered by the 78th meeting of the Committee and the additional studies submitted for review by the present meeting of the Committee, the Committee concluded that zilpaterol free base is an appropriate marker residue for muscle, liver and kidney.

Evaluation of zilpaterol residues in lungs and other edible offal

The twenty-second session of the CCRVDF requested the Committee to consider the potential risks of zilpaterol residues in animal lungs and other edible offal. To respond to this request, the definition of offal must be clarified. The definition of offal from two countries was determined by JECFA. In Australia, edible offal includes brain, heart, kidney, liver, pancreas, spleen, thymus, tongue and tripe. From Japan, all animal body parts except muscle, fat, kidney and liver are considered offal.

Residue data from some cattle tissues other than liver/kidney/muscle/fat are provided in a study evaluated by the Committee (Tulliez J., 1992). In this study, [^{14}C]zilpaterol hydrochloride was adsorbed onto a cellulose plug and administered by oral gavage at a dose of 0.2 mg zilpaterol hydrochloride per kg bw. Animals were sacrificed 12 hours, 48 hours and 8 days after dosing. Total residues were determined in the liver, muscle, visceral fat, perirenal fat; as well as in tripes (rumen, reticulum, omasum and abomasum). Total residues concentrations in the tripes were of the same order of magnitude as in kidneys at 12 and 48 hour withdrawal periods. Residues were not detected in the tripes at 8 days. See Table 8.6 for further details. There are

no data for residues in the other tissue matrices listed in the two described definitions (brain, heart, pancreas, etc).

Data from a study performed using male and female Sprague Dawley rats previously considered by the 78th meeting of the Committee also provided some information on tissue distribution (Tremblay D. et al., 1989). Radioactivity ratios from lung: plasma were provided for male rats, but radioactivity of lung tissue was not assessed in female rats (see Table 8.1). At 0.5 h after dosing, the radioactivity ratio for lung: plasma was 1.65:1, decreasing to 1.43:1 by 24 hours. Although there is a slightly higher total residue concentration in the lungs when compared to plasma (decreasing with time), this tissue: plasma radioactivity ratio is much lower than those for liver and kidneys (6 – 75 : 1 for liver; 17 – 34 : 1 for kidney). No data have been provided to JECFA on concentrations of zilpaterol residues in cattle lung tissue. However, based on the lung: plasma and liver/kidney: plasma ratios in the 24 h observation period in rats, the zilpaterol residue concentrations in bovine lungs may be much lower than residues in liver or kidney.

Methods of analysis for residues in tissues

Liquid chromatography – tandem mass spectrometry (LC-MS/MS)

A new method (Wrzesinski, C., et al 2015) was used for the analysis of free zilpaterol residues in the pivotal study submitted to the current meeting of the Committee (Crouch et. al., 2015). The Committee assessed the validation data against the analytical requirements as published in the Codex guidelines for analytical methods for residue control, CAC/GL 71-2009 (FAO/WHO, 2014c).

In brief, samples of homogenized bovine tissue (1.00 ± .0500 g) was fortified with a stable label internal standard (d7-zilpaterol free base) and extracted with 2 x 5 mL of methanol. A sub-sample of the extract was purified by cation exchange SPE and then analysed by a validated liquid chromatography/mass spectrometry/mass spectrometry (LC-MS/MS) using electrospray ionization in the positive ion mode. Quantification was performed using a solvent calibration curve with a range of 0.25 to 30 µg/kg tissue equivalents for all tissues. The limit of quantitation (LOQ) is 0.250 µg/kg for all tissues and the limit of detection (LOD) is 0.0479, 0.0673 and 0.0448 µg/kg for liver, muscle and kidney, respectively. The average recovery of zilpaterol in the methanol extracts was determined to be 76% (liver), 85% (kidney), and 73% (muscle). The analytical parameters of the method in liver, kidney and muscle tissues are summarized in Tables 8.16a-8.16c. The validated method provided by the Sponsor was considered to be adequate for effective residue control of zilpaterol.

Table 8.16a. Precision and accuracy for zilpaterol fortified in cattle liver.

	Mean ± CV(%) Concentration of zilpaterol free base in liver (µg/kg)			
Nominal concentration	QC 1 0.250	QC 2 1.00	QC 3 10.0	QC 4 24.0
Run 1	0.297±(7.3)	1.02±(7.3)	9.69±(4.5)	25.0±(3.4)
Run 2	0.295±(7.5)	0.975±(6.2)	10.4±(6.5)	25.7±(6.2)
Run 3	0.275±(12.2)	1.05±(2.6)	9.53±(3.9)	22.0±(4.1)
Within day	0.289±(9.3)	1.02±(6.2)	9.87±(6.3)	24.2±(8.2)

n = 6 per run. QC: quality control.

Table 8.16b. Precision and accuracy for zilpaterol fortified in cattle kidney.

	Mean ± CV(%) Concentration of zilpaterol free base in kidney (µg/kg)			
Nominal concentration	QC 1 0.250	QC 2 1.00	QC 3 10.0	QC 4 24.0
Run 1	0.30±(3.5)	1.02±(6.7)	10.1±(4.1)	25.4±(5.9)
Run 2	0.284±(8.1)	1.01±(5.5)	9.61±(12.1)	25.4±(3.7)
Run 3	0.299±(9.7)	1.08±(5.6)	10.4±(3.7)	24.8±(5.2)
Within day	0.294±(7.6)	1.14±(6.4)	10.0±(7.7)	25.2±(4.9)

n = 6 per run. QC: quality control.

Table 8.16c. Precision and accuracy for zilpaterol fortified in cattle muscle.

	Mean ± CV(%) Concentration of zilpaterol free base in muscle (µg/kg)			
Nominal concentration	QC 1 0.250	QC 2 1.00	QC 3 10.0	QC 4 24.0
Run 1	0.246±(9.8)	1.01±(5.1)	9.53±(4.3)	22.4±(3.8)
Run 2	0.271±(10.3)	0.990±(5.3)	8.59±(2.6)	21.4±(2.9)
Run 3	0.267±(6.2)	0.948±(7.6)	9.03±(2.7)	25.2±(39.4)
Within day	0.261±(9.5)	0.983±(6.3)	9.05±(5.4)	23.0±(24.7)

n = 6 per run. QC: quality control.

Sponsor comments to 78th JECFA monograph and 81st JECFA response

Comments from the Sponsor:

a) The sponsor identified several errors in some of the tables in the seventy-eighth JECFA monograph, which it believed may have had an impact on data interpretation and conclusions.

b) The sponsor stated that data gaps identified by the seventy-eighth JECFA were not fully justified, as available information in submitted studies had not been used by the Committee.

c) The sponsor stated that there were sufficient data sets (including the new studies – not available at the time of the seventy-eighth JECFA) to recommend MRLs.

JECFA response: The corrected tables have been included in the addendum to the residue monograph prepared by the current Committee. Assessment of the data has been performed using an approach based on all data available.

d) The Sponsor stated that only the residues of pharmacological concern are relevant for the dietary exposure assessment, as the ADI was based on a pharmacological end-point. In particular, the sponsor argued that insufficient attention was paid to the 10-fold difference in activity between zilpaterol and its main metabolite (deisopropyl zilpaterol) with respect to β2-agonist activity on the cardiovascular system.

JECFA response: The Committee has considered this comment, and the pharmacological activity of the various zilpaterol residues is reflected in the revised exposure assessment.

e) Regarding residues of pharmacological concern, the Sponsor proposed that the reduced bioavailability of zilpaterol residues (and thus not pharmacologically active) should be accounted for in the exposure assessment.

JECFA response: The bioavailability of the non-extractable portion of incurred bound residues was considered in the assessment, as per the Gallo-Torres model. A bioavailability correction factor of 0.05 was used for all non-extractable residues. All extractable residues were assumed to be fully bioavailable, as per current regulatory guidance in multiple jurisdictions, and the available data do not support the Sponsor's proposal.

JECFA response to request from 22nd CCRVDF

The CCRVDF at its 22nd session in April 2015 requested the next JECFA to consider potential risks of zilpaterol residues in animal lungs and other edible offal.

The Committee concluded that there were insufficient zilpaterol residue data to adequately consider exposure to residues in lungs and other edible offal of cattle apart from liver and kidney. No non-radiolabelled residue depletion data were provided for any cattle tissues other than liver, kidney and muscle. For lung tissue, there were no actual residue data available in cattle, just estimates based on ratios of plasma versus respiratory tissue radioactivity from preliminary radiolabel studies in rats. For edible offal, the only bovine data available were from a preliminary radiolabel study, with only two data points for tripe at each of the 12- and 48-hour withdrawal periods.

Before re-evaluation of zilpaterol with the aim of recommending MRLs in lungs and other edible offal of cattle, the Committee would require marker residue depletion data in such tissues over an appropriate withdrawal period (such as 72 – 96 hours). The Committee noted that the definitions of the tissues comprising offal were not consistent between countries.

Therefore, JECFA requests that CCRVDF provides a definition of edible offal before the risk assessment of zilpaterol residues in edible offal can be to be adequately considered by the JECFA.

Appraisal

Evaluation of pharmacological activity of zilpaterol residues

Comment from Sponsor

In response to the residue monograph prepared by the 78th meeting of the Committee, the sponsor considered that "the previous JECFA did not take into account the available data on relay pharmacology / bioavailability of residues which should be quantitatively considered in the risk assessment." The sponsor further concluded "that the pharmacological effect of incurred residues (relay pharmacology) should be quantitatively considered in the dietary intake assessment and the calculation of the maximum residue limits. This would be consistent with previous risk assessments where JECFA has considered poor oral bioavailability of residues in the dietary exposure assessment (FAO/WHO, 2008)."

Response from JECFA:

a) Assessment of relative pharmacological activity (potency) of zilpaterol metabolites

Information in the studies provided by the Sponsor indicates that the metabolism is mainly by N-deisopropylation and hydroxylation, leading to metabolites such as deisopropyl-zilpaterol and its N-acetyl product, hydroxy-zilpaterol and glucuronate conjugates of hydroxy-zilpaterol. N-deisopropylation was the major metabolic pathway in cattle and deisopropyl-zilpaterol was the only non-parent metabolite with >10% of the radioactivity found in edible tissues of cattle. The β2-agonist activity of deisopropyl zilpaterol was found to be about 10-fold lower than that of parent zilpaterol in rat studies. N-acetylation of the de-isopropyl zilpaterol further reduces the β2-agonist activity of this metabolite by disabling critical activity of the protonated form of the zilpaterol free base and is predicted to have no pharmacological activity based on an assessment of its structure–activity relationship. Hydroxy-zilpaterol and glucuronides thereof have not been detected in cattle tissues. The pharmacological potency of other unidentified metabolites is most likely significantly less than that that of parent compound after multi-step metabolism, leading to disruption of the pharmacophore for β2-adrenergic agonist activity. However, such metabolites do not represent a significant portion of the extractable TRR and of bioavailable bound metabolites. Hence, a conservative estimate for the pharmacological potency for such unidentified polar extractable residues would be 10% of the parent compound (similar to the potency of metabolite deisopropyl zilpaterol).

