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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

World Health Organization

Food and Agriculture Organization of the United Nations

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
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SPECIAL NOTE

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Evaluation of data on ractopamine residues in pig tissues

January – May 2010 (Electronic meeting)

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ABBREVIATIONS

ADI	Acceptable daily intake
AOAC	AOAC International
AUC	Area under the curve
BW or bw	Body weight
CAC	Codex Alimentarius Commission
CAS	Chemical Abstracts Service
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Foods
Cl	Clearance rate
Cmax	Maximum concentration
CR	Renal clearance
CV	Coefficient of variation
Cv_r	Repeatability
Cc_R	Reproducibility
ECD	Electron capture detector
EDI	Estimated daily intake
FAO	Food and Agriculture Organization of the UN
GC	Gas chromatography
GLP	Good laboratory practice
H or h	Hour
HPLC	High pressure liquid chromatography
IM	Intramuscular
IR	Infrared
IU	International Unit
IUPAC	International Union of Pure and Applied Chemistry
IV	Intravenous
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
Kg or kg	Kilogram (10^3grams)
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantitation
µg	microgram (10^{-6}grams)
mg	milligram (10^{-3}grams)
min	Minimum or minute
mL or ml	milliliter
MRL	Maximum Residue Limit
MS	Mass spectrometry
MW or mw	Molecular weight
N	Negative
NA or na	Not analyzed or not applicable
NC or nc	Not calculated
ND	Not detected
NOEL	No effect level
NQ	Non quantifiable
P	Positive
QA	Quality assurance
QC	Quality control
RP	Reverse phase
SC	Subcutaneous (injection)
SD	Standard deviation

S/N	Signal to noise ratio
SPE	Solid phase extraction
SD	Standard deviation
s.e.	Standard error
$t_{½}$	Half life
TR	Total residue
TMDI	Theoretical maximum daily intake
TRR	Total radiolabelled residues
TRS	Technical Report Series
TSP	Thermospray
UV	Ultraviolet
Vd	Volume of distribution
WHO	World Health Organization

INTRODUCTION

With reference to the request of the Codex Alimentarius Commission (CAC) at its thirty-second session (Rome, Italy 29 June – 4 July 2009, ALINORM 09/32/REP) to undertake a review of new data on residues of ractopamine in pig tissues, a summary of which was submitted to the eighteenth session of the Codex Committee on Residues of Veterinary Drugs in Food by the People's Republic of China, the Secretariat of JECFA at FAO and WHO requested the submission of these data and any other pertinent information related to depletion of residues of ractopamine in pig tissues. The Call for data was published on the JECFA websites 4 November 2009 with a deadline 15 December 2009 for submission of the full studies referred to by the People's Republic of China. Three study reports were submitted to FAO by the Codex contact point of the People's Republic of China on 20 December 2009. In addition, the full dossier previously submitted to the JECFA Secretariat for the evaluation of ractopamine was made available again by the sponsor of this veterinary drug. The People's Republic of China submitted one additional report of an experimental study on ractopamine residues in pig tissues on 13 May 2010 and asked the JECFA Secretariat to consider the data in that report.

Due to the urgency and specificity of the request for scientific advice from the 32nd CAC, and in view of the lack of time and resources to convene a regular JECFA meeting, the JECFA Secretariat at FAO and WHO agreed that a meeting in electronic format would be constituted to address the request.

Ractopamine has been evaluated by JECFA at the fortieth, sixty-second and sixty-sixth meetings of the Committee. An ADI of 0 – 1 µg/ kg bodyweight was established at the sixty-second meeting and full MRLs were recommended for tissues of cattle and pigs. The MRL recommendations were confirmed by the sixty-sixth meeting of the Committee.

The monograph addendum in this volume of the FAO JECFA Monographs on the residues of, exposure to and statements on the studies on ractopamine residues in pig tissues submitted were prepared by the invited experts for this electronic meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) during the period of January to May 2010. This was the twentieth meeting convened specifically to consider residues of veterinary drugs in food producing animal species. The Committee has evaluated residues of veterinary drugs at its 12th, 26th, 27th, 32nd, 34th, 36th, 38th, 40th, 42nd, 43rd, 45th, 47th, 48th, 50th, 52nd, 54th, 58th, 60th, 62nd, 66th and 70th meetings (ref. 1-15 and 18-23). The tasks for the Committee was to evaluate the three residue depletion studies on ractopamine in pig tissues submitted by the People's Republic of China, consider any other relevant studies previously assessed in this context by the Committee, provide recommendations on whether the information contained in the three studies would have an impact on the MRLs for ractopamine in pig tissues previously recommended by JECFA and consider any other scientific issues arising from the evaluation of the studies. The additional study received in May 2010 was considered separately due to its late submission.

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, November 1984 (ref. 16). 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 (ref. 17). 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. Nineteen such meetings of JECFA have been held prior to this meeting.

On-line edition of Residues of some veterinary drugs in animals and foods (from FAO JECFA Monographs and FAO Food and Nutrition paper Number 41)

The monographs and statements that have been published in the FAO JECFA Monographs 2 and 6 as well as those published in FAO Food and Nutrition Paper Series 41 (sixteen volumes since 1988) are all available online at http://www.fao.org/ag/agn/jecfa-vetdrugs/search.html. 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 the FAO homepage of JECFA at http://www.fao.org/ag/agn/agns/jecfa_index_en.asp . Readers are invited to address comments and questions on this publication and other topics related to the work of JECFA to:

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RACTOPAMINE HYDROCHLORIDE

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ADDENDUM

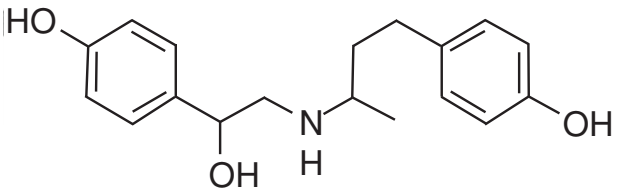
Addendum to the monographs prepared by the 40th, 62nd and 66th meetings of the Committee and published in FAO Food & Nutrition Paper 41/5, 41/16 and FAO JECFA Monographs 2, respectively.

IDENTITY

Chemical Name: 4-Hydroxy-α-[[[3-(4-hydroxyphenyl)-1-methylpropyl]amino]methyl] benzenemethanol hydrochloride {International Union of Pure and Applied Chemistry (IUPAC) name}
Benzenemethanol, 4-Hydroxy-α-[[[3-(4-hydroxyphenyl)-1-methylpropyl]amino]methyl]-hydrochloride {Chemical Abstracts Service (CAS) name; CAS number 90274-24-1}

Synonyms: Ractopamine hydrochloride (common name); proprietary names: Paylean®, Optaflexx®

Structural formula:



Background on ractopamine risk analysis process

At the 62nd meeting, in its review of ractopamine hydrochloride the Committee established an ADI of 0-1 µg per kg of body weight, rounded to one significant figure from the calculated value of 0-1.34 µg per kg of body weight, equivalent to 0 - 60 µg for a 60 kg person, and recommended the following MRLs for edible tissues of pigs and cattle, expressed as free ractopamine base: muscle, 10 µg/kg; liver, 40 µg/kg; kidney, 90 µg/kg; fat, 10 µg/kg. The calculated Theoretical Maximum Daily Intake, based on these MRLs and the ratios of marker to total residues at the 12 h depletion time point used to derive the MRLs, was estimated to be 50 µg, or 84% of the upper bound of the ADI.

The 66th meeting of the Committee reviewed and affirmed the practices used in establishment of an ADI, and the MRLs for ractopamine by the Committee at its 62nd meeting. The ADI was based on a NOEL of 67 µg/kg bw for acute cardiac responses in a human study, with the application of a safety factor of 50. This combined factor is comprised of a factor of 10 to account for individual variability and an additional factor of 5 to account for protection of sensitive individual and to account for the small sample size in the study. The MRLs recommended for liver and kidney of pigs and cattle by the 62nd Committee were based primarily on the large pool of data available from studies in pigs, supported by a smaller number of studies and data points for cattle. The 66th Meeting of the Committee confirmed the MRLs recommended by the Committee at its 62nd meeting. The Committee calculated an estimated daily intake of 9.0 µg per day per person using the median values from the residue data used in calculation of the MRLs for pigs at the 62nd Meeting of the Committee and the data-derived factors for marker to total residues for pig tissues. This

estimate is well below the ADI. Based on this evaluation, the 17th Session of CCRVDF, held in 2007, agreed to forward the proposed MRLs for ractopamine in pig and cattle tissues for adoption to the Codex Alimentarius Commission (CAC).

The government of the People's Republic of China had decided to conduct a comprehensive evaluation of the human health risk from food of animal origin through the use of ractopamine, because of concern about residue levels in different pig breeds and different farming methods in China. A summary of the test results from three residue depletion studies in pigs was submitted to the 18th Session of CCRVDF held in 2009. The Delegation of the People's Republic of China particularly expressed concern over the residue levels in lung, stomach, heart, large and small intestine as well as at early time points after withdrawal of medicated feed. The Delegation was of the view that such animal organs are important components of the diet for most consumers in Asian countries, including China.

The summary of the results of the three residue studies was also presented to the 32nd CAC held in 2009. Based at least in part on comments submitted by the People's Republic of China, the 32nd CAC requested FAO and WHO to request JECFA to undertake a review of new data on residues of ractopamine in pig tissues and to consider whether these data would have any implication on the recommended MRLs for ractopamine in pig tissues, accordingly. Subsequent to the 32nd Session of the CAC, People's Republic of China provided the detailed study reports of the three independent residue depletion studies to the JECFA Secretariat.

New residue studies in the pig

In the three new ractopamine residue depletion studies, different breeds of pigs and different feeding methods were used and they were carried out at three different national laboratories, located in Wuhan, Guangzhou and Beijing. The studies are referred to in this monograph as the three new studies and individually by the location in which the respective studies were carried out. The ractopamine residues in tissues collected were all analyzed using a deconjugation step prior to analysis.

In the study performed in Wuhan (Study no. 09-04 Elanco Animal Health: N), 40 Hubei White Swine were weighed and divided into 8 groups, each group comprising five animals. All pigs received a dose of approximately 20 mg ractopamine HCl per kg medicated animal feed daily for 30 days. (Summary results of the feeding regime are provided below in table 1). An additional group of eight animals served as controls. Five animals were slaughtered at 6, 12 hours, and 1, 2, 3, 5, 7 and 9 days following withdrawal of medicated feed. Muscle, liver, kidney, heart, lung, stomach, large intestine and small intestine were collected and analyzed. The results demonstrated that the ractopamine residue concentrations in liver, kidney, lung, and small intestine were greater than in the other tissues. Residue concentrations in the lung were greater than those in the liver and kidney, and were detected up to nine days following removal of medicated feed. Residues in muscle were below 10 µg/kg at 24 h, but were detectable for five days following removal of medicated feed. Residues in the liver were between 63 and 106 µg/kg at 12h, and one was greater than 40 µg/kg at 24 h following removal of medicated feed, and were detectable for up to five days. Residues in the kidney were highly variable, from 178 and 374 µg/kg at 12h, and one remained greater than 90 µg/kg at 24 h following the withdrawal time, and could be detected up to seven days following the withdrawal time.

In the study performed in Guangzhou (Study no. 2009-MOA-001), 30 Spotted Small-ear pigs were weighed and divided into six groups, each comprising five animals. All pigs received a daily dose at a rate of approximately 20 mg ractopamine HCl per kg medicated animal feed for 30 days. An additional group of six animals served as a control group. Animals were slaughtered 6, 12 hours, and 1, 2, 3 and 5 days after treatment. Samples of muscle, liver, kidney, heart, lung, stomach, large intestine and, small intestine, were collected from all treated and control animals. The results showed that the distribution of ractopamine demonstrated tissue selectivity in the pig, with the highest residue concentrations at 12 h in kidney, with the lung containing the second highest concentrations, followed by stomach, liver, small intestine, large intestine and muscle. The study showed that the mean ractopamine residue level in liver after 6 h and the mean ractopamine residue levels in kidney after 6 h and 12 h were all above 40 µg/kg for liver and 90 µg/kg for kidney. Ractopamine residues in the lung depleted slowly.

In the study carried out in Beijing (Study no. 2009001), seven groups of Landrace x Large Yorkshire binary cross pigs, with each group comprising five animals, received ractopamine HCl medicated feed at a dose rate of approximately 20 mg per kg daily for 30 days. An additional group of five animals served as a control group. Five animals were slaughtered at 12h and at 1, 2, 3, 5, 7 and 11 days after treatment. Samples of muscle, liver, kidney, heart, lung, stomach, small intestine, and large intestine were collected from all treated and control animals. Ractopamine residue concentrations were above the limit of quantification ($0.5\mu g.kg^{-1}$) in all the tissues collected 11 days after the treatment with the exception of muscle.

A summary of the studies considered in this monograph addendum are presented below in Table 1. It includes relevant studies in the pig from the original dossier evaluated by the Committee at its 62nd and 66th meetings and the three new residues studies in pigs.

Table 1. Summary of the design of the studies cited.

Study code	Breed	Body weight before first dose [kg]	Dose expressed as	Content in medicated feed in these units	Medicated feed consumption information[1]	Duration of dosing [days]	Withdrawal times [hours]	Tissues analysed	Analyte/ method	Result of tissue analysis expressed as
AAC8614	crossbred	77.1-90.7[2]	ppm ractopamine hydrochloride in feed	31.3-33.4	Not given	7	12	Liver, kidney	Parent compound/ HPLC	ppm ractopamine hydrochloride
							24	Liver, kidney		
AAC8924		99.5-111.5		8.1		5	12	Liver, kidney, muscle , fat		µg/kg (ppb) ractopamine hydrochloride
				12.9		5	12			
						6	48	Liver, kidney		
							96			
ABC-0231		approximately 50 kg		75.9	250g administered twice daily to each animal	7	6	Liver, kidney, muscle , fat	"total residue" /radioactivity counting	ppm free base
							36			
							120			
ABC-0273		approximately 50 kg		66.9 (radioassay)	500g administered twice daily to each animal	4	12		"total residue" and "nonextractable residue"/radioactivity counting	ppm ractopamine hydrochloride equivalents
						7				
						10				
ABC-0283		Approximately 45 kg		78.2 (radioassay)	400g administered twice daily to each animal	4	12	Muscle, liver, kidney, fat, blood, bile	"total residue" and "nonextractable[3] residue"/radioactivity counting	
							24			
							48			
ABC-0291a		Approximately 50 kg		60.8 (radioassay)	500g administered twice daily to each animal		12	Liver, kidney, muscle, fat	"total residue" /radioactivity counting	
							24			
							96			
							168			
ABC-0291b[4]							12	Liver, kidney	"nonextractable[5] residue"/radioactivity counting	
							24			
							96			
							168			
ABC-0368		Approximately 45 kg		58.0 (radioassay)			12	Liver, kidney	Total residue; methanol extractable parent drug after	

[1] Total amount of feed administered per day was twice 750g in study ABC-0231, and twice 1 kg in studies ABC-0273, ABC-0283, ABC-0291, and ABC-0368/369.