The current meeting of the Committee considered it scientifically valid and sufficiently conservative to assign a relative pharmacological potency (β2-adrenergic agonist activity) of 10% of parent zilpaterol for all extractable and bioavailable "bound" metabolites (i.e., all substances that are not parent zilpaterol).

b) Assessment of bioavailability of zilpaterol residues

When assessing the bioavailability of drug residues, the Gallo-Torres model (whereby the bioavailability of non-extractable or bound residues is considered in the human exposure assessment) has been utilized by numerous agencies, including the United States Food and Drug Administration (FDA-CVM, 2006) and the European Medicines Agency (EMA, 2008a; EMA, 2008b). JECFA follows this approach, as described in Environmental Health Criteria 240: Principles and Methods for the Risk Assessment of Chemicals in Food. Chapter 8: Maximum Residue Limits for Pesticides and Veterinary Drugs (FAO/WHO, 2009), first used by the 34th meeting of the Committee (FAO/WHO, 1989). The current Committee agreed that such an approach is appropriate for zilpaterol, given the data provided in the study **(**Girkin, 1999) in which rats were fed liver from zilpaterol-treated cattle (see Table 8.4). A bioavailability factor of approximately 5% for bound zilpaterol residues was considered appropriate by the Committee. The bioavailability of non-extractable residues in kidney and muscle was not determined in the study. The Committee however agreed that the same oral bioavailability of 5 % can conservatively be applied for bound residues in kidney and muscle.

The Committee does not typically account for potentially limited oral bioavailability of total (including non-bound plus extractable) residues in the dietary exposure assessment, consistent with the approach of regulatory agencies. A similar proposal to include a correction factor for the bioavailability of total drug residues was conclusively rejected by the European Committee for Medicinal Products for Veterinary Use (CVMP) when drafting the Reflection Paper on Assessment of Bioavailability of Bound Residues in Food Commodities of Animal Origin in the Context of Council Regulation (EEC) No 2377/90. The triclabendazole evaluation at the 70th JECFA cited by the sponsor appears to be the only case where such an approach has been used. Without evaluation of the triclabendazole raw data, the 81st JECFA could not ascertain the validity of this approach.

Furthermore, the sponsor's assertion that zilpaterol administered as dietary admixture (or as incurred residues in tissue) results in substantially lower bioavailability than when administered by oral gavage has not been conclusively demonstrated. The first argument, that zilpaterol bioavailability is approximately 10 times lower when administered as an admixture in feed compared to oral gavage, is based on data from male and female Sprague-Dawley rats (Sauvez, 1995). See Table 8.3 for complete results. The study report indicated relative bioavailability of 8.5 – 15.7% (depending on dose) for zilpaterol administered by oral admixture, compared to zilpaterol administered by oral gavage. However, these relative bioavailability values were based on oral C_{max} alone. When bioavailability was calculated using AUC (the typical method for bioavailability assessment), the relative bioavailability was 38.8 – 105.7%, depending on dose administered (0.055 or 1.1 mg/kg). Zilpaterol administered as part of dietary admixture may have prolonged drug absorption and resulted in lower peak plasma concentrations, but it did not result in significantly lower total drug exposure. Based on this data it is inappropriate to use a bioavailability correction factor for total (including both extractable and non-extractable) zilpaterol residues when mixed in food, as the data did not conclusively demonstrate that bioavailability of admixture-administered zilpaterol is lower than bioavailability of zilpaterol administered by oral gavage.

c) Assessment of pharmacological activity of incurred residues (relay pharmacology)

The sponsor proposed that zilpaterol's "pharmacological effect is reduced by a factor of approximately 10, if the substance is given together with food", based in part on a relay pharmacology study in conscious beagle dogs (Vacheron, 1995). Incurred zilpaterol residues in muscle or liver from zilpaterol-treated steers did not induce any effect on blood pressure or heart rate. When dogs were fed liver with incurred zilpaterol residues, the highest dose of ingested zilpaterol free base was 1.74 to 1.99 μg/kg of body weight. Doses achieved with incurred residues in muscle ranged from 0.25 to 0.28 μg/kg of body weight. A positive control group was treated with zilpaterol HCl at 3 μg/kg bw per day (as dietary admixture). In this group a slight increase in the global AUC and daily AUC was observed for heart rate, but not for blood pressure.

The Committee could not conclude that such data prove incurred zilpaterol residues in tissue lead to significantly lower pharmacological activity and/or bioavailability than zilpaterol administered by other oral means. For assessing the pharmacological potency of incurred residues, the following considerations were raised.

- Firstly, the study used only two beagle dogs (one male and one female), which is not sufficient to conclusively demonstrate that incurred zilpaterol residues produce less pharmacological effect than an equivalent dose of zilpaterol administered by other oral routes.
- Secondly, the effect of food preparation techniques (freeze, thaw, cooking) on the relative activity of incurred zilpaterol residues has not been demonstrated. For example, it is possible that cooking liver with incurred zilpaterol residues leads to increased bioavailability (and potential activity) than similar residues from uncooked liver.
- Thirdly, the relative pharmacological activity of incurred zilpaterol residues in muscle cannot be assessed from this study as the zilpaterol dose from muscle (0.25 – 0.28 μg/kg bw) was likely insufficient to produce pharmacological effects, regardless of oral administration method. It is inappropriate to conclude "reduced activity" based on the absence of pharmacodynamic response, when the dose administered was insufficient to generate a response (even if fully bioavailable).
- Fourthly, the relative pharmacological activity of incurred zilpaterol residues in kidney was not assessed in this study. Even if incurred residues in liver result in decreased potency, similarly limited activity cannot automatically be assumed for kidney or muscle.
- Finally, it cannot be concluded that incurred zilpaterol residues will have reduced pharmacological activity (possibly due to reduced oral bioavailability) in humans based solely on a very limited canine model. Differences in gastrointestinal pH and transit time between dogs and humans can result in differences in bioavailability, thus impacting pharmacological potency.

Regarding any potential reduction in oral bioavailability of incurred zilpaterol residues (compared with other oral means of administration), this study did not assess any zilpaterol concentrations in the plasma of treated dogs. Only pharmacological endpoints were measured

in this study. Without quantification of plasma zilpaterol concentrations, differences in relative oral bioavailability can only be *predicted* based on differences in relative pharmacological potency (which itself was not sufficiently demonstrated in this study). However, any potential differences in residue pharmacological activity do not provide definitive evidence of differences in bioavailability (though this is a likely hypothesis). Therefore even if the bioavailability of incurred zilpaterol residues is indeed lower than that of zilpaterol administered by other oral means, it is not possible to quantify such differences from the data provided.

In summary, the applicant's assertion that "pharmacokinetic studies in rats and dogs indeed suggest that co-administration of diet with zilpaterol has effects on pharmacokinetic parameters" cannot be conclusively demonstrated based on the data provided. Furthermore, attempting to quantify such a potential reduction in pharmacological activity or bioavailability is not possible based on the limited data provided.

Evaluation of the various zilpaterol residue depletion data sets

The zilpaterol residue depletion data from all submitted studies were assessed for suitability of application in the human exposure assessment and derivation of MRLs.

The following points apply to the most recent and extensive zilpaterol free base (marker residue) depletion study in cattle (Crouch, 2015).

- Only the 90 mg/head/day group was used in the exposure assessment, as this was the highest dose studied (and highest approved label dose). Pooling these data with the other dose group (60 mg/head/d) from the same study was not considered statistically appropriate due to differences in mean concentrations and numbers of concentrations above the LOQ at 120 h. The zilpaterol free base concentrations over time for each tissue are shown in Figures 8.1A-1C.

Figure 8.1A. Zilpaterol free base concentrations in muscle from S14078 (90 mg dose).

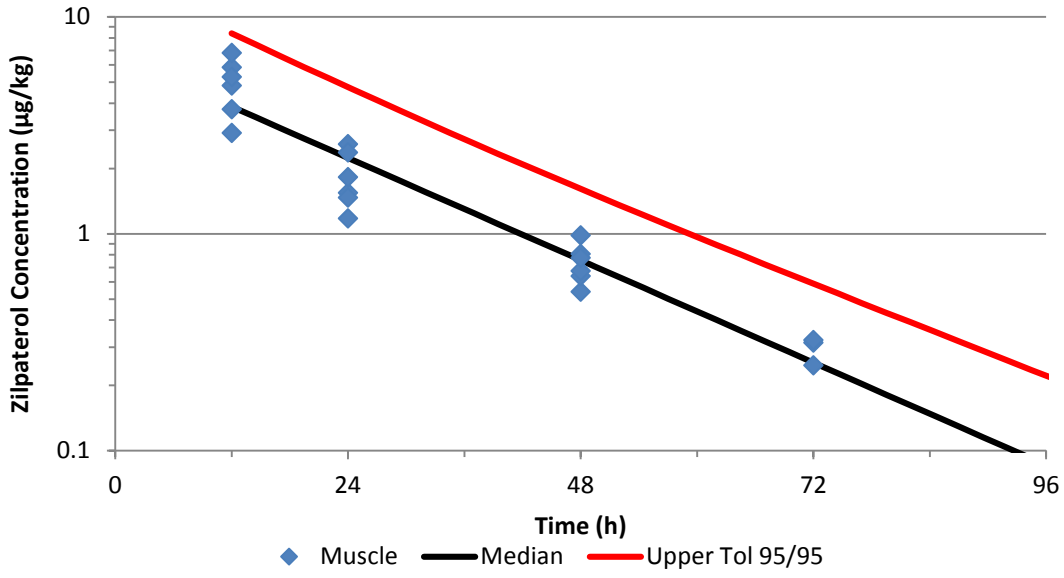


Figure 8.1B: Zilpaterol free base concentrations in kidney from S14078 (90 mg dose).

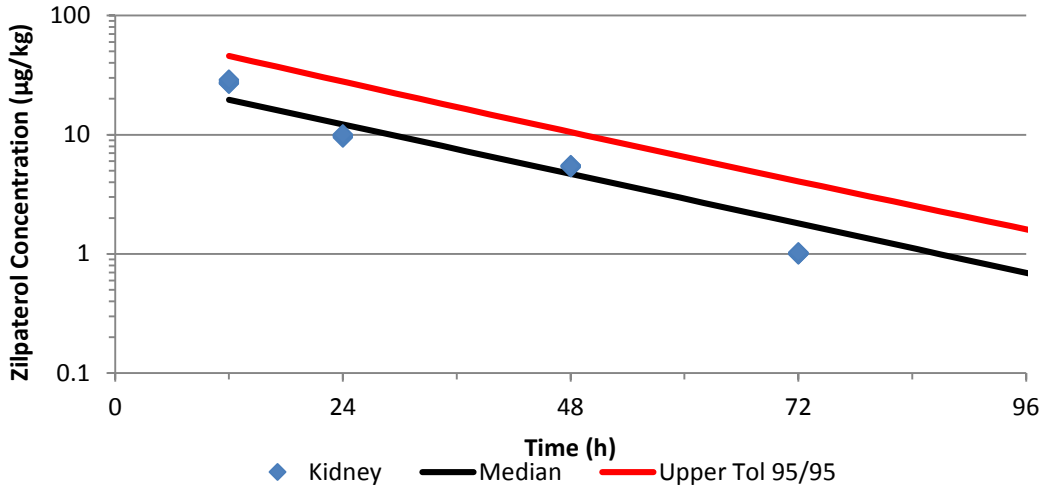
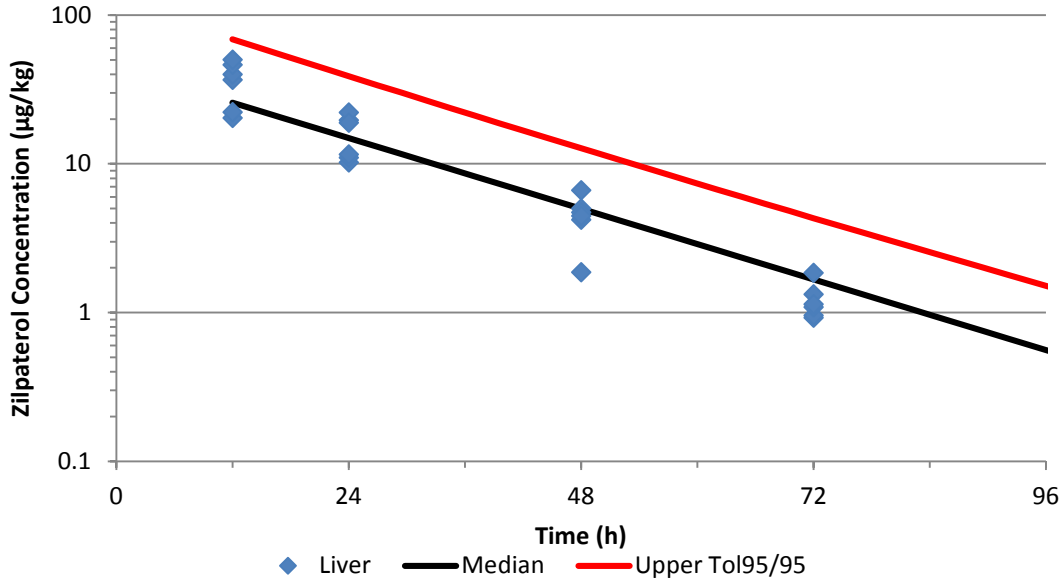


Figure 8.1C: Zilpaterol free base concentrations in liver from S14078 (90 mg dose).



- Although data were collected at 12, 24, 48, 72, 120, and 240 hour withdrawal times, only data up to and including 120 hours were used to estimate the rate of depletion of free zilpaterol (i.e., used in the regression analysis). As the depletion study with radiolabelled drug (Tulliez, 1999) covers the period up to 96 hours, it was considered acceptable to use the new depletion data using non-pradiolabelled drug (Crouch, 2105) dataset until 120 h to perform the linear regression.
- The data set was sufficient to calculate percentile concentrations and corresponding one-sided 95% confidence interval over the 95th percentile of residue concentrations (95/95 upper tolerance limit, or UTL) associated with the residue depletion profiles, and to assess residue exposure and MRLs consistent with approved uses (Good Veterinary Practices, GVP). The 95/95 UTL were estimated until 96 h, which is consistent with the withdrawal times applied according to current GVP.

- The marker residue data provided (Crouch, 2015) confirm that the depletion curves are parallel for liver, kidney, and muscle, indicating comparable depletion profiles. It was noted that zilpaterol concentrations below 1 µg/kg were observed in liver and kidney at 240 h which suggests a terminal elimination phase with a long half-life.
- The LOQ (0.25 µg/kg) of the analytical method used in this residue depletion study was sufficient to identify/monitor the residue depletion over an adequate time period after the last administration (up to 96 hours). Recoveries of residues from QC samples were typically close to 100 % and, therefore, no recovery correction was deemed necessary. The validated method provided by the Sponsor was considered to be adequate for effective residue control of zilpaterol.

The Committee also considered using all other GLP-compliant zilpaterol marker residue depletion studies in cattle previously submitted by the sponsor. It was noted that the residue depletion modelling of such a "pooled" data set provided results which were similar to the results from the most recent and extensive study (Crouch, 2015) alone. However, it was considered inappropriate to use a pooled data set due to the following design and methodological differences between the various residue depletion studies:

- Differences in sample sizes and dosage regimens;
- Differences in analytical methods (limits of quantification and recoveries);
- Lack of residue data for kidney in most of the previous studies;
- Differences in slaughter time points; and
- Use of pooled data would require considerable extrapolation of the earlier data depletion profiles.

Evaluation and quantification of the zilpaterol residues of concern

In its response to residue monograph prepared by the 78th meeting of the Committee, the sponsor proposed that only the pharmacologically active zilpaterol residues should be of concern in human exposure assessments. The current Committee concurs with this assessment. Total pharmacologically active residues (i.e., residues of concern, expressed as zilpaterol HCl-equivalents) were calculated by the Committee based on the zilpaterol free base concentration, sum of zilpaterol metabolite concentrations, relative potency of zilpaterol metabolites, bioavailability of non-extractable zilpaterol residues, and converted by the molecular weight ratio for zilpaterol free base: HCl.

The following equation was used to quantify the total active zilpaterol residues of concern:

Total pharmacologically active residue = Zilpaterol HCl + 0.1*[RR_{Ext} + (0.05*RR_{NonExt})]

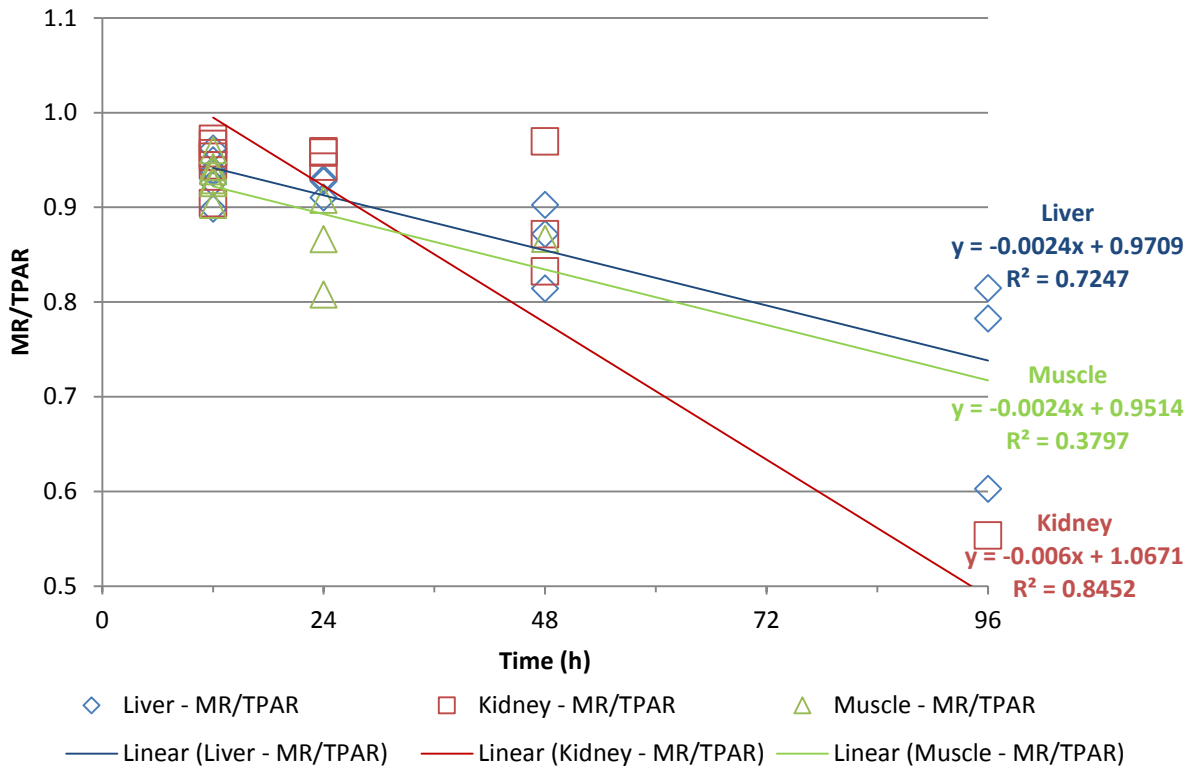
Where:

- Zilpaterol HCl = parent zilpaterol concentration, expressed as zilpaterol hydrochloride;
- 0.1 = relative pharmacological activity correction factor. The activity attributed to zilpaterol HCl was set as 1, whereas activity of all other extractable and bioavailable non-extractable residues was set as 0.1 (i.e., 10% of the parent zilpaterol activity);

- RRExt = sum of other extractable radioactive residue concentrations (including the major metabolite deisopropyl zilpaterol), expressed as zilpaterol HCl-eq;
- RRNonExt= non-extractable (bound) radioactive residue concentration, expressed as zilpaterol HCl-eq
- 0.05 = oral bioavailability of non-extractable residues (as per the Gallo-Torres model used by the 34th meeting of the Committee; FAO/WHO, 1989).

When determining marker residue to total pharmacologically active residue ratios for zilpaterol residues, only the pharmacologically active residues (as quantified above based on the data from the radiolabelled study (Tulliez 1999) were considered in determining the total pharmacologically active residues. Biologically inactive zilpaterol, or non-bioavailable "bound" residues, were not included as part of total residues. The ratios ($R_{tissue(t)}$) over time were plotted at each of the withdrawal periods (12, 24, 48, and 96 hours). Linear regression was performed on each data set to determine the $R_{tissue(t)}$ at any time between 12 – 96 hours. Figure 8.2 and Table 8.17 summarize the changing $R_{tissue(t)}$ ratios over time for each tissue. It was observed that the slope of the depletion curve for muscle is in the same range as those obtained for liver. Based on this observation, it was considered acceptable to extrapolate the ratio for muscle after 48 h until 96 h. This extrapolation is also supported by the parallel zilpaterol tissue depletion curves observed with the data from non-radiolabelled studies (same slope of -0.0024x). The ratios of zilpaterol free base (MR) to total pharmacologically active residue decrease from mean values of 94 %, 99 % and 92 % at 12 h to 74 %, 50 %, and 72% at 96 h for liver, kidney, muscle respectively.

Figure 8.2. Ratio (R_{tissue}) of zilpaterol HCl (marker residue, MR) to total pharmacologically active residues (TPAR expressed as Zilpaterol HCl) over time in liver, kidney, and muscle of cattle.



Marker residues (zilpaterol free base) in individual target tissues from the non-radiolabelled residue study (Crouch, 2015) were converted to total pharmacologically active residues (expressed as zilpaterol HCl-equivalents) using the following formula:

$$\text{Total pharmacologically active residue} = 1.1395^{*} [\text{Zilpaterol free base}]/R_{tissue(t)}$$

Where:

- 1.1395 = molecular weight conversion factor, required to convert all zilpaterol free base residues to zilpaterol HCl for comparisons with the ADI (zilpaterol HCl = 297.783 g/mol, zilpaterol free base = 261.325 g/mol);
- Zilpaterol free base = marker residue concentration;
- Rtissue(t) = ratio of marker residue and total pharmacologically active residue estimated at equivalent time point *(t)* for each tissue (liver, kidney, muscle) from the radioactive study.

The median and 95/95 upper tolerance limits (based on linear regression from the marker residue depletion study (Crouch, 2015), ratios of marker residue (MR): total pharmacologically active residue (TPAR), and resulting total pharmacologically active residues (as determined by the equation above) from 12 – 97 hours withdrawal are shown in Table 8.17.

Table 8.17. Median & 95/95 UTL zilpaterol free base (MR) concentrations, R_{tissue}, and median & 95/95 UTL total pharmacologically active residue (TPAR) concentrations as Zilpaterol HCl over time for edible tissues in cattle.