[2] Original data expressed in lbs: 183.2 $\pm$ 11.82

[3] In liver and kidney only

[4] Tissues obtained in study 0291a were used

[5] In liver and kidney only

									HPLC	
Study code	**Breed**	**Body weight before first dose [kg]**	**Dose expressed as**	**Content in medicated feed in these units**	**Medicated feed consumption information**[6]	**Duration of dosing [days]**	**Withdrawal times [hours]**	**Tissues analysed**	**Analyte/ method**	**Result of tissue analysis expressed as**
ABC-0369									Parent and metabolites A,B,C,D,E,F	
T4V739003	crossbred	Approximately 50 kg	ppm ractopamine hydrochloride in feed	40.6	500g administered twice daily to each animal	7	24	Liver, kidney, muscle , fat	Total residue; methanol extractable parent drug after HPLC	µg/kg (ppb) ractopamine hydrochloride equivalents
							48			
T4V739004		Approximately 50 kg		41.0	500g administered twice daily to each animal	7	24		Total residue; methanol extractable parent drug after HPLC	
							48			
							72			
T4V629001		86.5-97.3[7]		20[8]	3.0-4.2 kg/animal/day[9]	9	24	Liver, kidney	methanol extractable parent drug after HPLC with ECD	
							48			
							72			
							96			
							120			
T4V629501		94.5-118.3		21.3	3.19-3.89[10] kg/animal/day	10	12		methanol extractable parent drug after HPLC with fluorescence detection	
							24			
							36			
							48			
							60			
							72			
T4V759003		77-96[11]		19[12]	2.5-3.0 kg/animal/day[13]	14	12	Liver, kidney, muscle, fat, skin	methanol extractable parent drug after HPLC with ECD	
							24			
							48			
							72			
							96			
							120			

[6] Total amount of feed administered per day was twice 750g in study ABC-0231, and twice 1 kg in studies ABC-0273, ABC-0283, ABC-0291, and ABC-0368/369.

[7] Table 1 on page 8 provides start and end weights and states in a footnote:"Weights recorded at start and end of treatment". This statement is possibly not correct. Start weight may mean weight at the beginning of study, because different start weights are given in table 6 of Appendix A. These weights, which are given as averages of pens of three animals, are more plausible, because more realistic body weight gains result from these data. In the above table the range of pen averages is used.

[8] Initial content was determined as 20.01, final content as 19.61 ppm

[9] Based on pen averages (three animals/pen)

[10] Based on pen averages (three animals/pen)

[11] Basis is table 1 on page 8 of the report

[12] Two batches were analysed with the results 19.15 and 18.83 ppm, resp.

[13] Based on averages of 8 animals

Study code	Breed	Body weight before first dose [kg]	Dose expressed as	Content in feed in these units	Feed consumption information	Duration of dosing [days]	Withdrawal times [hours]	Tissues analysed	Analyte/ method	Result of tissue analysis expressed as
09-04 Elanco Animal Health: N[14]	Hubei White pigs	51-58	ppm ractopamine hydrochloride in feed	20	2.00[15] kg/animal/day	30	6, 12, 24, 48, 72, 120, 168, 216	Muscle, Liver, Kidney, Heart, Lung, Stomach, Small intestine, Large intestine	Ractopamine extracted with ethyl acetate after conjugate hydrolysis/HPLC-MS-MS with deuterated internal standard	Ractopamine hydrochloride
2009001[16]	Landrace× Large Yorkshire binary cross	51-59		20	2.18 kg/animal/day	30	12, 24, 48, 72, 120, 168, 264			
2009-MOA-001[17]	Guangdong Spotted Little-ear	42-52		20	1.63[18]	30	6, 12, 24, 48, 72, 120			

[14] "Wuhan Study"
[15] Average of all animals during treatment period
[16] "Beijing study"
[17] "Guangzhou study"
[18] Average of all animals of five treatment groups during treatment period; data for the sixth group not provided.

A "Meta-Analysis" of residue data for ractopamine in swine

Starting point

Three new residue depletion studies of ractopamine in pigs were submitted for evaluation to the Committee. The studies were conducted in the People's Republic of China in three different locations using three different breeds of pigs. Table 2 summarizes some of the design parameters of the studies together with the parameters of three pivotal residue depletion studies contained in the original dossier (T4V629001, T4V629501 and T4V759003) that formed the data base for the development of the MRLs recommended by the Committee.

Table 2. Design parameters of six residue depletion studies in pigs

Study code	Breed	Body weight before first dose [kg]	Ractopamine hydrochloride in feed [ppm]	Approximate feed consumption [kg/animal/day]	Approximate ractopamine dose [mg/kg bw/day]	Duration of dosing [days]
09-04 Original sponsor Animal Health: N[19]	Hubei White pigs	51-58	20	2.00	0.56	30
2009001[20]	Landrace × Large Yorkshire binary cross	51-59	20	2.18	0.61	30
2009-MOA-001[21]	Guangdong Spotted Little-ear	42-52	20	1.63	0.48	30
T4V629001	"Crossbred"	87-97	20	3.0-4.2	0.68	9
T4V629501		95-118	21.3	3.19-3.89	0.63	10
T4V759003		77-96	19	2.5-3.0	0.52	14

The three new studies differed from the original studies evaluated by the Committee in the following: The animal breeds, the body weight range, and the duration of treatment. The dose ranges were similar in all the studies considered in this review. In the studies originally submitted, the parent drug ractopamine was determined as the marker compound and was the residue definition for the MRLs recommended by the Committee. In the three new studies, the tissues were first subjected to conjugate hydrolysis before the parent drug was extracted and quantitatively determined. The relationship between this type of residue and the total residues is not precisely known from the original dossier and was not established in the new studies.

Thus, a comparison of the two groups of studies, a comparison of the results of the three new studies with the MRLs recommended by the Committee, and a new estimate of dietary intakes may not be possible unless links between the two sets of studies could be identified in a new review of the originally submitted studies. In the following, an attempt is made to perform a new "meta-analysis" of these studies in order to explore the possibility of linking the two groups of studies.

[19] "Wuhan Study"
[20] "Beijing study"
[21] "Guangzhou study"

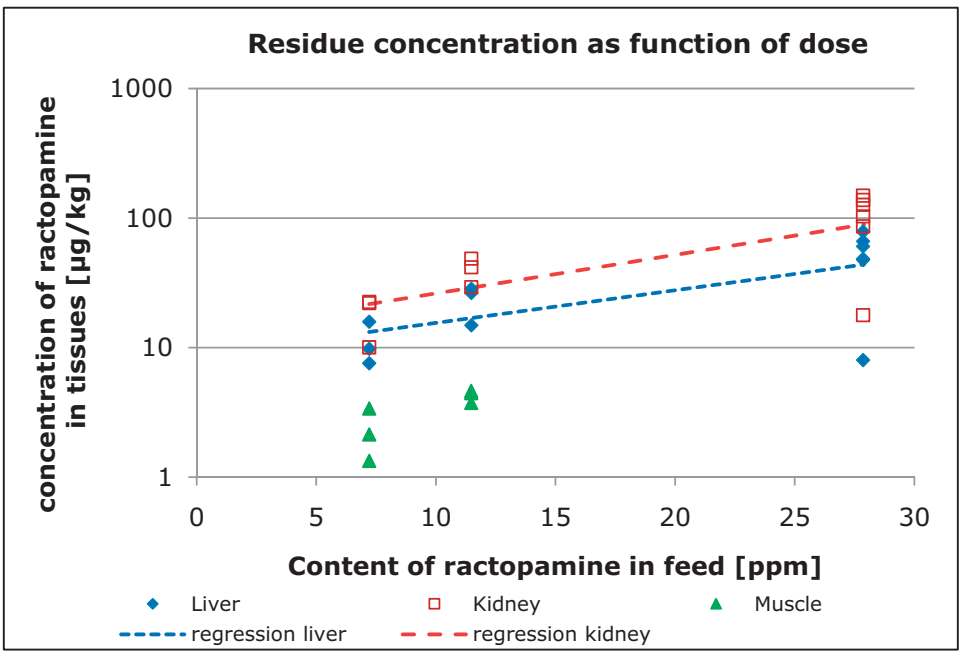
1. Is it possible to establish a mathematical relationship between the dose administered to animals and the observed residue concentrations in tissues?

1.1. Studies in the original dossier for which only the amount of ractopamine in animal feed is known.

In two different studies, groups of crossbred pigs of both sexes were exposed to ractopamine hydrochloride in feed and were slaughtered 12 h after a five day treatment with medicated feed. In the first study (AAC 8614) the level of ractopamine hydrochloride in feed was equivalent to approximately 27.9 mg/kg ractopamine base; in the second study (AAC8924) the two levels used were equivalent to 7.21 and 11.48 mg/kg ractopamine base. In both studies, medicated feed was offered *ad libitum*; data on feed consumption were not given. The six animals slaughtered in the first study had an average body weight of 83.3 kg before treatment and an average daily body weight gain of 1.23 kg. The six animals slaughtered in the second study had an average body weight of 103.3 kg and an average daily body weight gain of 1.47 kg.

The logarithms[22] of the concentrations of methanol-extractable ractopamine free base in liver and kidney were directly related (coefficient of correlation significant at approximately 1% level) to the ractopamine content in the medicated feed. The range of ractopamine content of the feed includes the highest recommended treatment level of 20 mg/kg ractopamine hydrochloride (equivalent to 17.8 mg/kg of free base). The ratio of the residue concentrations in kidney and liver was approximately 1.6 at the lowest level of ractopamine in feed and increased to approximately 2.0 at the highest level of ractopamine in feed. The results are summarized in figure 1.

Figure 1. Relationship between ractopamine levels in pig feed and the concentration of residues in liver and kidney at 12 hour withdrawal time.



1.2. Studies for which the *approximate dose* can be estimated in mg/kg of body weight of the animals

1.2.1. Minimum required exposure time at which steady state of residue concentrations in tissues is reached

[22] The logarithms were used because experience has shown that the distributions of residues found in animals treated with veterinary drugs are typically better represented by log-normal distributions than by normal distributions. In the above example linearity is also seen if the numerical values of the concentrations of the residues are directly used.

In several reports provided in the original dossier it is stated that steady state is reached after four days of exposure. This statement is based on the results obtained in study ABC-0273. In this study, groups of pigs of approximately 50 kg bw were exposed to ractopamine hydrochloride at doses equivalent to approximately 1.2 mg/kg bw/day of ractopamine base for 4, 7, and 10 days, respectively, and were slaughtered after 12 h withdrawal time. The results of the study are presented in figures 2a and 2b.

Figure 2a. Relationship between exposure time to a fixed dose of ractopamine and the concentration of total residue in tissues at 12 hours withdrawal.

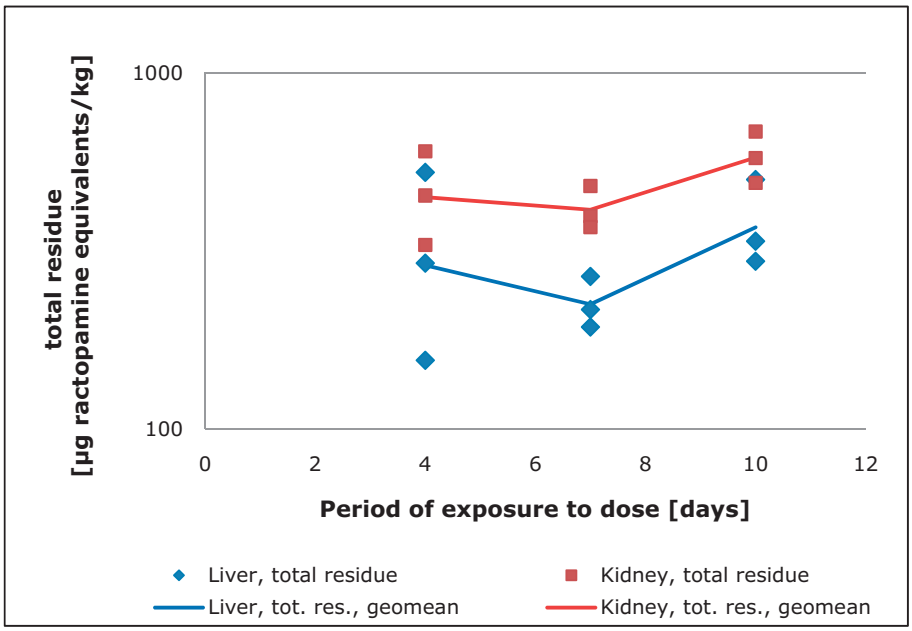
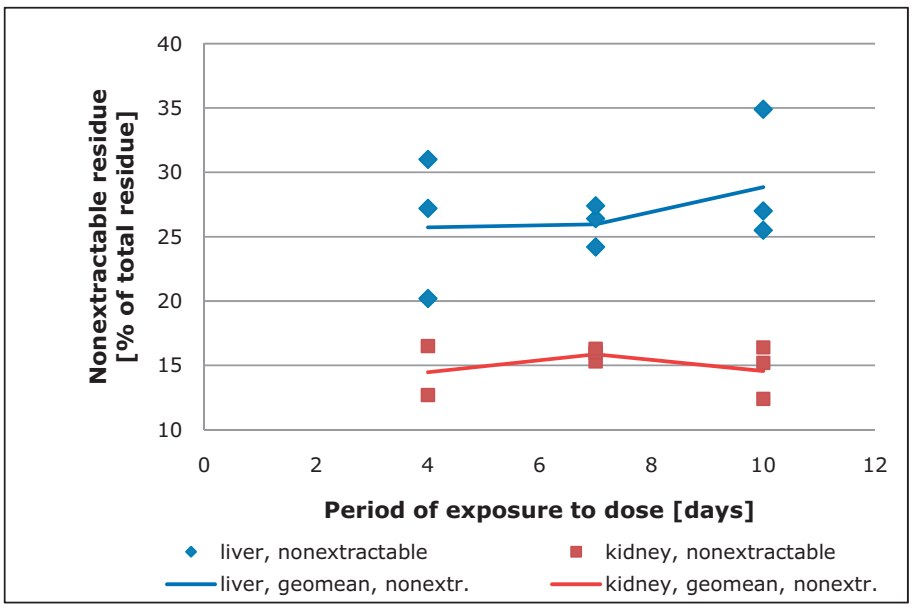


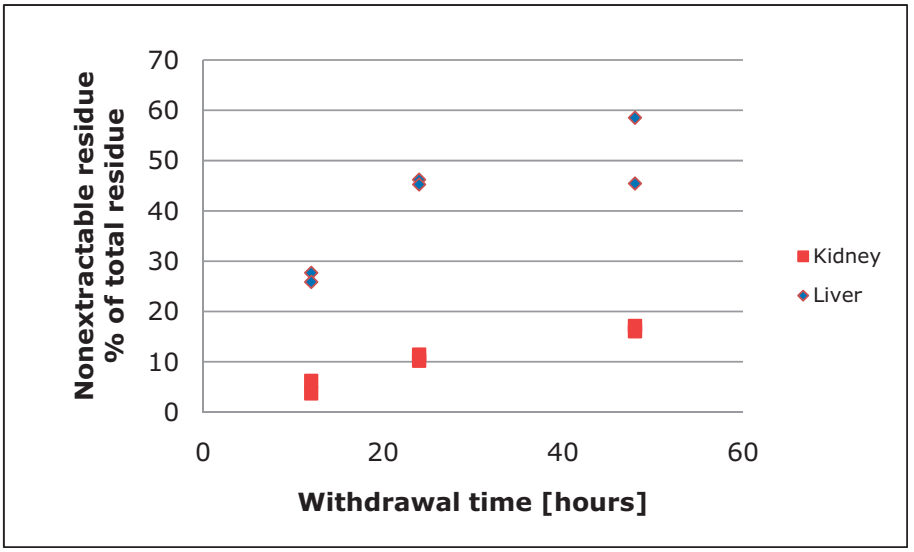
Figure 2b. Relationship between exposure time to a fixed dose of ractopamine and the fraction of non-extractable residues in tissues.