Time (h)	Median MR (µg/kg)			95/95 UTL MR (ug/kg)			MR: TPAR[1], $R_{tissue(t)}$			MedianTPAR[1] (µg zil HCl-eq/kg)			95/95 UTL TPAR[1] (µg zil HCl-eq/kg)		
	Liver	Kidney	Muscle	Liver	Kidney	Muscle	Liver	Kidney	Muscle	Liver	Kidney	Muscle	Liver	Kidney	Muscle
12	25.81	19.67	3.85	68.60	45.80	8.41	0.94	0.99	0.92	31.3	22.6	4.8	83.2	52.7	10.4
17	20.55	16.12	3.07	54.07	37.23	6.61	0.93	0.96	0.91	25.2	19.1	3.8	66.2	44.2	8.3
22	16.36	13.21	2.45	42.66	30.29	5.21	0.92	0.93	0.90	20.3	16.2	3.1	52.8	37.1	6.6
27	13.02	10.83	1.95	33.70	24.66	4.12	0.91	0.90	0.89	16.3	13.7	2.5	42.2	31.2	5.3
32	10.37	8.87	1.56	26.65	20.09	3.27	0.89	0.87	0.87	13.3	11.6	2.0	34.1	26.3	4.3
37	8.26	7.27	1.24	21.11	16.39	2.61	0.88	0.84	0.86	10.7	9.9	1.6	27.3	22.2	3.5
42	6.57	5.96	0.99	16.75	13.38	2.09	0.87	0.81	0.85	8.6	8.4	1.3	21.9	18.8	2.8
47	5.23	4.88	0.79	13.31	10.94	1.68	0.86	0.78	0.84	6.9	7.1	1.1	17.6	16.0	2.3
52	4.17	4.00	0.63	10.59	8.95	1.36	0.84	0.75	0.82	5.7	6.1	0.9	14.4	13.6	1.9
57	3.32	3.28	0.50	8.44	7.34	1.10	0.83	0.72	0.81	4.6	5.2	0.7	11.6	11.6	1.5
62	2.64	2.69	0.40	6.74	6.02	0.89	0.82	0.69	0.80	3.7	4.4	0.6	9.4	9.9	1.3
67	2.10	2.20	0.32	5.39	4.94	0.72	0.81	0.66	0.79	3.0	3.8	0.5	7.6	8.5	1.0
72[2]	1.67	1.81	0.25	4.32	4.06	0.59	0.80	0.63	0.78	2.4	3.3	0.4	6.2	7.4	0.9
77[3]	1.33	1.48	0.20	3.46	3.35	0.48	0.78	0.60	0.76	1.9	2.8	0.3	5.1	6.4	0.7
82	1.06	1.21	0.16	2.78	2.76	0.39	0.77	0.57	0.75	1.6	2.4	0.2	4.1	5.5	0.6
87	0.84	0.99	0.13	2.24	2.27	0.32	0.76	0.54	0.74	1.3	2.1	0.2	3.4	4.8	0.5
92	0.67	0.81	0.10	1.80	1.88	0.26	0.75	0.51	0.73	1.0	1.8	0.2	2.7	4.2	0.4
97	0.54	0.67	0.08	1.45	1.55	0.21	0.74	0.48	0.71	0.8	1.6	0.1	2.2	3.7	0.3

[1]Total pharmacologically active residue expressed as zilpaterol HCl = 1.1395* [Zilpaterol free base]/$R_{tissue(t)}$

[*]Median and ratios used to calculate chronic dietary exposure

[**]95/95 UTL and ratios used to calculate acute dietary exposure.

Dietary estimates and zilpaterol residue exposure

The Committee considered that there are insufficient residue data for zilpaterol to adequately consider exposure from consumption of lungs or offal of cattle. No non-radio-labelled residue depletion studies have been performed in any cattle tissues other than liver, kidney, and muscle. The radiolabelled residue data are extremely limited, with only 2 data points for tripes at each of 12 and 48 hour withdrawal periods. There are no actual residue data available for cattle lungs. The Committee therefore was unable to assess the potential contribution from consumption of offal to the dietary exposure.

A variety of acute and chronic dietary exposure estimates were calculated for zilpaterol residues (as measured in zilpaterol HCl-eq, see Figure 8.3). The present Committee noted that the basis of the previously established ADI was an acute effect in humans after a single dose of zilpaterol HCl; in line with evolving guidance on the need to consider the establishment of Acute Reference Doses (ARfD) for veterinary drugs, the Committee therefore considered it appropriate to establish an ARfD for zilpaterol HCl. The acute agonistic effect on β_2-adrenoceptor in humans was the most sensitive effect observed and therefore serves as the basis for both the ADI (0-0.04 μg/kg bw) and the ARfD (0.04 μg/kg bw).

Although the ADI for zilpaterol HCl is based on an acute endpoint, chronic exposure was estimated to provide context for the MRL derivation. To estimate chronic dietary exposure, both the Estimated Daily Intake (EDI) and the Global Estimated Chronic Dietary Exposure (GECDE) approaches were used. Where chronic exposure is expressed per person, bodyweights used for the calculations are 60 kg for the general population and 15 kg for children.

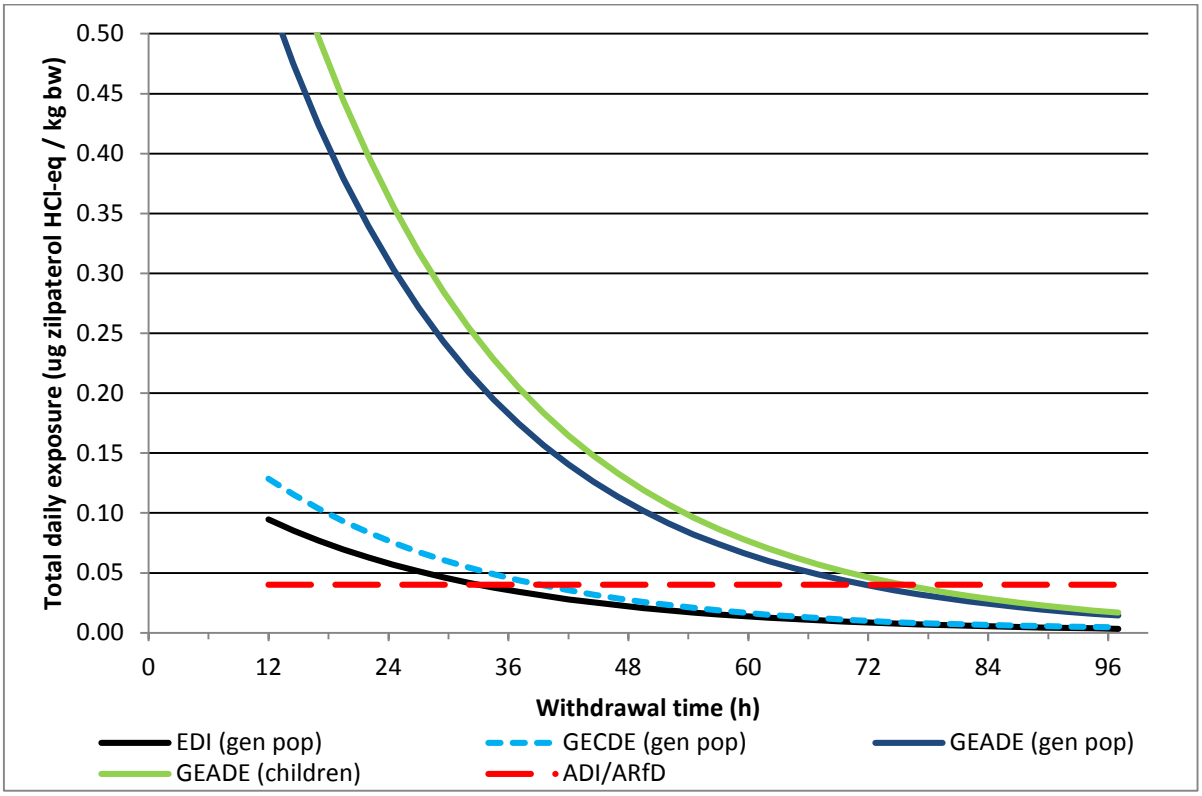
As the ADI for zilpaterol is based on an acute pharmacologic endpoint (immediate β-agonist activity), an acute exposure assessment was deemed most appropriate for the assessment of dietary exposure. The Global Estimated Acute Dietary Exposure (GEADE) approach was therefore used to estimate acute dietary exposure. The Committee noted that the TMDI approach had been used in the assessment of potential acute exposure to residues of carazolol (FAO/WHO, 2000), but considered the GEADE to now provide a more appropriate means to assess acute dietary exposure.

As noted by 66th meeting of the Committee, the EDI should not be applied when there is concern for acute toxicity or acute exposure, but is only applicable for evaluation of chronic dietary exposure (FAO/WHO, 2006). The EDI is not suitable for estimating acute dietary exposure, which must be based on the highest probable exposure from a single commodity on a single day. As with the EDI, the GECDE is based on chronic food consumption estimates and is not suitable for acute dietary exposure scenarios.

As the GEADE provides a robust estimate of potential acute residue exposure, the Committee considered this approach to be most appropriate for the assessment of dietary exposure to zilpaterol HCl. In contrast to the GECDE and EDI, the GEADE is based explicitly on acute dietary consumption estimates, and can therefore be used to calculate acute dietary exposures. For residues of zilpaterol HCl, dietary exposure estimates have been derived specifically for

children (as well as the general population), following the principle that dietary exposure assessments should cover the whole population.

Figure 8.3. Estimated exposure to zilpaterol residues after 12 - 96 hours withdrawal time.



Estimates of chronic dietary exposure

Consumption data used are based on a standard food basket for the EDI and on appropriate **dietary consumption** survey data (see FAO/WHO, 2011) for the GECDE calculation. The results of the calculations have been expressed per person for the whole population estimates to compare the EDI and GECDE exposure estimates, or per kilogram body weight, based on values reported in food consumption surveys.

In the chronic dietary exposure assessment, the contributors to dietary exposure to residues of zilpaterol HCl were the muscle tissue of beef and other bovines, mammalian liver and mammalian kidney. The chronic exposure to total pharmacologically active zilpaterol residues was estimated from the median residue concentrations determined by regression analysis at 72 hours withdrawal and their associated ratios (Table 8.17, Figure 8.3).

The estimated dietary exposure expressed as the EDI was 0.5 µg/person /day, which represents 21% of the upper bound of the ADI of 0-0.04 µg/kg bw/day (Table 8.18).

Using the median residue and consumption data for the most relevant food classifications as inputs, the GECDE for the general population was 0.010 µg/kg bw/day, which is equivalent to 24% of the upper bound of the ADI (Table 8.19). In children the GECDE was 0.011 µg/kg bw/day which represents 27% of the upper bound of the ADI. This estimate was slightly higher than the whole population estimate, as the lower bodyweight of children leads to comparatively higher exposure on a per bodyweight basis.

Table 8.18. Estimated Dietary Intake (EDI) of zilpaterol HCl residues at 72 hour withdrawal.

Tissue	Median MR concentration[1] (µg/kg)	MR: TPAR ratio[2]	MW ZHCL/ MW Z[3]	Median total pharmacologically active residue[4] (µg zil HCl-eq/kg)	Standard Food Basket (kg)	Daily intake (µg)
Muscle (Beef&other Bovines)	0.25	0.78	1.1395	0.4	0.3	0.1
Liver (mammalian)	1.67	0.80	1.1395	2.4	0.1	0.2
Kidney (mammalian)	1.81	0.63	1.1395	3.3	0.05	0.2
			TOTAL			0.5

[1]Median zilpaterol free base concentration at 72 hours.
[2]Ratio at 72 hours.
[3] Ratio of molecular weight Zilpaterol HCl to Zilpaterol free base = 1.1395.
[4]Total pharmacologically active residue = 1.1395* [Zilpaterol free base]/$R_{tissue(t)}$

Table 8.19. The global estimated chronic dietary exposure (GECDE) to adjusted zilpaterol HCl median residues (72 hours withdrawal) in the general population and in children.

Category	Type	Mean consumption[1] whole population, g/day	97.5th consumption[2] consumers only, g/day	Exposure (µg/kg bw/day) Mean	Exposure (µg/kg bw/day) 97.5th	GECDE[3] µg/kg bw/day	%ADI
General population							
Mammalian muscle	Beef/other Bovines	63	291	0.00038	0.00177	0.00038	1.0
Mammalian offal	Mammalian liver	2	111	0.00008	0.00440	0.00008	0.2
Mammalian offal	Mammalian kidney	0.5	166	0.00003	0.00906	0.00906	22.6
TOTAL				0.00046	0.00906	0.00952	24
Children							
Mammalian muscle	Beef/other Bovines	37	159	0.00090	0.00387	0.00090	2.3
Mammalian offal	Mammalian liver	0.5	62	0.00008	0.00983	0.00983	24.6
Mammalian offal	Mammalian kidney	0.05	19	0.00001	0.00415	0.00001	0.0
TOTAL				0.00098	0.00983	0.01074	27

[1] highest mean consumption figures based on whole population considered from the available dataset.
[2] highest 97.5th food consumption figures based on consumers only considered from the available dataset.
[3] GECDE is the sum of the highest exposure at the 97.5th percentile of consumption for a food and the mean dietary exposures of the other foods.

Estimates of acute dietary exposure

The definition of high-level consumers is crucial to the outcome of an acute exposure estimate. The reliability of high percentile consumption data is related to the number of subjects used to calculate them; percentiles calculated on a limited number of subjects should be treated with caution as the results may not be statistically robust. When the number of observations is not large enough, the coverage probability may not attain the nominal value, and drops below, for example, 95%. This is more likely to occur at high percentiles such as the 97.5th. Therefore, the coverage probability can be used to set guidelines to determine the minimum number of samples for which 97.5th percentiles can be computed. In the case of significance level (α) being set at 0.05 to determine a 95% confidence interval, the coverage probability should target 95%. This is achieved for observations where n >70 for the 97.5th percentile. Therefore, a cut-off of n =70 has been used for consumption data used as inputs into acute dietary exposure assessment for zilpaterol HCl.

For the purpose of undertaking the acute dietary exposure assessment for residues of zilpaterol HCl, an up-to-date individual food consumption database of animal tissues and food of animal origin expressed on a large portion (LP) sizes values, based on the 97.5th percentile of food consumption, were used by the Committee. The data were derived from records of individual consumer days (i.e. survey days on which the food or foods of interest were consumed) reported in individual-level survey data from 25 countries (Australia, Brazil, China and 22 European countries) and summarized in the EFSA Comprehensive European Food Consumption Database (EFSA, 2015). Those data were previously collected following a request to Member countries as part of the Joint FAO/WHO Expert Meeting on Dietary Exposure Assessment Methodologies for Residues of Veterinary Drugs (WHO, 2012). The following rules were followed for selecting consumption amounts as inputs:

- For the complete database, where the highest reported 97.5th percentile tissue consumption reported for a country had consumer numbers larger than 70 this value was selected as an input for acute dietary exposure.
- Where the maximum 97.5th percentile reported had consumer numbers less than 70, the reported observations from the complete database were pooled and treated as independent observations.
- If the total number of consumers of the pooled observations was more than 70, the 97.5th percentile was calculated and used as the input. If the total number of consumers was less than 70, the median was calculated and used as the input.

Table 8.20 shows the consumption data selected for the assessment. The highest 97.5th percentiles reported for individual countries were used as inputs for consumption of muscle for the general population and children. For liver, the highest reported 97.5th percentile for an individual country was used for adults but samples were pooled to derive the 97.5th percentile for children. For kidney, the observation numbers were low, so pooled observations were used to derive the 97.5th and 50th (median) percentiles for the general population and children respectively.

The acute exposure to total pharmacologically active zilpaterol residues (expressed as zilpaterol HCl equivalents) was estimated from the 95/95 UTLs determined by regression analysis after 72 hours withdrawal (see Tables 8.17 & 8.22, Figure 8.3). The following 95/95 UTLs were derived: 4.1 µg/kg in kidney, 4.3 µg/kg in liver, and 0.6 µg/kg in muscle. Using acute dietary exposure assessments (GEADE), these 95/95 UTLs could lead to an acute dietary exposure of ~ 99% of the Acute Reference Dose (ARfD) in the general population and ~ 117% of the ARfD in children. Note that the Committee established the Acute Reference Dose (ARfD) for zilpaterol at 0.04 µg/kg bw, the same value as the upper bound of the previously-established ADI.

Because the acute exposure in children exceeded the ARfD using the residue depletion data at 72 hrs., the Committee considered a refined assessment with 95/95 UTLs derived at 77 hours post-dose: 3.3 µg/kg in kidney, 3.5 µg/kg in liver, and 0.5 µg/kg in muscle. The GEADE for the general population was approximately 0.032 µg/kg bw/day for the tissue with the highest exposure (beef liver). Exposure from beef kidney was lower (0.019 µg/kg bw/day) and potential exposure from consuming muscle tissue was much lower (0.006 µg/kg bw/day, or 14% of the ARfD) than that from consuming beef liver. For the general population, the GEADE (beef liver) represented 81% of the ARfD of µg/kg bw (Table 8.21).

For children, the GEADE was approximately 0.038 µg/kg bw per day for beef liver. As with the general population, potential exposure from muscle tissue was much lower (0.0001 µg/kg bw/day or 10% of the ARfD). Exposure to beef kidney was also much lower than for the general population because the comparatively lower consumption amount used for the children sub-population (refer to Table 8.20). For children, the acute dietary exposure estimate (beef liver) was 95% of the ARfD (Table 8.21).

Table 8.20. Consumer statistics calculated from 97.5th tissue consumptions (expressed in grams tissue/kg bw/day).

Cattle Tissue	97.5th General population consumption			97.5th Children consumption		
	max	**p97.5**	**Median**	**max**	**p97.5**	**Median**
Muscle	7.7	6.6	3.9	12.7	12.0	7.1
Liver	6.4	5.8	2.0	8.3*	7.5	2.8
Kidney	3.2*	3.0	1.5	12.9*	12.3*	2.1

Bold numbers used as inputs for exposure calculation.

*Number of total consumers <70.

Table 8.21. The global estimated acute dietary exposure (GEADE) to adjusted zilpaterol 95/95 UTL residues (77 hours withdrawal) in the general population and in children.

Category	Type	97.5th Consumption[1] g tissue/kg bw/day	GEADE[2] µg/kg bw/day	%ARfD
General Population				
Mammalian muscle	Beef and other Bovines	7.7	0.0055	14
Mammalian offal	Beef liver	6.4	0.032	81
Mammalian offal	Beef kidney	3.0	0.019	48
Children				
Mammalian muscle	Beef and other Bovines	12.7	0.0091	23
Mammalian offal	Beef liver	7.5	0.038	95
Mammalian offal	Beef kidney	2.1	0.013	33

[1] highest 97.5th food consumption figures considered from the available dataset representing a single eating occasion

[2] GEADE is the product of the 97.5th level of consumption multiplied with the 95/95 UTL pharmacologically active residue (see Table 8.17)

Derivation of 95/95 upper tolerance limits

At the 77 hour withdrawal time point, the 95/95 UTLs for zilpaterol free base as marker residue are 3.5, 3.3, and 0.5 µg/kg in liver, kidney, and muscle, respectively (Table 8.22). These 95/95 UTLs are appropriate for Maximum Residue Limits (MRLs) for zilpaterol.

It is noted that this time point is in the range of approved withdrawal periods for currently approved zilpaterol formulations (2 – 4 days).

Table 8.22. Zilpaterol MRL derivation for acute and chronic dietary exposure estimates.*****

Data from EDI S-14078 (90 mg dose)							**ADI = 0-0.04 µg zilpaterol HCl-eq/day** **ARfD = 0.04 µg zilpaterol HCl-eq/day**			
	Median zilpat free base (µg/kg)			**95/95 UTL (µg/kg)**			***CHRONIC* estimate (µg zilpaterol HCl-eq/kg bw/d)**		***ACUTE* estimate (µg zilpaterol HCl-eq/kg bw/d)**	
Time (h)	**Liver**	**Kidney**	**Muscle**	**Liver**	**Kidney**	**Muscle**	**EDI (median)**	**GECDE (adult)**	**GEADE (adult)**	**GEADE (child)**
12	25.81	19.67	3.85	68.6	45.8	8.4	0.095	0.129	0.531	0.622
14.5	23.03	17.81	3.44	60.9	41.3	7.4	0.085	0.115	0.475	0.556
17	20.55	16.12	3.07	54.1	37.2	6.6	0.077	0.104	0.424	0.497
19.5	18.33	14.59	2.74	48.0	33.6	5.9	0.070	0.093	0.379	0.444
22	16.36	13.21	2.45	42.7	30.3	5.2	0.063	0.084	0.339	0.397
24.5	14.60	11.96	2.19	37.9	27.3	4.6	0.057	0.075	0.303	0.355
27	13.02	10.83	1.95	33.7	24.7	4.1	0.051	0.067	0.271	0.318
29.5	11.62	9.80	1.75	30.0	22.3	3.7	0.046	0.061	0.243	0.285
32	10.37	8.87	1.56	26.7	20.1	3.3	0.042	0.054	0.218	0.255
34.5	9.25	8.03	1.39	23.7	18.1	2.9	0.038*	0.049	0.195	0.228
37	8.26	7.27	1.24	21.1	16.4	2.6	0.034	0.044	0.175	0.205
39.5	7.37	6.58	1.11	18.8	14.8	2.3	0.031	0.040*	0.157	0.184
42	6.57	5.96	0.99	16.7	13.4	2.1	0.028	0.036	0.141	0.165
44.5	5.86	5.39	0.89	14.9	12.1	1.9	0.025	0.032	0.126	0.148
47	5.23	4.88	0.79	13.3	10.9	1.7	0.023	0.029	0.113	0.133
49.5	4.67	4.42	0.71	11.9	9.9	1.5	0.021	0.026	0.102	0.119
52	4.17	4.00	0.63	10.6	9.0	1.4	0.019	0.023	0.091	0.107

54.5	3.72	3.62	0.56	9.5	8.1	1.2	0.017	0.021	0.082	0.096
57	3.32	3.28	0.50	8.4	7.3	1.1	0.015	0.019	0.074	0.087
59.5	2.96	2.97	0.45	7.5	6.6	1.0	0.014	0.017	0.067	0.078
62	2.64	2.69	0.40	6.7	6.0	0.9	0.013	0.015	0.060	0.070
64.5	2.36	2.43	0.36	6.0	5.5	0.8	0.011	0.014	0.054	0.063
67	2.10	2.20	0.32	5.4	4.9	0.7	0.010	0.012	0.049	0.057
69.5	1.88	1.99	0.29	4.8	4.5	0.7	0.009	0.011	0.044	0.051
72	1.67	1.81	0.25	4.3	4.1	0.6	0.009	0.010	0.040*	0.046
74.5	1.49	1.63	0.23	3.9	3.7	0.5	0.008	0.009	0.036	0.042
77	1.33	1.48	0.20	3.5	3.3	0.5	0.007	0.008	0.032	0.038*
79.5	1.19	1.34	0.18	3.1	3.0	0.4	0.006	0.008	0.029	0.034
82	1.06	1.21	0.16	2.8	2.8	0.4	0.006	0.007	0.026	0.031
84.5	0.95	1.10	0.14	2.5	2.5	0.4	0.005	0.006	0.024	0.028
87	0.84	0.99	0.13	2.2	2.3	0.3	0.005	0.006	0.021	0.025
89.5	0.75	0.90	0.12	2.0	2.1	0.3	0.004	0.006	0.019	0.023
92	0.67	0.81	0.10	1.8	1.9	0.3	0.004	0.005	0.018	0.021
94.5	0.60	0.74	0.09	1.6	1.7	0.2	0.004	0.005	0.016	0.019
97	0.54	0.67	0.08	1.4	1.5	0.2	0.003	0.005	0.014	0.017

*Colour denotes first time point at which the exposure estimate falls to the ADI/ARfD for zilpaterol and data used in calculation.