The variability of the results was high and the number of data points limited in this study. It is difficult to judge – on the basis only of this study whether the statement of four days being sufficient to reach steady state is correct. The percentage of non-extractable residue was also determined in study ABC-0283. Body weight of the animals was approximately 45 kg. The dose was equivalent to 1.24 mg/kg bw/day ractopamine base and animals were exposed for four days. The results obtained for the non-extractable

residues and at a withdrawal time of 12 hours were similar in the two studies ABC-0273 and ABC-0283. The percentage of non-extractable residue increased as a function of withdrawal time and with a slightly, but possibly insignificantly higher rate in kidney, however no statistics were performed. The results of the study are presented in figure 3.

Figure 3. Non-extractable residues in liver and kidney as function of withdrawal time.



1.2.2. Relationship between the doses administered to the animals and the observed concentrations of residues

Radiolabelled ractopamine hydrochloride was administered to pigs of both sexes in a variety of studies using a range of doses, exposure times, and withdrawal times before of the animals. Body weights of the animals were similar (45-50 kg) in all studies. The studies noted below were evaluated to investigate the relationship between administered dose and resulting total radioactive residue (information in brackets refers to the table in the Annex where the original data used can be found): ABC-0231 (*table A3*), ABC-0273 (*table A4*), ABC-0283 (*table A5*), ABC-0291 (*table A6*), ABC-0368 (*table A7*), T4V739003 and T4V739004 (*table A9*). The comparison was made on the basis of data at 12 h withdrawal time. These data were either directly available from the studies or were obtained by linear interpolation/extrapolation. The details are given in table 3.

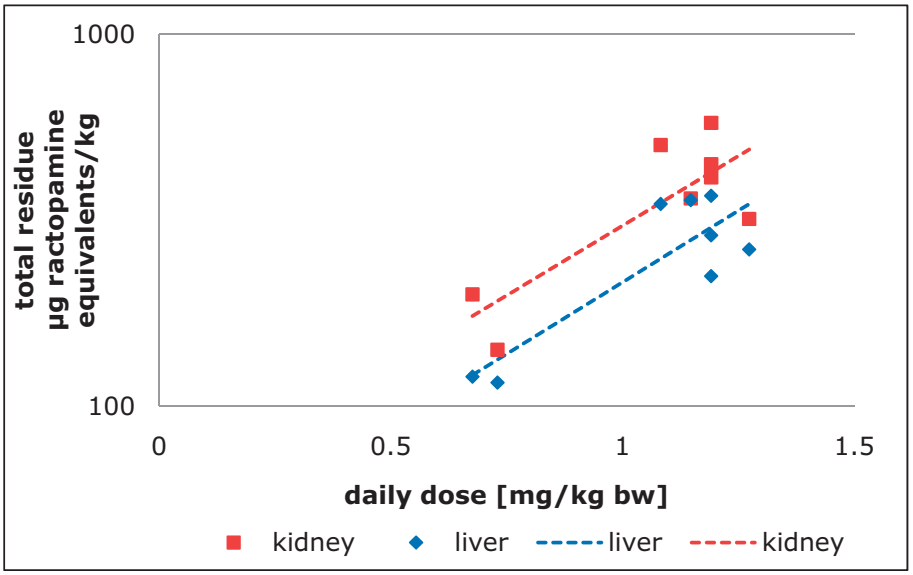
Table 3. Data base for figure 4[1].

Daily dose [mg ractopamine/kg bw]	Period of exposure [days]	Total residue [ppb ractopamine equivalents/kg]		Study code	Method used to obtain 12 h withdrawal time data
0.676	7	199.8	120	ABC-0231	semi-log interpolation between 6 and 36 hours
1.191	4	447.00	287.90	ABC-0273	geometric mean of 12 h data
1.191	7	412.40	223.70		
1.191	10	577.90	367.90		
1.273	4	318.90	264.00	ABC-0283	from semi-log regression line 12-48 hour data
1.082	4	503.70	349.70	ABC-0291	geometric mean of 12 h data
1.147	4	362.00	358.20	ABC-0368	geometric mean of 12 h data
0.73	7	141.80	115.70	T4V739003	Back extrapolation from 24-72 hour on semi-log regression line
				T4V739004	

1) Column 3 refers to residues in kidney; column 4 refers to residues in liver

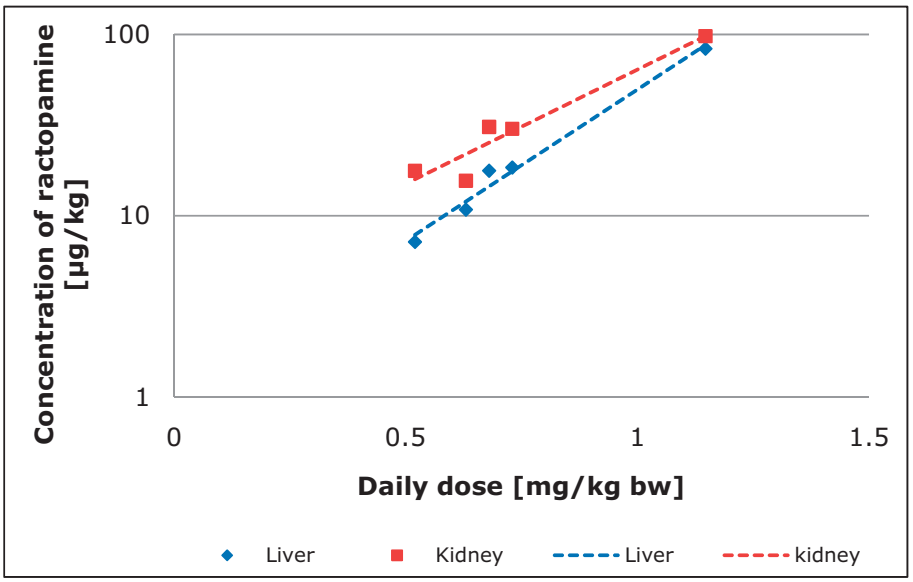
Figure 4 shows the relationship between administered dose and total residue in liver and kidney. A log-linear relationship is possible. The coefficient of correlation is significant at a level < 1%. However, since only two clusters of data points are available, a number of other curves could be fitted to these data.

Figure 4. Total residues in tissues as function of dose.



Similarly, studies were evaluated in which the parent drug ractopamine had been determined, either through radioactivity counting in purified fractions (studies A-0368 and T4V739003/T4V739004) or through HPLC-analysis (studies T4V629001, T4V629501, and T4V759003). A log-linear relationship was obtained for the dose administered and the residue of parent drug in liver and kidney. The coefficient of correlation was significant at about the 0.1% level. The results are presented in figure 5.

Figure 5. Residues of parent ractopamine in tissues as function of dose.

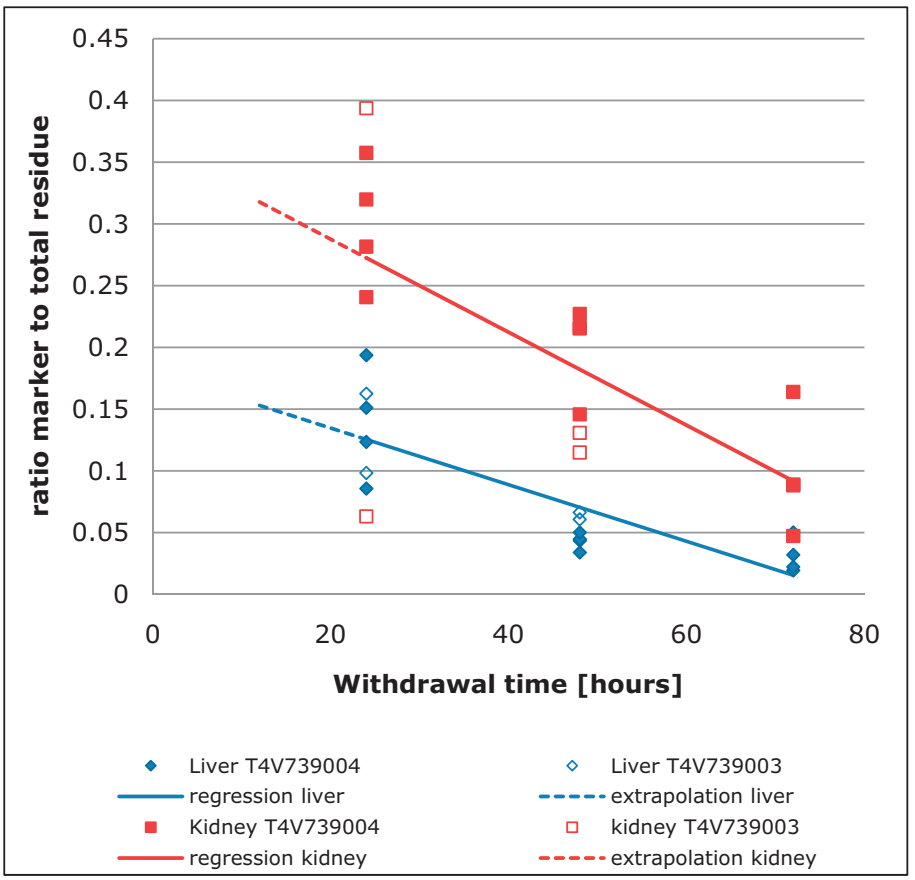


From the results of studies described above it can be concluded that the logarithms of the concentrations of ractopamine residues in liver and kidney of the crossbred animals used in the original sponsor studies were linearly related to the administered dose of the drug.

2. Relationship between different marker residues and total residue in tissues in the three new studies

There are two closely related studies in which the ratio of the established residue marker (free ractopamine base) and total residue was determined. These are the studies T4V739003 and T4V739004. The data from these studies formed the basis for the determination of the numerical value of this ratio in the previous evaluation by the Committee. The studies cover withdrawal times from 24 to 72 hours. The results, including a back extrapolation to 12 hours withdrawal time, are presented in figure 6. The extrapolated ratios at 12 h were 0.153 for liver and 0.318 for kidney.

Figure 6. Ratio of marker to total residue concentrations in liver and kidney.



Ratios of marker to total residues were also determined in study ABC-0368 at a higher dose and using six animals slaughtered at 12 h withdrawal time. The ratios for liver and kidney were 0.234 and 0.272, respectively. The tissues from the ABC-0368 study were used in study ABC-0369 in order to isolate and quantify metabolites of ractopamine. Parent ractopamine and six metabolites A, B, C, D, E, and F were separated. Summing up the percent of total residue provided by the authors for the separated substances, as well as of the non-extractable residues and unidentified polar matter, 77.7 to 93.3% of the total radioactivity was recovered. Table 4 summarizes certain options for the selection of numerical values for the ratios of marker to total residues based on a statistical analysis of the above referenced studies. The values given in the first row from the basis for the factors of the marker residue free ractopamine base to total residues applied in intake assessment. The factors given in the last row could be considered for an intake assessment of the measured residues to total residues for the three new studies. Since the ratio of marker to total residues clearly depends on the withdrawal time, and other possible influencing factors such as administered dose and duration of treatment have not been studied, only the results of similarly designed studies can be compared with some confidence to obtain reliable results. This excludes *a priori* a comparison of the original studies submitted by the sponsor with the three new studies at any withdrawal time other than at 12 hours.

Table 4. Options for the selection of marker to total residue ratios in three new studies.

Study Code	BW of animals [kg]	Ractopamine dose [mg/kg bw/day]	Additional assumptions	Number of data points for each tissue	Ratio marker to total liver	Ratio marker to total kidney
T4V739003 and T4V739004	50	0.723	Parent is marker	16	0.153	0.318
		0.730				
ABC-0368	45	1.147		6	0.234	0.272
ABC-0369					0.287	0.234
			All metabolites A-F are hydrolysable conjugates. Sum is marker		0.620	0.793
			Metabolites A-D are hydrolysable conjugates. Sum is marker		0.565	0.760

3. Kinetics of marker residue depletion

3.1. Depletion studies of the previously submitted studies evaluated by the Committee

3.1.1. Study T4V629001

The report of the study provides individual body weight data for the start of the study and for the start of the treatment period of nine days. End-of-treatment body weights and feed consumption, however, are given on a pen average basis (3 animals per pen). Based on these pen averages the range of body weights before treatment was from 86.5 to 97.3 kg and from 95.0 to 110 kg at the end of treatment. Daily feed consumption was 3.00 to 4.21 kg and daily body weight gain was from 0.89 to 1.44 kg. Feed contained 17.8 mg/kg ractopamine free base equivalents in the form of its hydrochloride. On this basis the average daily dose was estimated to 0.62 to 0.77 mg/kg of body weight. Concentrations of residues were given as ractopamine hydrochloride and were recalculated as ractopamine free base for the purpose of the evaluations carried out in this document. Some results were below the limit of detection. However, since the raw data were available, the original results were used for graphical and statistical purposes instead of LOD/2 substitutes. The data used for the preparation of this document are given in the *Annex in table A10 of the original study*.