Maximum Residue Limits

In recommending MRLs for zilpaterol, the Committee considered the following factors:

- An ARfD of 0.04 µg/kg bw was established. This is the same value as the upper bound of the ADI previously established by the seventy-eighth Committee and reaffirmed by the present Committee.
- Zilpaterol HCl is registered to be mixed into feed at a level of 7.5 mg/kg on a 90% dry matter basis. This level provides a dose of approximately 0.15 mg/kg bw or 60–90 mg zilpaterol HCl per animal per day.
- Where information on authorized uses was provided, withdrawal periods ranged from 2 to 4 days.
- Zilpaterol HCl is not approved for use in lactating dairy cattle.
- The major metabolite in cattle tissues is deisopropyl zilpaterol.
- Zilpaterol HCl administration in cattle results in non-extractable residues that are poorly bioavailable in laboratory animals. This low oral bioavailability (~5%) demonstrated for liver was assumed to be similar for non-extractable residues in muscle and kidney.
- The most sensitive toxicological end-point is an acute pharmacological effect. It was assumed that zilpaterol HCl has a reference activity of 1. Deisopropyl zilpaterol was shown to have ~10% of the pharmacological activity of parent zilpaterol, with the activity of all other extractable and bioavailable non-extractable residues being equivalent to, or less than, that of deisopropyl zilpaterol.
- Parent zilpaterol (free base) was an appropriate marker residue in muscle, liver and kidney. Fat was not considered relevant for residue monitoring purposes.
- The ratios of zilpaterol (MR) to the total residues of concern (total pharmacologically active residues) for muscle, liver and kidney could be determined with sufficient confidence over a 96-hour period after the last drug administration. The MR:total pharmacologically active residue ratios were between 0.9 and 1.0 for liver, kidney and muscle at 12 hours withdrawal. By 96 hours withdrawal, the MR:total pharmacologically active residue ratios were 0.7 (liver and muscle) and 0.5 (kidney).
- A validated analytical procedure for the determination of zilpaterol in edible bovine tissues (liver, kidney, muscle) is available and may be used for monitoring purposes. The LOQ is 0.25 µg/kg for all tissues.

The MRLs recommended for bovine tissues are based on an acute dietary exposure scenario (GEADE). The Committee initially derived the following one-sided 95% confidence interval over the 95th percentile of residue concentrations (95/95 upper tolerance limit, or UTL) in bovine tissues at the 72-hour time point: 4.1 µg/kg in kidney, 4.3 µg/kg in liver and 0.6 µg/kg

in muscle. Using acute dietary exposure assessments (GEADE), these 95/95 UTLs could lead to an acute dietary exposure of ~ 99% of ARfD in the general population and ~ 117% of the ARfD in children.

Because the exposure in children exceeded the ARfD using the 72-hour data, the Committee considered a refined assessment with 95/95 UTLs derived at 77 hours post-dose: 3.3 µg/kg in kidney, 3.5 µg/kg in liver and 0.5 µg/kg in muscle. These values would result in acute dietary exposure (GEADE of 1.9 µg/day for the general population and 0.57 µg/day for children) of ~94% of the ARfD in children and ~80% of the ARfD in the general population and are recommended as MRLs. It is noted that the time point at which the MRLs are calculated (77 hours) is consistent with currently approved withdrawal times (GVP).

The Committee recognizes that the approach used in this evaluation differs from that of previous evaluations for similar types of veterinary compounds. However, this was appropriate due to the acute nature of the pharmacological end-point and the availability of an appropriate model for acute exposure. Detailed chronic and acute dietary exposure assessments are included in the addendum to the residue monograph to provide additional information to risk managers.

The Committee concluded that there were insufficient zilpaterol residue data to adequately consider exposure to residues in lungs and other edible offal of cattle apart from liver and kidney. No non-radiolabelled residue depletion data were provided for any cattle tissues other than liver, kidney and muscle. For lung tissue, there were no actual residue data available in cattle, just estimates based on ratios of plasma versus respiratory tissue radioactivity from preliminary radiolabel studies in rats. For edible offal, the only bovine data available were from a preliminary radiolabel study, with only two data points for tripe at each of the 12- and 48-hour withdrawal periods.

Recommendation

The Committee noted that the definitions of the tissues comprising offal were not consistent between countries. Therefore, JECFA requests that CCRVDF provide a definition of edible offal.

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ANNEX 1 -Summary of Recommendations from the 81st JECFA on Compounds on the Agenda and Further Information Required

Diflubenzuron (insecticide)

Acceptable daily intake: In the absence of adequate information on exposure to 4-chloroaniline (PCA), a genotoxic and carcinogenic metabolite and/or degradate of diflubenzuron, the Committee was unable to establish an ADI for diflubenzuron because it was not possible to assure itself that there would be an adequate margin of safety from its use as a veterinary drug. The Committee also noted that it was not possible to calculate a margin of exposure for PCA in the absence of adequate information on exposure to PCA.

Residue definition: The Committee was unable to recommend MRLs for diflubenzuron, as an ADI could not be established. The Committee considered that the the following information would assist in the further evaluation of the compound:

- A comparative metabolism study of diflubenzuron in humans and rats (e.g. in hepatocytes).
- Information on PCA exposure associated with the consumption of treated fish.
- Information on the amount of PCA present (if any) as an impurity in the product formulation.
- Information on the amount of PCA generated during food processing.
- A method suitable for monitoring diflubenzuron residues in fish muscle and fillet (muscle plus skin in natural proportion).

Ivermectin (antiparasitic agent)

Acceptable daily intake: The Committee established an ADI of 0–10 µg/kg body weight on the basis of a no-observed-adverse-effect level (NOAEL) of 0.5 mg/kg body weight per day for neurological effects (mydriasis) and retardation of weight gain in a 14-week dog study, with application of an uncertainty factor of 50 (5 for interspecies differences based on pharmacokinetic studies in dogs and humans and 10 for intraspecies differences). The previous ADI of 0–1 µg/kg body weight was withdrawn.

Acute reference dose: The Committee established an ARfD of 0.2 mg/kg body weight, based on a NOAEL of 1.5 mg/kg body weight, the highest dose tested in a safety, tolerability and pharmacokinetics study in healthy human subjects, with application of an uncertainty factor of 10 for intraspecies variability.

Residue definition: Ivermectin B1a

Recommended maximum residue limits (MRLs)[1] for Ivermectin B1a

Species	Fat (µg/kg)	Kidney (µg/kg)	Liver (µg/kg)	Muscle(µg/kg)
Cattle	400	100	800	30

[1] No new data were provided for use of ivermectin in dairy cattle; therefore, the Committee did not recommend any revision to the MRL of 10 µg/kg for ivermectin in milk.

Lasolocid sodium (antibiotic)

Acceptable daily intake: Following consideration of the issues raised in concern forms from CCRVDF, the Committee concluded that there would be no concern for colonization barrier disruption in the colon from acute exposure to residues of lasalocid. The ADI established at the seventy-eighth meeting of JECFA (WHO TRS No. 988, 2014) remain unchanged.

Residue definition: The MRLs that were recommended by the 66th meeting of the Committee (WHO TRS No. 988, 2014) were maintained.

Sisapronil (ectoparasiticide)

Acceptable daily intake: The Committee concluded that a toxicological ADI could not be established because the Committee had no basis upon which to determine a suitable uncertainty factor to accommodate the lack of a long-term toxicity study.

Residue definition: The Committee could not recommend MRLs, as an ADI could not be established. The Committee considered that the the following information would assist in the further evaluation of the compound:

- Data to address long-term toxicity relevant to humans (e.g. 1-year dog study).
- Comparative pharmacokinetics studies and an explanation of interspecies differences in the pharmacokinetic profiles.

Teflubenzuron (insecticide)

Acceptable daily intake: The Committee established an ADI of 0–5 μg/kg body weight on the basis of a lower 95% confidence limit on the benchmark dose for a 10% response (BMDL10) of 0.54 mg/kg body weight per day for hepatocellular hypertrophy in male mice observed in a carcinogenicity study, with application of an uncertainty factor of 100 to account for interspecies and intraspecies variability.

Residue definition: Teflubenzuron

Recommended maximum residue limits (MRLs) for Teflubenzuron

Species	Fillet[a] (μg/kg)	Muscle (μg/kg)
Salmon	400	400

[a] Muscle plus skin in natural proportion.

Zilpaterol hydrochloride (β_2-adrenoceptor agonist)

Acceptable daily intake: The Committee reaffirmed the ADI of 0–0.04 μg/kg body weight established at the seventy-eighth meeting (WHO TRS No. 988, 2014).

Acute reference dose: The Committee established an ARfD of 0.04 μg/kg body weight based on a lowest-observed-adverse-effect level (LOAEL) of 0.76 μg/kg body weight for acute pharmacological effects observed in a single-dose human study, with application of an uncertainty factor of 20, comprising a default uncertainty factor of 10 for human individual

variability and an additional uncertainty factor of 2 to account for use of a LOAEL for a slight effect instead of a NOAEL.

Residue definition: Zilpaterol (free base) in muscle, liver and kidney.

Recommended maximum residue limits (MRLs) for Zilpaterol (free base)

Species	Kidney (µg/kg)	Liver (µg/kg)	Muscle (µg/kg)
Cattle	3.3	3.5	0.5

[a] There were insufficient zilpaterol residue data to adequately consider exposure to residues in lungs and other edible offal of cattle apart from liver and kidney. The Committee noted that the definitions of the tissues comprising offal were not consistent between countries. Therefore, JECFA requests that CCRVDF provide a definition of edible offal.

Annex 2 - Summary of JECFA evaluations of veterinary drug residues from the 32nd meeting to the present

The following table summarises the veterinary drug evaluations conducted by JECFA at the 32nd (1987), 34th (1989), 36th (1990), 38th (1991), 40th (1992), 42nd (1994), 43rd (1994), 45th (1995), 48th (1997), 50th (1998), 52nd (1999), 54th (2000), 58th (2002), 60th (2003), 62nd (2004), 66th (2006), 70th (2008), 75th (2011), 78th (2013) and 81st (2015) meetings. These meetings were devoted exclusively to the evaluation of veterinary drug residues in food. This table must be considered in context with the full reports of these meetings, published as WHO Technical Report Series.

Some notes regarding the table:

- The “ADI/ArfD” column provides the ADI and, when applicable, the ArfD established by the Committee. When no ARfD is stated, an ArfD has not been established.
- The “ADI Status” column refers to the ADI and indicates whether an ADI was established; if a full ADI was given, or if the ADI is temporary (T).
- Where an MRL is temporary, it is indicated by “T”.
- Where a compound has been evaluated more than once, the data given are for the most recent evaluation, including the 78th meeting of the Committee.

Substance	ADI/ARfD (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Abamectin	ADI: 0-1 (JMPR 1995)	Full	47 (1996)	100 50	Liver, Fat Kidney	Cattle	Avermectin B_{1a}
Albendazole	ADI: 0-50	Full	34 (1989)	100 5000	Muscle, Fat, Milk Liver, Kidney	Cattle, Sheep	MRLs analysed as 2-amino-benzimidazole, expressed as albendazole equivalents
Amoxicillin	ADI: 0-0.7	Full	75 (2011)	50 4	Muscle, Liver, Kidney, Fat Milk	Cattle, Pig, Sheep Cattle, Sheep	Amoxicillin
Apramycin	ADI: 0-30	Full	75 (2011)	5000	Kidney	Cattle, Chicken	Apramycin
Avilamycin	ADI: 0-2000 (as avilamycin activity)	Full	70 (2008)	200 300	Muscle, Kidney, Skin/Fat Liver	Pig, Chicken, Turkey, Rabbit Pig, Chicken, Turkey, Rabbit	Dichloroisoeverninic acid (DIA), expressed as avilamycin equivalents
Azaperone	ADI: 0-6	Full	52 (1999)	60 100	Muscle, Fat Liver, Kidney	Pig	Sum of azaperone and azaperol
Benzylpenicillin	ADI: <30µg/person/ day of the penicillin moiety	Full	36 (1990)	50 4	Muscle, Liver, Kidney Milk	All species	Benzylpenicillin
Bovine Somatotropins	ADI: Not specified	Full	78 (2013)	Not specified	Muscle, Liver, Kidney, Fat, Milk	Cattle	
Carazolol	ADI: 0-0.1 ARfD: 0.1	Full	52 (1999)	5 25	Muscle, Fat/Skin Liver, Kidney	 Pig	Carazolol. The Committee noted that the concentration of carazolol at the injection site may exceed the ADI that is based on the acute pharmacological effect of carazolol.

Substance	ADI/ARfD (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Carbadox	No ADI or ARfD		60 (2003)	No MRL			The Committee decided that quinoxaline-2-carboxylic acid is nc an appropriate marker residue
Ceftiofur	ADI: 0-50	Full	48 (1997)	1000 2000 6000 100	Muscle Liver, Fat Kidney Milk	Cattle, Pig Cattle	Desfuroylceftiofur
Cefuroxime	No ADI or ARfD		62 (2004)	No MRL			
Chloramphenicol	No ADI or ARfD		62 (2004)	No MRL			
Chlorpromazine	No ADI or ARfD		38 (1991)	No MRL			
Chlortetracycline, Oxytetracycline, Tetracycline	ADI: 0-30 (Group ADI)	Full	58 (2002)	200 600 1200 400 100 200	Muscle Liver Kidney Eggs Milk Muscle	Cattle, Pig, Sheep, Poultry Poultry Cattle, Sheep Fish,Giant prawn	Parent drugs, either singly or in combination Oxytetracycline only
Clenbuterol	ADI: 0-0.004	Full	47 (1996)	0.2 0.6 0.05	Muscle, Fat Liver, Kidney Milk	Cattle, Horse Cattle, Horse Cattle	Clenbuterol
Closantel	ADI: 0-30	Full	40 (1992)	1000 3000 1500 5000 2000	Muscle, Liver Kidney, Fat Muscle, Liver Kidney Fat	Cattle Sheep	Closantel

Substance	ADI/ARfD (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Colistin	ADI: 0-7	Full	66 (2006)	150	Muscle, Liver,Fat	Cattle, Sheep, Goat, Chicken, Turkey, Pig, Rabbit	Residue definition is the sum of Colistin A and colistin B. The MRL includes skin + fat where appropriate (chicken, turkey, pigs).
				200	Kidney		
				50	Milk	Cattle, Sheep	
				300	Eggs	Chicken	
Cyfluthrin	ADI: 0-20	Full	48 (1997)	20	Muscle, Liver, Kidney		Cyfluthrin
				200	Fat	Cattle	
				40	Milk		
Cyhalothrin	ADI: 0-5	Full	62 (2004)	20	Muscle, Kidney	Cattle, Sheep, Pig	Cyhalothrin
				400	Fat	Cattle, Sheep, Pig	
				20	Liver	Cattle, Pig	
				50	Liver	Sheep	
				30	Milk	Cattle, Sheep	
Cypermethrin α-Cypermethrin	ADI: 0-20 (Group ADI)	Full	62 (2004)	50	Muscle, Liver, Kidney	Cattle, Sheep	Total of cypermethrin residues (resulting from the use of cypermethrin or α-cypermethrin as veterinary drugs)
				1000	Fat	Cattle,Sheep	
				100	Milk	Cattle	
Danofloxacin	ADI: 0-20	Full	48 (1997)	200	Muscle	Cattle, Chicken	Danofloxacin For chicken fat/skin in normal proportions
				400	Liver, Kidney		
				100	Fat		
				100	Muscle	Pig	
				50	Liver		
				200	Kidney		
				100	Fat		
Deltamethrin	ADI: 0-10 (1982 JMPR)	Full	60 (2003)	30	Muscle	Cattle, Chicken, Sheep, Salmon	Deltamethrin

Substance	ADI/ARfD (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
				50	Liver, Kidney	Cattle, Sheep, Chicken	
				500	Fat		
				30	Milk	Cattle	
				30	Eggs	Chicken	
Derquantel	ADI: 0-0.3	Full	78 (2013)	0.3	Muscle	Sheep	Derquantel
				0.4	Kidney		
				7.0	Fat		
				0.8	Liver		
Dexamethasone	ADI: 0-0.015	Full	70 (2008)	1	Muscle, Kidney	Cattle, Pig, Horse	Dexamethasone
				2	Liver	Cattle, Pig, Horse	
				0.3	Milk	Cattle	
Diclazuril	ADI: 0-30	Full	50 (1998)	500	Muscle	Sheep, Rabbit, Poultry	Diclazuril
				3000	Liver		Poultry skin + fat
				2000	Kidney		
				1000	Fat		
Dicyclanil	ADI: 0-7	Full	60 (2003)	150	Muscle		Dicyclanil
				125	Liver, Kidney	Sheep	
				200	Fat		
Diflubenzuron	No ADI or ARfD		81 (2015)	No MRL			
Dihydro-streptomycin Streptomycin	ADI: 0-50 (Group ADI)	Full	58 (2002)	600	Muscle, Liver, Fat	Cattle, Pig, Chicken, Sheep	Sum of dihydrostreptomycin and streptomycin
				1000	Kidney		
				200	Milk	Cattle, Sheep	
Dimetridazole	No ADI or ARfD		34 (1989)	No MRL			
Diminazene	ADI: 0-100	Full	42 (1994)	500	Muscle	Cattle	Diminazene

Substance	ADI/ARfD (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
				12000	Liver		
				6000	Kidney		
				150	Milk		
Doramectin	ADI: 0-1	Full	62 (2004)	10	Muscle	Cattle	Doramectin
				5	Muscle	Pigs	
				100	Liver	Cattle, Pigs	
				30	Kidney	Cattle, Pigs	
				150	Fat	Cattle, Pigs	
				15	Milk	Cattle	
Emamectin benzoate	ADI: 0–0.5	Full	78 (2013)	100	Muscle Fillet (muscle with skin)	Salmon Trout	Emamectin B1a
Enrofloxacin	ADI: 0-2	Full	48 (1997)	No MRL			
Eprinomectin	ADI: 0-10	Full	50 (1998)	100	Muscle	Cattle	Eprinomectin B_{1a}
				2000	Liver		
				300	Kidney		
				250	Fat		
				20	Milk		
Erythromycin	ADI: 0-0.7	Full	66 (2006)	100	Muscle, Liver, Kidney, Fat/Skin	Chicken, Turkey	Erythromycin A
				50	Eggs	Chicken	
Estradiol-17β	A|DI: 0-0.05	Full	52 (1999)	Not specified	Muscle, Liver, Kidney, Fat	Cattle	
Febantel Fenbendazole Oxfendazole	ADI: 0-7 (group ADI)	Full	50 (1998)	100	Muscle, Kidney, Fat	Cattle, Goat, Horses,	Sum of febantel, fenbendazole and oxfenbendazole, expressed as oxfendazole sulfone equivalents
				500	Liver	Pig, Sheep	
				100	Milk	Cattle, Sheep	

Substance	ADI/ARfD (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Fenbendazole (see Febantel)							
Fluazuron	ADI: 0-40	Full	48 (1997)	200 500 7000	Muscle Liver, Kidney Fat	Cattle	Fluazuron
Flubendazole	ADI: 0-12	Full	40 (1992)	10 200 500 400	Muscle, Liver Muscle Liver Eggs	Pig Poultry Poultry Poultry	Flubendazole
Flumequine	ADI: 0-30	Full	66 (2006)	500 1000 500 3000 500 500T	Muscle Fat Liver Kidney Muscle Muscle	Cattle, Sheep, Pig, Chicken Trout Black Tiger Shrimp, Shrimp	Flumequine. The MRLs are temporary for Black Tiger Shrimp and Shrimp. The MRLs for shrimp applies to all fresh water and marine shrimp.
Furazolidone	No ADI or ARfD		40 (1992)	No MRL			
Gentamicin	ADI: 0-20	Full	50 (1998)	100 2000 5000 200	Muscle, Fat Liver Kidney Milk	Cattle, Pig Cattle	Gentamicin
Gentian violet	No ADI or ARfD		78 (2013)	No MRL			
Imidocarb	ADI: 0-10	Full	60 (2003)	300	Muscle	Cattle	Imidocarb, free base

Substance	ADI/ARfD (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
				1500	Liver		
				2000	Kidney		
				50	Fat, Milk		
Ipronidazole	No ADI or ARfD		34 (1989)	No MRL			
Isometamidium	ADI: 0-100	Full	40 (1992)	100	Muscle, Fat, Milk	Cattle	Isometamidium
				500	Liver		
				1000	Kidney		
Ivermectin	ADI: 0–10 ARfD: 200	Full	81 (2015)	30	Muscle	Cattle	Ivermectin B_{1a}. The Committee considers that the presence of high concentrations of ivermectin residues at the injection site is product dependent and must be assessed on a case-by-case basis during marketing authorization by comparison of suitable acute dietary exposure estimates with the ARfD.
				800	Liver	Cattle	
				100	Kidney	Cattle	
				400	Fat	Cattle	
				15	Liver	Pig, Sheep	
				20	Fat	Pig, Sheep	
				10	Milk	Cattle	
Lasalocid sodium	ADI: 0–5	Full	81 (2015)	400	Muscle	Chicken, Turkey, Quail, Pheasant	Lasalocid A
				1200	Liver		
				600	Kidney		
				600	Fat/Skin		
Levamisole	ADI: 0-6	Full	42 (1994)	10	Muscle, Kidney, Fat	Cattle, Sheep, Pig, Poultry	Levamisole
				100	Liver		
Lincomycin	ADI: 0-30	Full	62 (2004)	200	Muscle	Chicken, Pig	Lincomycin
				500	Liver	Chicken, Pig	