3.1.2 Study T4V629501

The report of the study provides individual body weight data for the start and the end of the treatment period of ten days. Feed consumption, however, is given on a pen average basis (3 animals per pen). The range of body weights before treatment was from 94.5 to 118.3 kg and from 104.9 to 132.6 kg at the end of treatment. Daily body weight gain was from 0.72 to 1.61 kg. Pen averages of the daily feed consumption were 3.19 to 3.89 kg. Feed contained 18.96 mg/kg ractopamine free base equivalents in the form of its hydrochloride. On this basis the average daily dose was estimated to 0.57 to 0.67 mg/kg of body weight. Concentrations of residues were given as ractopamine hydrochloride and were recalculated as ractopamine free base for the purpose of the evaluations carried out in this document. The data used for the preparation of this document are given in the *Annex in table A11 of the original study*.

3.1.3 Study T4V759003

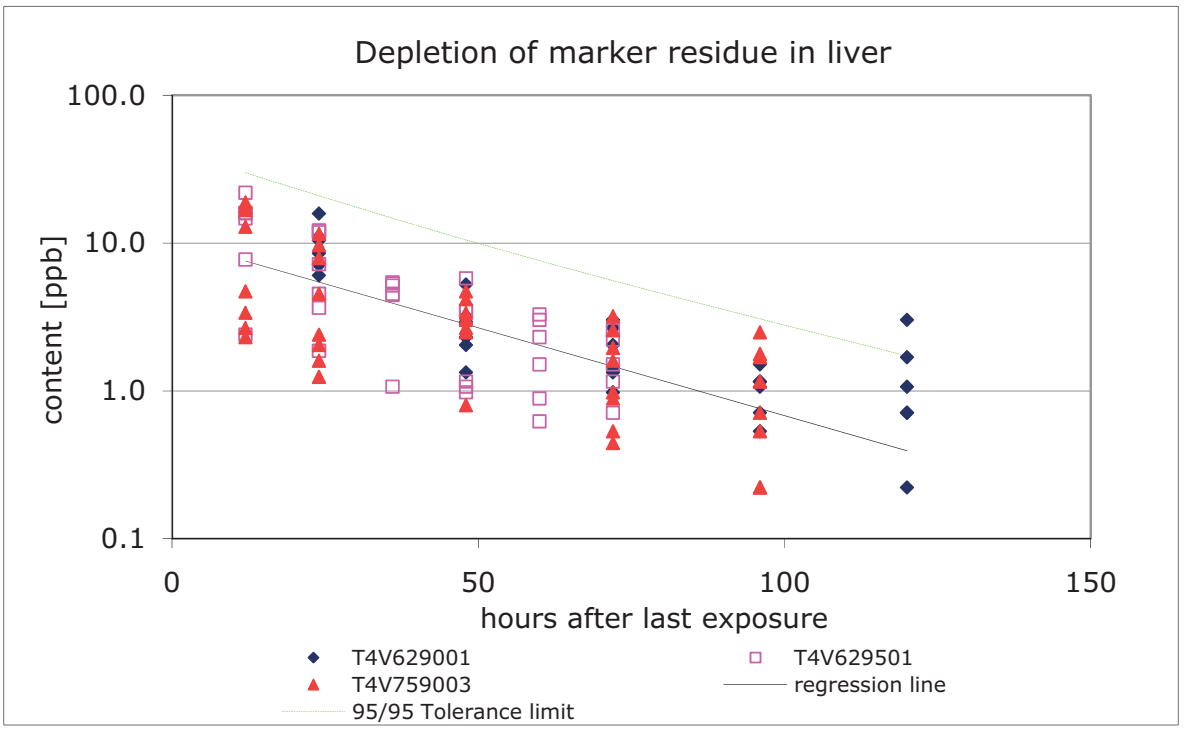
The report of the study provides individual body weight data for the start and the end of the treatment period of fourteen days. Feed consumption, however, is given on a group average basis (8 animals per group). The range of body weights before treatment was from 77 to 96 kg and from 89 to 113.5 kg at the end of treatment (one animal with a negative body weight gain and exhibiting other signs was excluded from the evaluation). Daily body weight gain was from 1.25 to 1.46 kg. Group averages of the daily feed consumption were 2.4 to 3.00 kg. Feed contained 16.9 mg/kg ractopamine free base equivalents in the form of its hydrochloride. On this basis the average daily dose was estimated to 0.48 to 0.58 mg/kg of body weight. Concentrations of residues were given as ractopamine hydrochloride and were recalculated as ractopamine free base for the purpose of the evaluations carried out in this document. In addition to residues in liver and kidney the report

also provides some data on residues in muscle, fat, and skin. The data used for the preparation of this document are given in the *Annex in table A12 of the original study*.

3.1.4 Combined statistical evaluation of the previously submitted studies for evaluation

The 66th meeting of the Committee concluded that it was justified to pool the data from the above three studies (Studies T4V629001, T4V629501 and T4V759003). Graphs of the depletion of the marker residue in liver and kidney are given in figures 7 and 8 including (on a semi-logarithmic scale): the data points, a linear regression line and the upper limit of the one-sided 95% confidence interval over the 95th percentile ("95/95 tolerance limit").

Figure 7. Statistical analysis of three marker residue depletion studies in liver.

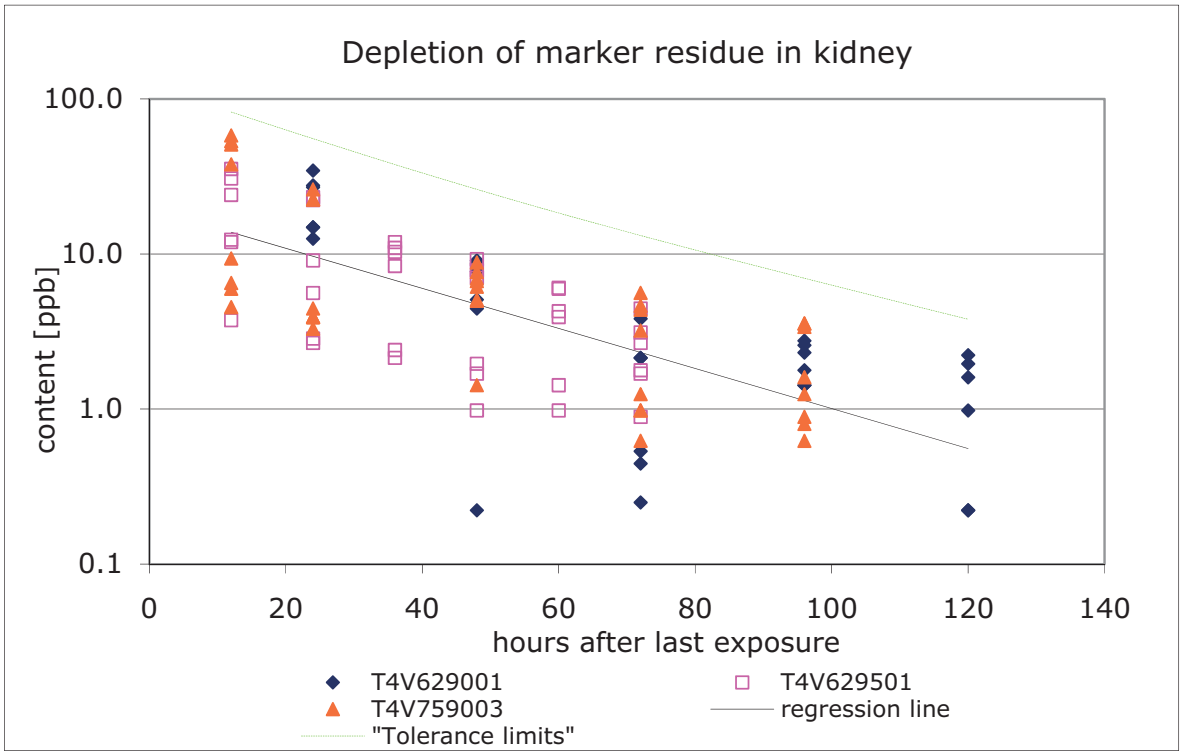


The curves for figure 7 and 8 can be constructed using the following parameters:

Parameter	liver	kidney
a:	1.29569	1.02159
b:	-0.01293	-0.01189
r:	-0.68007	-0.74234
$s_{y.x}$:	0.3852	0.2967
n:	100	100

Parameter a [ppb] is the intercept on the content axis at zero withdrawal time, b is the depletion rate constant [ppb/hour], r is the sample coefficient of correlation; $s_{y.x}$ is the residual variance and n is the number of data points used.

Figure 8. Statistical analysis of three marker residue depletion studies in kidney.



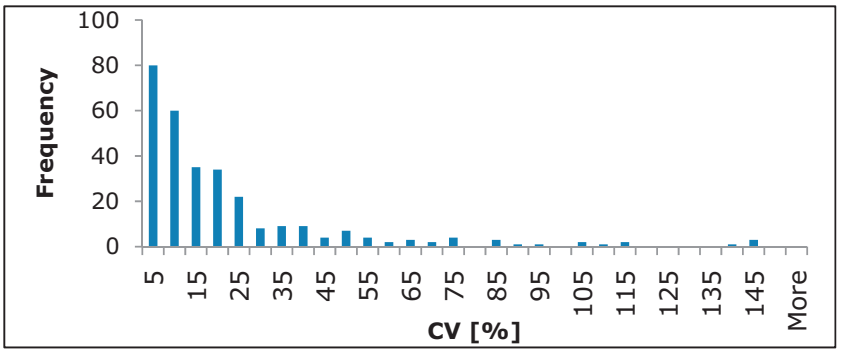
3.2 New residues depletion studies

3.2.1 The Wuhan study

Hubei white pigs were used in this study. The report of the study provides individual body weight data for the start and end of the 30 day treatment period, but no information on the sex of the animals. According to the original report the animals were 28 weeks old. This was corrected to 18 weeks in later correspondence. The range of body weights before treatment was from 51 to 58 kg and from 67 to 81 kg at the end of treatment. Feed consumption data are given for groups of 10 animals. Based on these group averages, daily feed consumption was 2.00 kg per animal and daily body weight gain was from 0.40 to 0.93 kg. Feed contained 17.8 mg/kg ractopamine free base equivalents as the hydrochloride. On this basis the average daily dose was estimated at 0.55 to 0.58 (average 0.56) mg/kg of body weight. Concentrations of residues were given for muscle, liver, kidney, heart, lung, stomach, small and large intestine. Some additional results were provided for fat, skin and tongue. Sample material was hydrolyzed with β-glucuronidase (containing also significant amounts of sulfatase). However, no data were provided for validation of the hydrolytic procedure[23]. Many results of duplicate analyses varied appreciably between the two replicates. Figure 9 shows a frequency distribution of the observed percent coefficient of variation. The coefficient of variation was >20% for 30% of all analytical results. The results were highly variable even at the very high concentrations of the marker residue at short withdrawal times. It is stated in the report that the limit of quantification was 0.5 $\mu g.kg^{-1}$.

[23] Validation experiments were carried out with samples fortified with ractopamine. Therefore, the performance of the hydrolytic step was not tested.

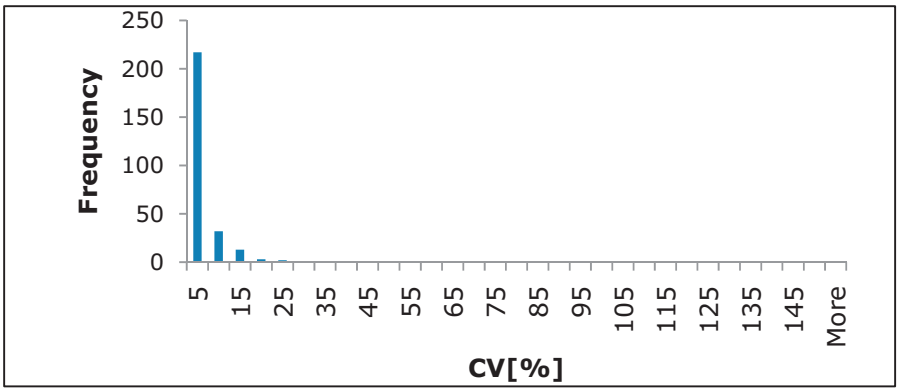
Figure 9. Frequency distributions of the coefficient of variation in sample analyses carried out in the Wuhan study.



3.2.2 The Beijing study

Landrace x Large Yorkshire binary cross breeding pigs were used in this study. The report of the study provides individual body weight data for the start and the end of the treatment period of 30 days. The animals were approximately 90 days old at the beginning of the study. The range of body weights before treatment was from 51 to 59 kg and from 63 to 81 kg at the end of treatment[24]. Feed consumption data are given for groups of 5 animals. An error occurred in the calculation of feed consumption in the original report, which was corrected in later correspondence. Based on corrected group averages, daily feed consumption was 2.18 kg per animal and daily body weight gain was from approximately 0.37 to 0.77 kg. Feed contained 17.8 mg/kg ractopamine free base equivalents as the hydrochloride. On this basis the average daily dose was estimated to 0.59 to 0.62 mg/kg of body weight. Concentrations of residues were given for muscle, liver, kidney, heart, lung, stomach, small and large intestine. Sample material was hydrolyzed with a β-glucuronidase/arylsulfatase preparation. However, the hydrolytic procedure was not validated. Results of duplicate analysis varied slightly between the two replicates. Figure 10 shows a frequency distribution of the observed percent coefficient of variation using the same scale for the x – axis as in figure 9. The coefficient of variation was >20% for only 1% of all analytical results. The limit of detection was reported as 0.2 $\mu g.kg^{-1}$ and the limit of quantification as 0.5 $\mu g.kg^{-1}$.

Figure 10. Frequency distributions of the coefficient of variation in sample analyses in the Beijing study.



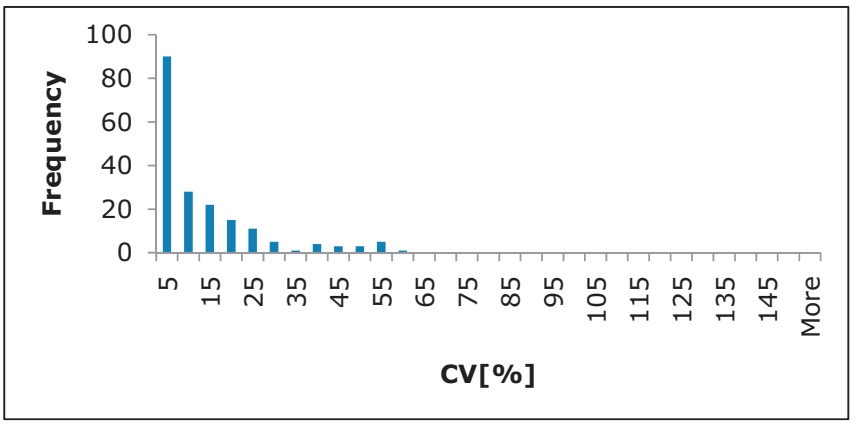
3.2.3 The Guangzhou study

Guangdong spotted little ear pigs were used in this study. The report of the study provides individual body weight data for the start of the treatment period of 30 days and for the time of slaughter. The age of the animals was not given. The range of body weights before treatment was from 42 to 62 kg and from 58 to 87 kg at time of slaughter. End-of-treatment body weights were not given. Feed consumption data are given for groups of 5 animals. Based on group averages, daily feed consumption was 1.63 kg per animal and daily body weight gain was from approximately 0.43 to 0.92 kg from start of treatment to time of slaughter. Feed

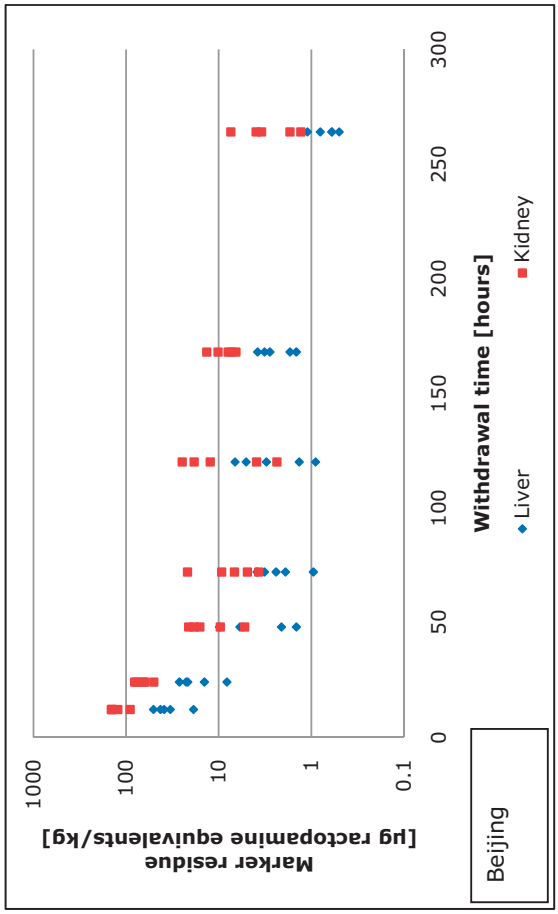
[24] These data are not contained in the original report, but were later provided.

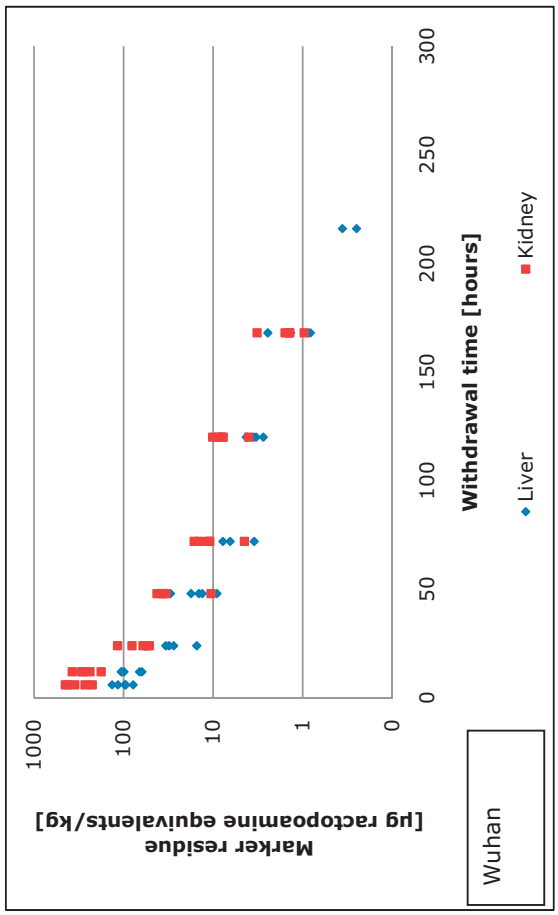
contained 17.8 mg/kg ractopamine free base equivalents as its hydrochloride. On this basis the average daily dose was estimated to 0.40 - 0.52 mg/kg of body weight. Concentrations of residues were given for muscle, liver, kidney, heart, lung, stomach, small and large intestine. Sample material was hydrolyzed with a β-glucuronidase/arylsulfatase preparation. However, the hydrolytic procedure was not validated. For many samples, duplicate analysis was performed. For some samples only one numerical result was provided and the second was "ND". Since this occurred even when the first result was a high value, significantly above the LOQ and the authors had not averaged the two figures, it was assumed that the sample had been analyzed only once. Variability of duplicate analyses was intermediate between the Wuhan study and the Beijing study. Figure 11 shows a frequency distribution of the observed percent coefficient of variation using the same scale for the x – axis as in figures 9 and 10. The coefficient of variation was >20% for a significant proportion of analytical results (approximately 14%). The report stated that the limit of detection was 0.2 $\mu g.kg^{-1}$ and the limit of quantification was 0.5 $\mu g.kg^{-1}$.

Figure 11. Frequency distributions of the coefficient of variation in sample analyses in the Guangzhou study.



Figures 12 to 14 provide a graphical representation of the analytical results of the total ractopamine residues in the three studies. Concentrations are expressed according to the marker residue chosen in these studies (which is different from the residue definition established by the Committee). Figure 12 shows the results for liver and kidney. The results for lung, stomach, and small intestine are given in figure 13. The results for large intestine, heart and muscle are given in figure 14. The additional data on fat, skin, and tongue which were communicated by the authors of the Wuhan study are summarized in figure 15.





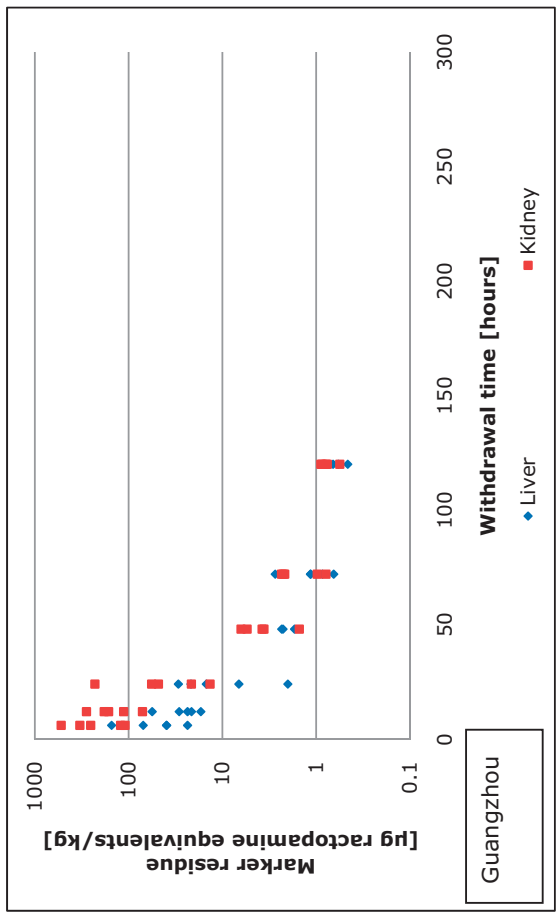
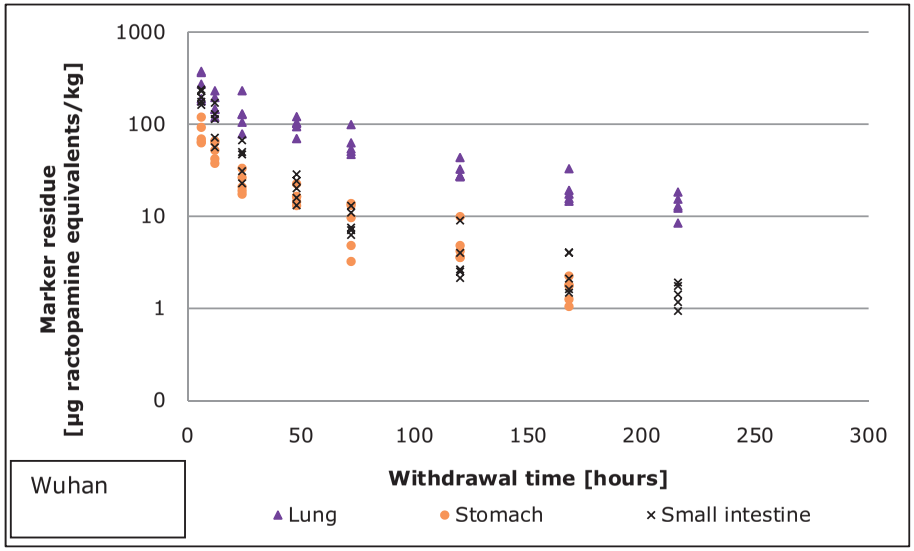


Figure 12. Depletion kinetics of ractopamine marker residue in liver and kidney.



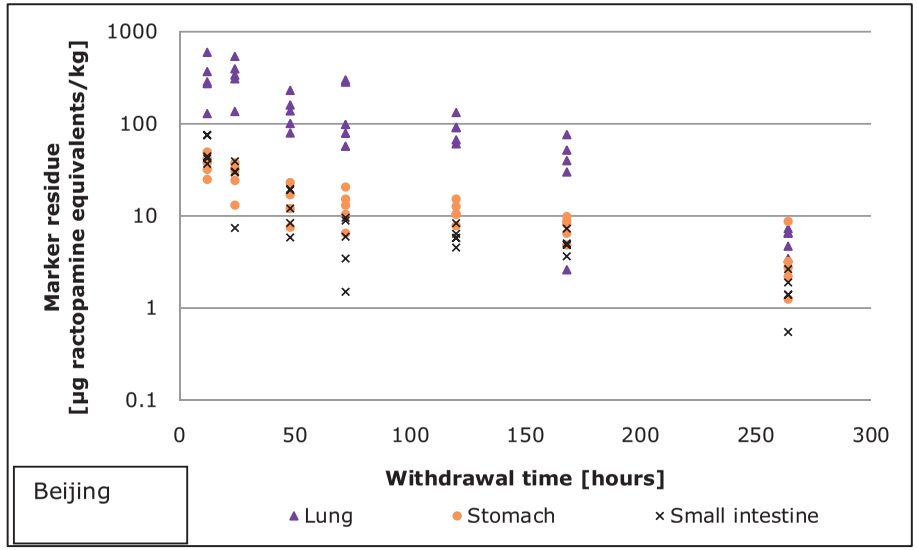
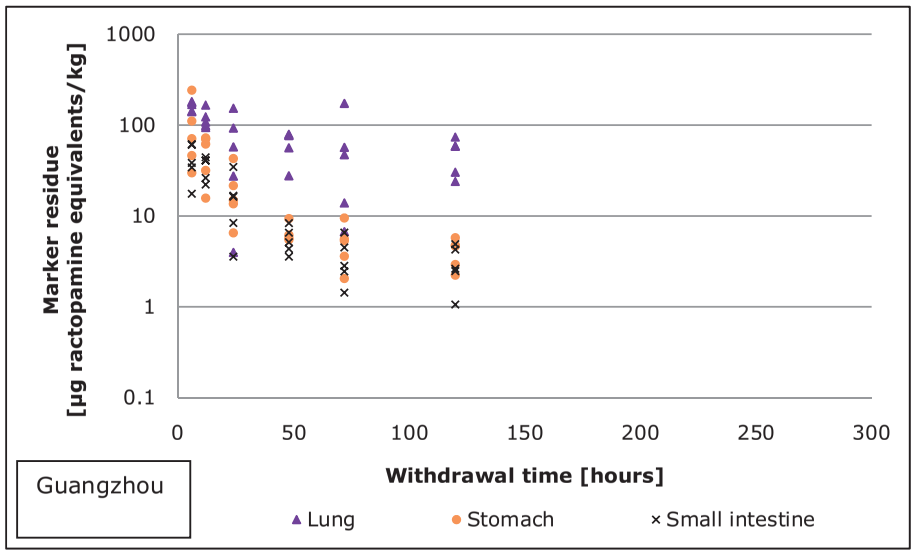
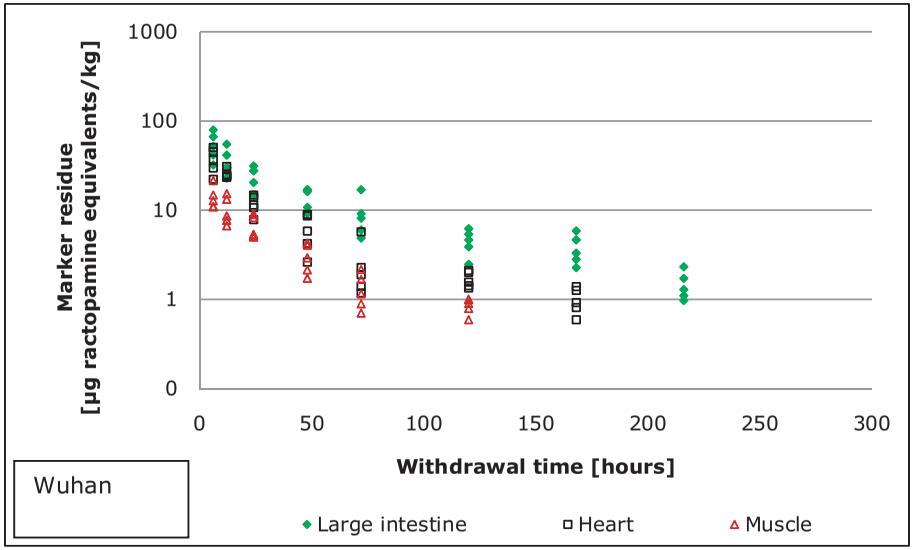
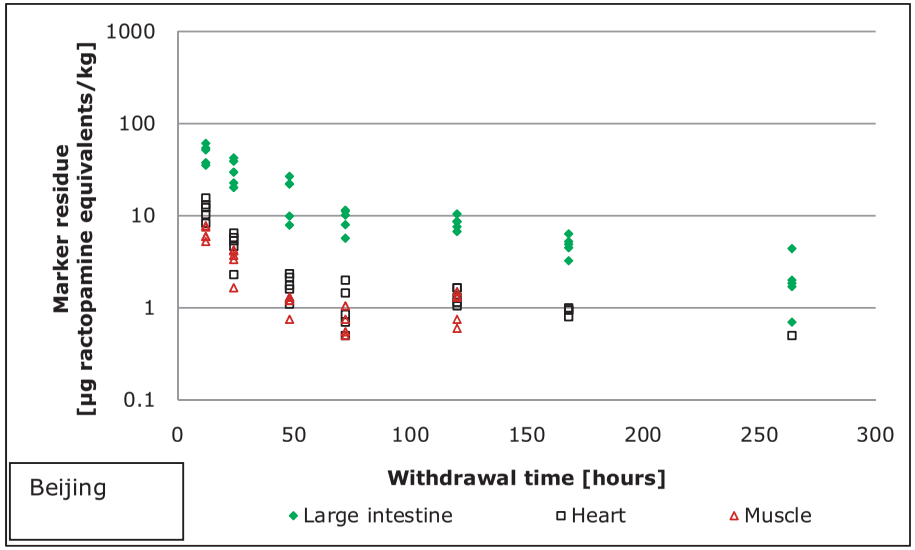


Figure 13. Depletion kinetics of ractopamine marker residue in lung, stomach and small intestine.