Substance	ADI/ARfD (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
				1500	Kidney	Pig	A separate MRL of 300 µg/kg for skin with adhering fat for pigs was recommended in order to reflect the concentrations found in skin of pigs and this MRL was also extended skin/fat for chicken.
				500	Kidney	Chicken	
				100	Fat	Chicken, Pig	
				150	Milk	Cattle	
Melengestrol Acetate	ADI: 0-0.03	Full	66 (2006)	1	Muscle	Cattle	Melengestrol acetate
				10	Liver		
				2	Kidney		
				18	Fat		
Metronidazole	No ADI or ARfD		34 (1989)	No MRL			
Monensin	ADI: 0-10	Full	70 (2008)	10	Muscle, Liver, Kidney	Chicken, Turkey, Quail	Monensin
			75 (2011)	10	Muscle, Kidney	Cattle, Sheep, Goat	
				20	Liver	Sheep, Goat	
				100	Liver	Cattle	Cattle liver MRL revised at 75 JECFA
				100	Fat	Cattle, Sheep, Goat, Chicken, Turkey, Quail	
				2	Milk	Cattle	
Monepantel	ADI: 0-20	Full	78 (2013)	500	Muscle		Monepantel sulfone
				7000	Liver	Sheep	
				1700	Kidney		

Substance	ADI/ARfD (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
				13000	Fat		
Moxidectin	ADI: 0-2	Full	50 (1998)	20 50 100 50 500	Muscle Muscle Liver Kidney Fat	Cattle, Deer Sheep Cattle, Deer, Sheep Cattle, Deer, Sheep Cattle, Deer, Sheep	Moxidectin The Committee noted very high concentrations and great variation in the residue levels at the injection site in cattle over a 49-day period after dosing.
Narasin	ADI: 0-5	Full	70 (2008) 75 (2011)	15 50 15 50	Muscle, Kidney Liver, Fat Muscle, Kidney Liver, Fat	Chicken, Pig Chicken, Pig Cattle Cattle	Narasin A Temporary MRLs for cattle, replaced with full MRLs in cattle tissue
Neomycin	ADI: 0-60	Full	60 (2003)	500 10000 1500 500	Muscle, Fat, Liver Kidney Milk Eggs	Cattle, Chicken, Sheep, Turkey Goat, Pig, Duck Cattle Chicken	Neomycin
Nicarbazin	ADI: 0-400	Full	50 (1998)	200	Muscle, Liver, Kidney, Fat/Skin	Chicken (broilers)	N,N'-bis(4-nitrophenyl)urea
Nitrofurazone/ Nitrofural	No ADI		40 (1992)	No MRL			
Olaquindox	No ADI or ARfD		42 (1994)	No MRL			The Committee recommended no MRLs but noted that 4µg/kg in muscle of pigs of the metabolite MQCA (3-Methylquinoxaline-2-carboxylic acid) is consistent with Good Veterinary Practice.
Oxfendazole (See Febantel)							
Oxolinic acid	No ADI or ARfD		43 (1994)	No MRL			

Substance	ADI/ARfD (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Oxytetracycline See chlortetracycline							
Permethrin	No ADI or ARfD		54 (2000)	No MRL			
Phoxim	ADI:0-4	Full	62 (2004)	50 400	Muscle, Liver, Kidney Fat	Goat, Pig, Sheep	Phoxim
Pirlimycin	ADI: 0-8	Full	62 (2004)	100 1000 400 100	Muscle, Fat Liver Kidney Milk	Cattle	Pirlimycin
Porcine Somatotropin	ADI: Not Specified		52 (1999)	Not Specified	Muscle, Liver, Kidney, Fat	Pig	
Procaine benzylpenicillin	ADI: < 30µg/person/ day of the penicillin moiety	Full	50 (1998)	50 4	Muscle, Liver, Kidney Milk	All species	Benzylpenicillin
Progesterone	ADI: 0-30	Full	52 (1999)	Not Specified	Muscle, Liver, Kidney, Fat	Cattle	
Propionyl-promazine	No ADI or ARfD		38 (1991)	No MRL			
Ractopamine hydrochloride	ADI: 0-1	Full	66 (2006)	10 40 90	Muscle, Fat Liver Kidney	Cattle, Pig	Ractopamine
Ronidazole	No ADI or ARfD		42 (1994)	No MRL			
Sarafloxacin	ADI: 0-0.3	Full	50 (1998)	10 80 20	Muscle Liver, Kidney Fat/skin	Chicken, Turkey	Sarafloxacin

Substance	ADI/ARfD (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Spectinomycin	ADI: 0-40	Full	50 (1998)	500 2000 5000 2000 200(µg/L)	Muscle Liver, Fat Kidney Eggs Milk	Cattle, Chicken, Pig, Sheep Chicken Cattle	Spectinomycin
Spiramycin	ADI: 0-50	Full	48 (1997)	200 600 300 800 300 200(µg/L)	Muscle Liver Kidney Kidney Fat Milk	Cattle, Chicken, Pig Cattle, Chicken, Pig Cattle, Pig Chicken Cattle, Chicken, Pig Cattle	For cattle and chicken, MRLs are expressed as the sum of spiramycin and neospiramycin. For pigs, the MRLs are expressed as spiramycin equivalents (antimicrobial active residues).
Streptomycin (See dihydro-treptomycin)							
Sulfadimidine (Sulfamethazine)	ADI: 0-50	Full	42 (1994)	100 25	Muscle, Liver, Kidney, Fat Milk	Cattle, Sheep, Pig, Poultry Cattle	Sulfadimidine
Sulfathiazole	No ADI or ARfD		34 (1989)	No MRL			
Teflubenzuron	ADI: 0–5	Full	81 (2015)	400 400	Muscle Muscle plus skin in natural proportion	Salmon Salmon	Teflubenzuron
Testosterone	ADI: 0-2	Full	52 (1999)	Not specified	Muscle, Liver, Kidney, Fat	Cattle	
Tetracycline (See chlortetracycline)							
Thiamphenicol	ADI: 0-5	Full	58 (2002)	No MRL			
	ADI: 0-100	Full	58 (2002)	100			

Substance	ADI/ARfD (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Tiabendazole (Thiabendazole)					Muscle, Liver, Kidney, Fat	Cattle, Pig, Goat, Sheep	Sum of tiabendazole + 5-hydroxy tiabendazole
				100	Milk	Cattle, Goat	
Tilmicosin	ADI: 0-40	Full	70 (2008)	100	Muscle, Fat	Cattle, Pig, Sheep	Tilmicosin
				1000	Liver	Cattle Sheep	
				1500	Liver	Pig	
				300	Kidney	Cattle, Sheep	
				1000	Kidney	Pig	
				150	Muscle	Chicken	
				100	Muscle	Turkey	
				2400	Liver	Chicken	
				1400	Liver	Turkey	
				600	Kidney	Chicken	
				1200	Kidney	Turkey	
				250	Skin/Fat	Chicken, Turkey	
Trenbolone acetate	ADI: 0-0.02	Full	34 (1989)	2	Muscle	Cattle	β Trenbolone for muscle
				10	Liver		α-Trenbolone for liver
Trichlorfon (Metrifonate)	ADI: 0-2	Full	66(2006)	50	Milk	Cattle	Trichlorfon
				50	Muscle, Liver, Kidney, Fat		Guidance MRLs at the limit of quantitation of the analytical method for monitoring purposes. No residues should be present in tissues when used with Good Veterinary Practice.
Triclabendazole	ADI: 0-3	Full	70 (2008)	250	Muscle	Cattle	Keto-triclabendazole
				850	Liver	Cattle	

Substance	ADI/ARfD (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
				400	Kidney	Cattle	
				200	Muscle	Sheep	
				300	Liver	Sheep	
				200	Kidney	Sheep	
				100	Fat	Sheep,Cattle	
Tylosin	ADI: 0-30	Full	70 (2008)	100	Muscle, Liver, Kidney	Cattle, Pig, Chicken	Tylosin A
				100	Fat	Cattle, Pig	
				100	Skin/Fat	Chicken	
				100	Milk	Cattle	
				300	Eggs	Chicken	
Xylazine	No ADI or ARfD		47 (996)	No MRL			
Zeranol	ADI: 0-0.5	Full	32 (1987)	2	Muscle	Cattle	Zeranol
				10	Liver		
Zilpaterol hydrochloride	ADI: 0–0.04 ARfD: 0.04	Full	81 (2015)	0.5	Muscle	Cattle	Zilpaterol (free base). The GEADE is 1.9 µg/day for the general population, which represents approximately 80% of the ARfD.
				3.5	Liver		
				3.3	Kidney		

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2. Residue evaluation of certain veterinary drugs – Joint FAO/WHO Expert Committee on Food Additives, 66th meeting 2006

3. Compendium of food additive specifications – Joint FAO/WHO Expert Committee on Food Additives, 67th meeting 2006

4. Compendium of food additive specifications – Joint FAO/WHO Expert Committee on Food Additives – 68th meeting 2007

5. Compendium of food additive specifications – Joint FAO/WHO Expert Committee on Food Additives, 69th meeting 2008

6. Residue evaluation of certain veterinary drugs – Joint FAO/WHO Expert Committee on Food Additives. 70th meeting 2008

7. Compendium of food additive specifications – Joint FAO/WHO Expert Committee on Food Additives, 71st meeting 2009

8. Safety evaluation of certain contaminants in food – Joint FAO/WHO Expert Committee on Food Additives, 72nd meeting 2010. Joint FAO/WHO publication: WHO Food Additives Series No. 63/FAO JECFA Monographs 8

9. Residue evaluation of certain veterinary drugs – Joint FAO/WHO Expert Committee on Food Additives, Meeting 2010 – Evaluation of data on ractopamine residues in pig tissues

10. Compendium of food additive specifications – Joint FAO/WHO Expert Committee on Food Additives, 73rd meeting 2010

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15. Residue evaluation of certain veterinary drugs, Joint FAO/WHO Expert Committee on Food Additives.78th meeting 2014

16. Compendium of food additive specifications, Joint FAO/WHO Expert Committee on Food Additives. 79th meeting 2014

17. Compendium of food additive specifications, Joint FAO/WHO Expert Committee on Food Additives. 80th meeting 2015

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RESIDUE EVALUATION OF CERTAIN VETERINARY DRUGS

Joint FAO/WHO Expert Committee on Food Additives

81st Meeting 2015

This document contains food additive specification monographs, analytical methods, and other information prepared at the seventy-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which was held in Rome, Italy, from 17–26 November 2015. The specification monographs provide information on the identity and purity of food additives used directly in foods or in food production. The main three objectives of these specifications are to identify the food additive that has been subjected to testing for safety, to ensure that the additives are of the quality required for use in food or in processing and to reflect and encourage good manufacturing practice. This publication and other documents produced by JECFA contain information that is useful to all those who work with or are interested in food additives and their safe use in food.

ISBN 978-92-5-109210-1 ISSN 1817-7077

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I5590E/1/04.16