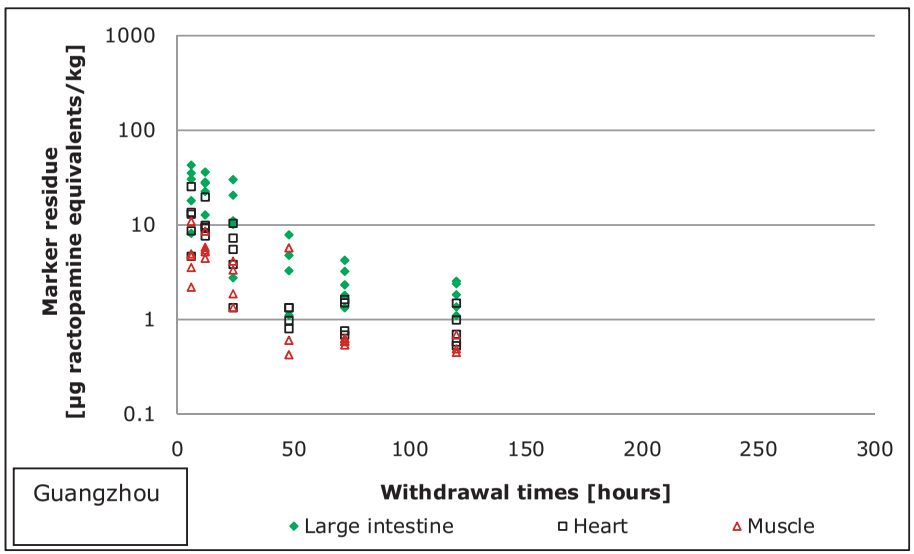
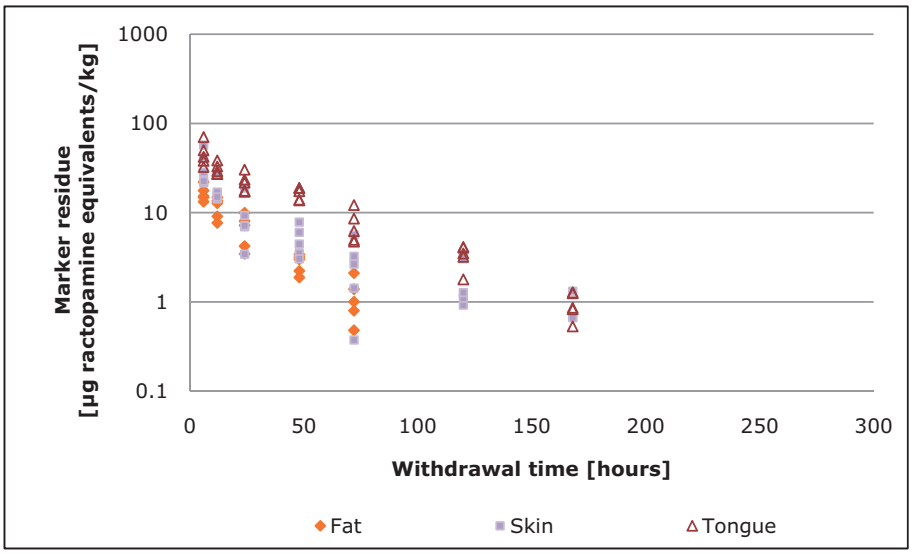


Figure 14. Depletion kinetics of ractopamine marker residue in large intestine, heart and muscle.

Figure 15. Depletion kinetics of the ractopamine marker residue in fat, skin and tongue (Wuhan study).



Statistical analysis of the data and comparison of the results of the studies

The results presented in the above figures 12 – 15 clearly show that there were (sometimes large) differences in the results of the three studies, particularly with regard to maximum concentrations reached at short withdrawal times, slope of depletion, variability of the data obtained for the individual animals, and number of depletion phases. The logarithmically transformed concentration values were used for linear regression analysis. Generally the data points describing the first 48-72 hours of depletion were used (approximately 20 data points). This model gave an acceptable fit of the linear model to most data sets except the data describing the kinetics in lung tissue. The parameters of the linear regression were used to estimate the upper limits of the one-sided 95% confidence interval over the 95th percentile ("95/95-tolerance limits"). The results of these calculations are summarised in table 5.

Table 5 also provides estimates of the median concentration values and tolerance limits predicted for a withdrawal time of 12 hours. The ratio between the tolerance limit and the median can be used as an indicator of the variability of the results obtained for the groups of animals used in the studies.

The variability of the results of the Guangzhou study was greatest. The following observations may only partly explain the variability. The variability of the initial body weights was significantly greater in this study compared to the other two studies. Unfortunately, the end-of-treatment body weights of the Guangzhou study were not given and feed intake information for one treatment group was also missing. However, it is possible that the variability of body weight gain was also greatest in this study.

The feed/body weight gain ratio was probably the lowest in the Guangzhou study. This result would correspond to the fact that typically the residue concentrations found in the Guangzhou study were the lowest of the three studies.

An attempt was made to re-calculate selected results of the three new studies in equivalents of the marker residue definition. The factors were obtained from the above table 4. It is certainly prudent to consider this as an approach with unknown but possibly great inherent uncertainties. However, as is shown below, it helps to highlight some of the major differences between the Chinese studies and the original sponsor studies. All numbers were calculated for 12h withdrawal times and expressed in µg ractopamine equivalents/kg tissue. The calculations were only possible for liver and kidney due to the absence of comparable data for the other tissues.

Study	Median residue concentrations expressed as "Chinese marker residue"		Coversion factor from "Chinese marker residue" to JECFA marker residue		Median residue concentrations expressed as "JECFA marker residue"	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Wuhan	73.2	216			19.8	90.4
Beijing	30.3	112	0.271	0.418	8.21	46.9
Guangzhou	29.1	137			7.88	57.3
Original studies					13.8	7.57

Study	Tolerance limits expressed as "Chinese marker residue"		Coversion factor from "Chinese marker residue" to JECFA marker residue		Tolerance limits expressed as "JECFA marker residue"	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Wuhan	194	648			52.6	271
Beijing	133	416	0.271	0.418	36	174
Guangzhou	165	767			44.7	321
Original studies					29.9	82.3

It appears that the residue concentrations in liver, expressed as marker residue definition used by the Committee in recommending MRLs ("JECFA marker residue" – free ractopamine base) were similar in all studies and for both parameters, the median and the "95/95-tolerance limits". Contrary to this, residue concentrations in kidney were much higher in the three new studies. Considerable variability of the data of the new studies – indicated by the distance between median and tolerance limits – was also seen.

Table 5. Summary of the results of the statistical evaluation of kinetic ractopamine residue depletion data.

Kinetic Parameters/Data	Muscle	Liver	Kidney	Fat	Skin	Heart	Lung	Tongue	Stomach	Small intestine	Large intestine
ORIGINAL SPONSOR studies *(marker ractopamine)*											
a [log/ppb]:		1.29569	1.02159								
b[log ppb/hour]:		-0.01293	-0.01189								
r:		-0.68007	-0.74234								
sy.x [log ppb]:		0.3852	0.2967								
n:		100	100								
Median residue at 12 h withdrawal time [ppb]		7.57	13.8								
95/95 Tolerance limit [ppb]		29.9	82.3								
Wuhan study *(marker ractopamine after conjugate hydrolysis)*											
a [log/ppb]:	1.20212	2.10587	2.64850	1.31398	1.49759	1.62002	2.38260	1.63688	1.89864	2.31068	1.72970
b[log ppb/hour]:	-0.01583	-0.02012	-0.02612	-0.02098	-0.01873	-0.01919	-0.00906	-0.00956	-0.01578	-0.02271	-0.01299
r:	-0.88867	-0.89180	-0.91538	-0.92014	-0.83372	-0.90697	-0.72449	-0.83244	-0.87593	-0.90159	-0.84286
$s_{y.x}$ [log ppb]:	0.1385	0.1731	0.1948	0.1514	0.2103	0.1511	0.1462	0.1079	0.1474	0.1848	0.1407
n:	20	20	20	20	20	20	20	20	20	20	20
Median residue at 12 h withdrawal time [ppb]	10.3	73.2	216	11.5	18.7	24.5	188	33.3	51.2	109	37.5
95/95 Tolerance limit [ppb]	22.4	194	648	27.1	61.3	57.5	428	61.1	117	309	82.8
Beijing study *(marker ractopamine after conjugate hydrolysis)*											
a [log/ppb]:	0.94048	1.72689	2.30559			1.17040	2.58120		1.62134	1.85384	1.78145
b[log ppb/hour]:	-0.01649	-0.02043	-0.02128			-0.01745	-0.00717		-0.00791	-0.01644	-0.01171
r:	-0.93981	-0.89153	-0.91781			-0.91416	-0.57304		-0.74658	-0.84718	-0.87945
$s_{y.x}$ [log ppb]:	0.1415	0.2522	0.2236			0.1880	0.2489		0.1713	0.2504	0.1539
n:	19	20	20			20	20		20	20	20
Median residue at 12 h withdrawal time [ppb]	5.53	30.3	112			9.14	313		33.6	45.4	43.8
95/95 Tolerance limit [ppb]	12.8	133	416			27.5	1344		91.6	197	108
Guangzhou study *(marker ractopamine after conjugate hydrolysis)*											
a [log/ppb]:	0.83436	1.85967	2.65454			1.08188	2.13651		1.95180	1.71129	1.55919
b[log ppb/hour]:	-0.01631	-0.03301	-0.04307			-0.01668	-0.01013		-0.02519	-0.02128	-0.02105
r:	-0.65388	-0.85725	-0.92258			-0.70568	-0.42808		-0.82310	-0.83691	-0.75272
$s_{y.x}$ [log ppb]:	0.2969	0.3027	0.3054			0.3347	0.3628		0.2825	0.2360	0.2992
n:	17	18	20			19	20		19	20	19
Median residue at 12 h withdrawal time [ppb]	4.35	29.1	137			7.62	103		44.6	28.6	20.3
95/95 Tolerance limit [ppb]	24.4	165	767			50.5	799		222	108	111

4. Intake assessment – Calculation of Estimated Daily Intake (EDI)

4.1. Using the data of the Wuhan study and the model diet employed by the Committee

The Wuhan study is the only study providing kinetic residue data for all tissues of the model diet employed by the Committee. At the same time the concentrations found in muscle, liver and kidney are the highest of all three new studies. Therefore, using the data of this study would result in the highest intake estimates that could be calculated from any of the three studies. The predicted concentrations after 12 h withdrawal time are used, as only for this time point is the ratio of (parent + conjugates)/total residue known from the studies of the original sponsor dossier. Sufficient information is available to interpolate any concentration data or marker/total ratios for this time point. Thus, 12 h withdrawal time represents the only time point for which all data sets can be compared.

4.1.1. Intake of "Chinese marker residue" from the Wuhan study data using two different intake assumptions

Food item	Daily consumed amounts [kg]	Median concentration of Chinese marker residue [µg/kg]	Daily intake of Chinese marker residue [µg/person]
Muscle	0.3	10.3	3.1
Liver	0.1	73.2	7.3
Kidney	0.05	216	10.8
Fat	0.05	11.5	0.6
Skin	0.05	18.7	0.9
Total intake 1 (muscle, liver, kidney, fat)	0.5		21.8
Total intake 2 (muscle, liver, kidney, skin)	0.5		22.1

4.1.2. Factors for conversion of marker to total residue

Factors for muscle, skin and fat

Study AAC 8924 provides 6 data pairs relating concentration of ractopamine in feed to residue concentration in muscle. Animals in this study had a body weight range of 99.5 - 111.5 kg and the exposure time was 5 days. Animals were slaughtered at 12 h withdrawal time. The relevant results are:

mg/kg in feed	µg/kg residue in muscle
7.21	1.34
7.21	2.14
7.21	3.38
11.48	4.45
11.48	3.74
11.48	4.63

If the concentration in muscle is linearily extrapolated to a feed content of 17.8 mg/kg (see analogy to figure 1, where a linear relationship was demonstrated for liver and kidney) a residue concentration of 11.9 µg/kg ractopamine in muscle is obtained.

In study ABC-0231, pigs of approximately 50 kg bw were exposed for 7 days to daily doses of approximately 0.676 mg radiolabelled ractopamine/kg of body weight (it is estimated that the dose in the Wuhan study was approximately 0.56 mg/kg bw/day). The observed total residue concentrations in muscle and fat are given in the table below. If these data are interpolated for the 12

h withdrawal time, 14.5 and 10.9 µg/kg of **total** residue concentrations are obtained for muscle and fat, respectively. These total residue concentrations are also in the same order of magnitude of the "Chinese marker residue" concentrations in the Wuhan study.

Withdrawal time [hr]	Animal ID	Sex	Concentration of total residue [µg/kg]	
			Muscle	Fat
6	151	M	22	14
6	232	F	38	17
36	205	M	2	5
36	088	F	9	8
120	126	M	3	7
120	165	F	2	4

In study ABC-0273, animals were exposed to approximately 1.191 mg/kg bw/day (twice the dose of the Wuhan study) of radioactive ractopamine. Exposure was for 4, 7, and 10 days, respectively. Animals were slaughtered after 12 h withdrawal time in each group. The concentrations of **total** residue in muscle and fat were as follows:

Exposure time [days]	Animal ID	Sex	Concentration of total residue [µg/kg]	
			Muscle	Fat
4	773	f	16.02	11.57
4	774	m	16.02	8.01
4	776	f	24.03	8.01
7	775	m	14.24	11.57
7	770	f	16.91	9.79
7	771	m	18.69	8.9
10	767	f	17.8	14.24
10	768	f	23.14	12.46
10	766	m	24.03	13.35

The geometric mean of the individual data was 18.7 and 10.7 µg/kg for muscle and fat, respectively.

An even higher dose of approximately 1.237 mg/kg bw/day of radiolabelled ractopamine was administered to animals of an approximate body weight of 45 kg for four days (original sponsor study ABC-0283). The following data on **total** residue in muscle and fat were provided:

Withdrawal time [h]	Animal ID	Sex	Concentration of total residue [µg/kg]	
			Muscle	Fat
12	923	f	12.46	13.35
12	926	m	12.46	11.57
24	922	f	5.34	6.23
24	924	m	4.45	5.34
48	916	m		2.67
48	917	f		2.67

These data correspond to a geometric mean at 12 h withdrawal time of 12.46 and 12.43 µg/kg for muscle and fat, respectively.

Doses of approximately 1.082 mg/kg bw/day were administered to animals of approximately 50 kg bw for four days in original sponsor study ABC-0291a. The concentrations of **total** residue

found in muscle and fat are noted below. These data correspond to a geometric mean at 12 h withdrawal time of 18.76 and 18.09 µg/kg for muscle and fat, respectively.

Withdrawal time [h]	Animal ID	Sex	Concentration of total residue [µg/kg]	
			Muscle	Fat
12	175	m	11.57	12.46
12	179	m	26.7	22.25
12	188	f	21.36	21.36
48	173	f		6.23
48	185	f		1.78
48	187	m		2.67
96	181	m	1.78	3.56
96	183	f		
96	184	m	0.89	
168	174	f		4.45
168	182	f		1.78
168	186	m		2.67

All these data suggest that the total residue concentrations in muscle and fat are in the same order of magnitude as the concentrations of the parent compound and of the marker residue measured in the Wuhan study. That means that at short withdrawal times the only relevant residue in muscle and fat is parent ractopamine. The proposed factor for the conversion of marker to total residue, therefore is 1. For skin almost no data exist to relate the marker residue to total residue. However, the contribution of residues in skin to the total intake is very low. Therefore, within reason, the choice of which factor to use is essentially irrelevent. For this estimate, a factor of 1 was used.

Factors for liver and kidney

The only available data base is the original sponsor study ABC-0369. If it is assumed that only metabolites A,B,C, and D can be hydrolysed to yield ractopamine and that all the other remaining residues are of equal toxicological concern, then the ratio of the Chinese marker residue to total residue would be 0.565 for liver and 0.760 for kidney (see table 4) corresponding to conversion factors of 1.770 for liver and 1.316 for kidney.

4.1.3. Calculation of the EDI on the basis of the Wuhan study

Using the above factors the EDI could be calculated on the basis of the one of the three new studies yielding the highest marker residue concentrations, i.e. the Wuhan study, and the most conservative conversion factors derived from the studies of the original dossier. This calculation is presented in table 6.

Table 6. Calculation of the EDI for ractopamine on the basis of the Wuhan study using two intake estimates.

Food item	Daily consumed amounts [kg]	Median concentration of Chinese marker residue [µg/kg]	Daily intake of Chinese marker residue [µg/person]	Coversion factor	Daily intake of total residue [µg/person]
Muscle	0.3	10.3	3.09	1	3.1
Liver	0.1	73.2	7.32	1.770	13.0
Kidney	0.05	216	10.8	1.316	14.2
Fat	0.05	11.5	0.575	1	0.6
Skin	0.05	18.7	0.935	1	0.9
Total intake 1-(muscle, liver, kidney, fat)	0.5		21.8		30.8
Total intake 2 (muscle, liver, kidney, skin)	0.5		22.1		31.2

Doubling of the conversion factor for skin would result in a 3% increase of the intake estimate. The above two EDI estimates correspond to 38.5 - 39.0% of the upper limit of the ADI for a person with a body weight of 60 kg.

Around 87-88% of this intake results from consumption of 150 g edible offal (liver, kidney). Information provided in the correspondence with scientists from the Chinese Centre for Disease Control (data from the 2002 Chinese Survey on Nutrition, Diet and Health Status) suggested that the 97.5 percentile consumption of all eaters could range around 200 g of kidney, 250 g of liver, 300 g of lung, 300 g of all offal including liver, kidney, heart, lung, stomach, and intestine[25]. Replacement of the 150 g offal of the model diet by 250 g liver would result in an intake estimate of approximately 31 to 32 µg/person/day. Replacement of the 150 g offal (100 g liver and 50 g kidney) of the model diet by 200 g kidney would result in an intake estimate of approximately 61 µg/person/day.

The coefficient of variation of duplicate estimates of tissue concentrations was particularly unsatisfactory for the Wuhan study. Therefore, in a second run all values with a CV above 20% were omitted in the linear regression; all other data points until 72 hours withdrawal time were used as provided. This improved the goodness of fit, but had almost no effect on the estimated median residue concentrations. The values changed from 10.3 to 10.1 for muscle, 73.2 to 66.9 for liver, and 216.0 to 215.2 for kidney. As a result the EDI on the basis of the model diet was lowered by approximately 3%. This confirms that the observed variability of the analytical method performance had only a small influence on residue depletion and intake estimates.

4.1.4. Using muscle, liver and kidney data of the Beijing and Guangzhou studies

Neither the Bejing or the Guangzhou study provide any residue data for fat tissue. However, since the contribution of fat consumption to the total residue intake is minimal, an intake estimate on the basis of the data sets of the two other studies is possible. The results are summarised in tables 7a and 7b.

[25] Apparently these figures have been rounded in steps of 50g. Some figures are unusual, for example that the subgroup of 2-6 years old children have a higher consumption of kidney than all eaters.

Table 7a. Calculation of the EDI for ractopamine on the basis of the Beijing study.

Food item	Daily consumed amounts [kg]	Median concentration of Chinese marker residue [µg/kg]	Daily intake of Chinese marker residue [µg]	Coversion factor	Daily intake of total residue [µg/person]
Muscle	0.3	5.53	1.66	1	1.6
Liver	0.1	30.3	3.03	1.77	5.4
Kidney	0.05	112	5.6	1.32	7.4
Fat	0.05	No data available		1	
Skin	0.05			1	
Total intake (muscle, liver, kidney,)	0.45				14.4

Table 7b. Calculation of the EDI for ractopamine on the basis of the Guangzhou study.

Food item	Daily consumed amounts [kg]	Median concentration of Chinese marker residue [µg/kg]	Daily intake of Chinese marker residue [µg]	Coversion factor	Daily intake of total residue [µg/person]
Muscle	0.3	4.35	1.31	1	1.3
Liver	0.1	29.1	2.91	1.77	5.2
Kidney	0.05	137	6.85	1.32	9.0
Fat	0.05	No data available		1	
Skin	0.05			1	
Total intake (muscle, liver, kidney,)	0.45				15.5

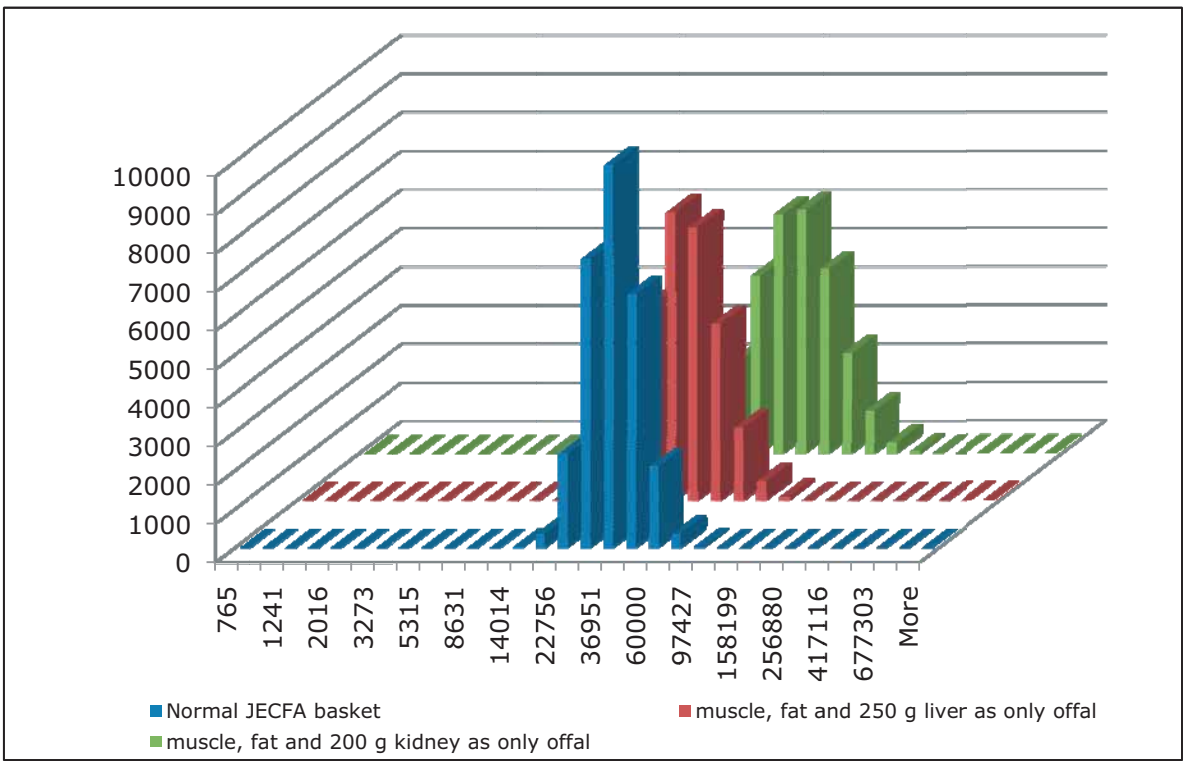
The above applied factors for the marker to total residue conversion are conservative. If it is assumed that the unidentified metabolites E and F of study ABC-0369 and the combined residues of the separation experiments also represent hydrolysable conjugates, then the conversion factors for liver and kidney would decrease to 1.127 and 1.034, respectively. This would reduce the EDI to approximately 23, 11, and 12 µg/person/day using the data of the Wuhan, Beijing and Guangzhou study, respectively. In this case the 150 g offal in the model diet could be replaced by any other offal (except lung; see section 4.3) and in any amount up to the maximum consumption without the EDI exceeding the the upper bound of the ADI.

4.2. Modeling on the basis of the model diet employed by the Committeeand with increased consumption data for liver and kidney

The kinetic parameters **a** and **b** given in table 5, the residual variance $\mathbf{s}_{y,x}$ of the data, and the factors for marker to total residue conversion were used to calculate model intakes for every day of 80 years of a human lifespan, assuming daily consumption of 300 g of muscle, 100 g of liver, 50 g of kidney and 50 g of fat. For this purpose for each tissue 29,220 (i.e. number of days in 80 years) log-normally distributed random residue concentration values were generated for a withdrawal time of 12 hours and numerically ranging from the value predicted by the regression line of the tissue concentrations ***plus or minus four times*** the residual variance to the same predicted residual variance. The results were expressed in µg intake/day of total ractopamine related residues. Figure 16 shows the results of three of such modeling experiments. The upper class limit of 60,000 ng/person/day separates all intakes below and up to the upper bound of the ADI. Using the data of the Wuhan study and the model diet (described as the normal JECFA basket in figure 16), the distribution shown in the blue (front) columns were obtained. If the experiment was repeated many times, the median was always similar to the above estimated EDI. However, typically 1.2 to 1.8% of the results exceeded the ADI with the

highest results ranging around 1.5 times the upper bound of the ADI. If the 100 g of liver and 50 g of kidney of the model diet were replaced by 250 g liver, the distribution was slightly shifted to higher intake values with 8.3 to 8.8% of the results numerically above the upper limit of the ADI with maxima in the order of 2.5 times the upper limit of the ADI (middle, red columns). If the 100 g of liver and 50 g of kidney were replaced by 200 g of kidney, the distribution was further shifted to higher intake values. 50.6 to 51.7% exceeded the upper limit of the ADI with maxima ranging in the order of 5 times the upper limit of the ADI (back, green columns).

Figure 16. Predicted frequency distrubution of 29,220 daily intakes of ractopamine.



x-axis: ng intake/person/day; y-axis: frequency of observations (total = 29220)

4.3 The specific problem of the high residue concentrations in lung

No conversion factors for converting marker to total residue concentrations are available. If one conducted modeling experiments like the ones described above replacing liver and kidney by 300g of lung and using conversion factors of 1 for lung, the EDI would already exceed the ADI for the Wuhan and Beijing studies. However, using the the intake modeling approach described above, a considerable fraction of the 29,220 estimated intake figures would exceed the ADI with maximum values up to 20 times the ADI in the Wuhan study. The EDI estimated for the Guangzhou study would remain significantly below the ADI. However, approximately 15% of the 29,220 estimated intake figures would exceed the ADI, and the maxima could well reach 15 times the ADI because the data set for residues in lung in the Guangzhou study exhibits the greatest variability of all 27 data sets provided for individual tissues in the three studies.

Analytical methods

Prior to initiating the three studies, the analytical method for determination of ractopamine was established and validated in each laboratory according to a protocol described in the study reports. In general, samples were hydrolyzed with β-glucuronidase/aryl sulfatase and extracted with ethyl acetate-25% ammonium hydroxide (95-5) then purified with solid phase extraction and analyzed by

LC/MS/MS using deuterium labelled (D6)-ractopamine hydrochloride as an internal standard. The limit of quantification (LOQ) reported for this method was 0.5µg.kg^{-1}; the limit of detection (LOD) was 0.2µg.kg^{-1}. This analytical procedure is different from the methods considered in previous evaluations by the Committee where enzymatic hydrolysis was not used in the analytical determination of ractopamine residues. Details on the implications of this hydrolysis step are presented below.

Globally, the analytical strategy can be considered as fit for the purpose. It is based on the use of isotopic dilution (deuterium labelled internal standards) and LC-MS/MS (reverse phase separation, electrospray ionization, and acquisition of the signals in the selected reaction monitoring mode on a triple quadrupole instrument) for characterization of the analytes. However, the main weaknesses concerned the first steps of the method and especially the phase II metabolite deconjugation process with different enzymatic sources and the first extraction step used to recover hydrolyzed metabolites. Conditions used for the deconjugation differed appreciably. The enzyme source was not the same (varying sulfatase and glucuronidase composition, and also from different commercial sources). In addition, the enzyme activity, and the conditions used for the hydrolysis differed to some extent (buffer, pH, temperature, duration).

Specific Remarks

Target residues - isomers

The first question of importance is the definition of the real target markers assessed. Ractopamine includes 2 asymmetric carbons and can therefore be considered as a racemic mixture containing four isomers (RR, RS, SR, SS). As the RR isomer is by far the chemical form with the highest affinity for the β1 and β2 adrenergic receptors, the question of the separate quantitation of these isomers can be raised. However, there was no information on the proportion of the various isomers in the residues analyzed.

Internal standards

Deuterated labelled ractopamine has been used (six D/H substitutions used) as the internal standard (IS). The positioning of the labelled atoms on the ractopamine chemical structure is on the aliphatic component. It is an important point as these positions are less easily exchangeable than on the aromatic ring. However, it would be better to use the glucuronide of deuterated ractopamine (GDR) to be as mimetic as possible with the target analytes. This option is particularly important when concentration characterisation has to be performed on tissue (solid material, where extractability is often critical). In the study reports, the calculated amount of ractopamine has probably been slightly underestimated. Moreover, the variability would have been decreased using a glucuronide of deuterated ractopamine as IS.

Counter ion

Ractopamine standards were in the hydrochloride (counter ion) salt form. Correction of ractopamine concentration by 1.121^{NB} is of considerable importance, depending on whether or not the HCl counter ion has been taken into account in the calculation. The situation is different considering the internal standard as its concentration does not appear in the calculation of the target compound.
NB: Ractopamine HCl: MW=337.85 (CAS number = 97825-25-7),
Ractopamine: MW=301.38 (CAS number = 90274-24-1)
Correction factor = 1.121

Chromatography and spectrometry

Two laboratories used HPLC (High performance liquid chromatography), one used UPLC (ultra performance liquid chromatography). There is no expected consequence on the quality of the data, nor their comparability. The three studies used both positive electrospray and triple quadrupoles (QqQ) for quantification; this approach can be considered as the *de facto* strategy for ractopamine characterization in biological samples.

Conditions of the hydrolysis

As noted above, conditions used for the deconjugation differed appreciably. This is probably the most critical stage in the methods that can lead to differences in the results. Moreover, it is common practice to conduct the hydrolysis step after a first extraction of the solid matrix; furthermore, the original tissue sample is usually either digested (e.g. using a protease), or lyophilised and grinded. In these studies, the deconjugation was performed directly on homogenized tissue. The accessibility of the enzyme to the substrate is obviously less facilitated, and it is possible that less of the ractopamine conjugates would be hydrolysed. The consequence would be a slight underestimation of the quantities of conjugated and unconjugated residues combined.

The composition of the enzyme preparation is either purified (in this case, there are few additional enzymatic activities, so bound residues are not released), or the preparation is not purified (in this case, it is possible that some endogenous protease in the enzymatic preparation can release some bound residues, but no proof, and little literature on this point is available). In the latter case, it is possible that concentrations of target analytes could be affected by the crude enzymatic preparation, leading once again to an underestimation of ractopamine concentration.

Limits of performance

LODs and LOQs were found comparable in the three laboratories (LODs better than 0.2 $\mu g.kg^{-1}$ and LOQs better than 0.5 $\mu g.kg^{-1}$). There are no major negative comments on the calibration curves used, and the applied quantitation strategies.

Summary of analytical methods

In summary, even if some shortcomings were identified for the different analytical methods used to detect residues in the three new studies, it was noted that:
1. The analytical data provided by the Chinese are of acceptable quality.
2. Even if the strategies used by the three different laboratories were slightly different, and even if in the final analysis, the performance between the three studies is differ to some degree, it can be concluded that the data are valid for use in the analysis presented in this report.

Residue depletion study submitted by the People's Republic of China in May 2010

The Secretariat of JECFA received from the People's Republic of China on 13 May 2010, a fourth ractopamine residue depletion study in pigs. Given the late receipt of the study, sufficient time was not available to fully analyze and integrate results with the three earlier studies. The Committee noted that no authorship was provided in submitted study report.

The study consisted of 25 Duroc × large White × Landrace pigs of approximately 50 kg body weight at the start of the study. The feed regimen used medicated feed at 19.2 mg/kg ractopamine hydrochloride daily for thirty days. Feed and weight gain were measured with an automated feed intake and daily weight gain system. Median feed intake per day for this study was higher than in Wuhan, Beijing and Guangzhou studies with pigs consuming 2.61 kg medicated feed per day, with individual animal intake varying from 1.64 to 3.26 kg per day. The Wuhan, Beijing and Guangzhou studies daily intake of medicated feed was 1.63, 2.0 and 2.18 kg per day. Slaughter withdrawal times for the treated pigs were 6, 24, 48 and 72 hours. Sampling times in the Wuhan, Beijing and Guangzhou studies all contained a 12 hour withdrawal time and two of the three studies included a 6 h withdrawal time. The tissues sampled were the same as those in the Wuhan, Beijing and Guangzhou studies.

The sample preparation and analytical method was similar to that used in the other three studies with two important differences. This study included sample preparation with and without enzymatic digestion of the tissue samples prior to extraction and purification and the use of a different internal standard in the residue analysis (a tri-deuterium labelled ractopamine (D3) rather than the

hexa-deuterium (D6) labelled ractopamine). The Wuhan, Beijing and Guangzhou studies did not include residue analysis without enzyme hydrolysis. The analytical method was reported to have the same limits of quantitation (LOQ) and detection (LOD). Analysis was performed in three different laboratories, although the names of these laboratories were only provided as acronyms. Ractopamine residue concentrations in liver, kidney, lung, and small intestine were greater than in the other tissues. Residue concentrations in the lung were greater than those in the liver and kidney, and were detected up to nine days following removal of medicated feed. Overall, results were comparable to the Wuhan, Beijing and Guangzhou studies when comparing results involving the enzyme hydrolysis procedure.

The concentration of residues determined using the enzymatic step are all greater than those determined without the enzymatic step except for lung tissue where the ratio was approximately 1. i.e. the residues in lung tissue consisted of mostly free ractopamine. For stomach, large intestine and small intestine tissues the ratios were 1.09 to 1.20. Large coefficients of variation (CV) for the intra-laboratory and inter-laboratory results shows concern with the enzymatic step regarding reproducibility for fat, kidney and liver tissues, in agreement observations made for the residue data for these tissues in the other three studies. More typically, studies that include an enzyme hydrolysis procedure include an initial extraction with an appropriate solvent prior to buffered enzyme hydrolysis. In addition, there are no data provided on the validation of the enzyme hydrolysis procedure in this study.

The analytical data on the residues in all tissues, with and without enzymatic hydrolysis, do provide information indicating that residue analysis using enzymatic treatment of the crude tissue samples yields higher amounts of measurable residues than the same sample without enzymatic hydrolysis in all tissues at all time points with the exception of some of the lung tissue samples. However, a high variability in the residue levels in this tissue was noted. Levels of residues in all tissue samples using the enzymatic hydrolysis step are of the same order of magnitude as in the Wuhan, Beijing and Guangzhou studies. The new study does indicate, in general, a relatively consistent ratio between the enzymatic and non-enzymatic results for all four sampling times for each individual tissue and organ type. A summary of the residue data from each laboratory, each tissue and each sampling time is noted below in Table 8. The coefficients of variation (CV) for liver, kidney and fat are remarkably high both in absolute values and in comparison with the other tissue results.

The analytical methods were reviewed to assess if the use of the different internal standard in the residue analysis (a tri-deuterium labelled ractopamine (D3) would influence the comparability of the data between the studies and to evaluate the analytical details of the two approaches consisting in the measurement of ractopamine after an enzymatic hydrolysis step or without hydrolysis (no deconjugation step). The evaluation focused on instruments, analytical method used, validation data, internal standards, enzyme source, extraction and purification procedures, mass spectrometric ions monitored, criteria for analyte identification and overall performance of the method. It was concluded that the method used is fit for purpose of the study with no major analytical concerns regarding the new set of data.

In summary, an overall analysis of the data in this additional study show levels of residues in all tissue samples using the enzymatic hydrolysis step of the same order of magnitude as in the Wuhan, Beijing and Guangzhou studies. No direct comparison to the dietary exposure assessments was performed using the three studies as no sampling was performed at a 12 hour withdrawal time in this new study.

Table 8. Ratio of ractopamine residues using enzymatic (E) and non-enzymatic (NE) conditions for each of the three sets of laboratory results.

Muscle	IVDC E/NE	CAU E/NE	SCAU E/NE	All
Mean	1.84	1.27	1.66	1.60
SD	0.91	0.25	1.00	0.82
CV	49%	19%	60%	51%
Liver	IVDC E/NE	CAU E/NE	SCAU E/NE	All
Mean	1.50	1.40	4.14	2.35
SD	0.25	0.26	4.63	2.93
CV	17%	18%	112%	125%
Kidney	IVDC E/NE	CAU E/NE	SCAU E/NE	All
Mean	2.83	2.86	3.60	3.09
SD	1.50	1.78	3.63	2.47
CV	53%	62%	101%	80%
Lung	IVDC E/NE	CAU E/NE	SCAU E/NE	All
Mean	1.01	1.04	1.01	1.02
SD	0.12	0.09	0.07	0.09
CV	12%	8%	7%	9%
Heart	IVDC E/NE	CAU E/NE	SCAU E/NE	All
Mean	1.37	1.32	1.30	1.33
SD	0.32	0.30	0.35	0.32
CV	24%	23%	27%	24%
Fat	IVDC E/NE	CAU E/NE	SCAU E/NE	All
Mean	0.98	1.77	2.01	1.59
SD	0.48	1.67	1.78	1.48
CV	49%	95%	88%	93%
Small Intestine	IVDC E/NE	CAU E/NE	SCAU E/NE	All
Mean	1.09	1.15	1.34	1.20
SD	0.23	0.11	0.34	0.26
CV	21%	9%	25%	22%
Stomach	IVDC E/NE	CAU E/NE	SCAU E/NE	All
Mean	1.11	1.10	1.12	1.11
SD	0.10	0.06	0.09	0.08
CV	9%	6%	8%	8%
Large Intestine	IVDC E/NE	CAU E/NE	SCAU E/NE	All
Mean	1.11	1.09	1.07	1.09
SD	0.13	0.09	0.10	0.11
CV	12%	8%	10%	10%

Note: IVDC, CAU and SCAU refer to the three laboratories in the study. No information was available as to the name and location of these laboratories.

Summary and appraisal

Background

The monograph addendum to the residue monograph on ractopamine hydrochloride in this volume of the FAO JECFA Monographs on the residues of, exposure to and statements on the studies on ractopamine residues in pig tissues submitted were prepared by the invited experts for this electronic meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) during the period of January to May 2010. The tasks for the Committee was to evaluate three residue depletion studies on ractopamine in pig tissues performed by the People's Republic of China, consider any other relevant studies previously assessed in this context by the Committee, provide recommendations on whether the information contained in the three new studies would have an impact on the MRLs recommended for ractopamine and consider any other scientific issues arising from the evaluation of the studies. The additional study received in May 2010 was considered separately due to its late submission.

This request originated from the 32nd Session of the CAC, which had asked FAO and WHO to review the three residue studies in pigs using ractopamine hydrochloride medicated feed conducted by the Government of the People's Republic of China. These studies on ractopamine residue depletion used three different breeds in of pigs and were carried out at three different national laboratories, located in Wuhan, Guangzhou and Beijing. The Delegation of People's Republic of China had at the 32nd CAC expressed concern over the ractopamine residue levels in lung, stomach, heart, large and small intestine as well as in the tissues for which MRLs were proposed (muscle, liver, kidney and fat), particularly at early time points after withdrawal of the medicated feed.

Description of the three new residue studies in the pig

The Wuhan study used 40 Hubei White Swine. All pigs received a dose of approximately 20 mg ractopamine hydrochloride per kg medicated animal feed daily for 30 days. Average feed consumption was 2.0 kg/animal/day. Animals were slaughtered at 6, 12 hours, and 1, 2, 3, 5, 7 and 9 days following withdrawal of medicated feed. Muscle, liver, kidney, heart, lung, stomach, large intestine and small intestine were collected and analyzed. Ractopamine residue concentrations in liver, kidney, lung, and small intestine were greater than in the other tissues. Residue concentrations in the lung were greater than those in the liver and kidney, and were detected up to nine days following removal of medicated feed.

The Beijing study used Landrace x Large Yorkshire binary cross pigs. Animals received ractopamine hydrochloride in medicated feed at a dose rate of approximately 20 mg per kg daily for 30 days. Average feed consumption was 2.18 kg/animal/day. Samples of muscle, liver, kidney, heart, lung, stomach, large intestine, and small intestine were collected from all treated and control animals at 12 hours, 1, 2, 3, 5, 7 and 11 days. Ractopamine residue concentrations were above the limit of quantification (0.5 $\mu g.kg^{-1}$) in all the tissues collected 11 days after the treatment with the exception of muscle.

The Guangzhou study used 30 Spotted Small-ear pigs. All pigs received a daily dose at a rate of approximately 20 mg ractopamine hydrochloride per kg medicated animal feed for 30 days. The average feed consumption was 1.63 kg/animal/day. Animals were slaughtered 6, 12 hours, and 1, 2, 3 and 5 days after treatment. Samples of muscle, liver, kidney, heart, lung, stomach, large intestine and small intestine, were collected from all treated and control animals. The distribution of ractopamine demonstrated tissue selectivity in the pig, with the highest residue concentrations at 12 h in kidney, with the lung containing the second highest concentrations, followed by stomach, liver, small intestine, large intestine and muscle. Ractopamine residues in the lung depleted slowly.

Additional residue study submitted in May 2010

The additional study used 25 Duroc × large White × Landrace pigs. All pigs received a daily dose at a rate of approximately 20 mg/kg ractopamine hydrochloride daily for thirty days. The average feed

consumption was 2.61 kg/animal/day, higher than in the other three studies. Animals were slaughtered 6, 24, 48 and 72 hour after treatment. The tissues sampled were muscle, liver, kidney, fat, lung, heart, stomach, large and small intestine. Analysis was performed with and without enzymatic digestion of the tissue samples prior to extraction and purification The concentration of residues determined using the enzymatic step are all greater than those determined without the enzymatic step except for lung tissue where the ratio was approximately 1. i.e. the residues in lung tissue consisted of mostly free ractopamine. For stomach, large intestine and small intestine tissues, the ratios were 1.09 to 1.20. Large coefficients of variation (CV) for the intra-laboratory and inter-laboratory results shows concern with the enzymatic step regarding reproducibility for fat, kidney and liver tissues, in agreement observations made for the residue data for these tissues in the other three studies. The levels of residues in all tissue samples analyzed using the enzymatic hydrolysis step were of the same order of magnitude as in the other studies. No direct comparison to the dietary exposure assessments was performed using the three other studies, as no sampling was performed at a 12 hour withdrawal time in this new study.

Comparison of new data with previously evaluated data

A detailed analysis was undertaken of the three new studies in comparison with previously assessed studies and used to recommend MRLs. There were differences in study protocols and methodology for determination of ractopamine residues as well as in the determination of ractopamine residues in organ tissues other than liver and kidney. A comprehensive analysis was conducted to estimate the relationship of ractopamine residues as analyzed in the new studies using the enzymatic hydrolysis step with the ractopamine residues as determined in the studies in the previous evaluation by the Committee. The previous evaluation employed the relationship of marker residue to total residues determined without enzyme hydrolysis. Information on the ractopamine metabolites (A, B, C and D representing known ractopamine conjugates) from the previously reviewed ractopamine studies was used for this purpose.

In this analysis, the ratio between kidney and liver free ractopamine residues to total residues was 0.318 and 0.153, respectively, as estimated from data in the studies previously evaluated the by Committee. The most comparable values of marker to total residues for the data from the three new studies was estimated to 0.76 and 0.565, respectively, based on data from a 12 h withdrawal time point. These estimated values were derived with the aid of residue metabolism data from the previous evaluations. The 12 h time point was used as only the results of similarly designed studies can be compared with some confidence to obtain reliable results. This excludes *a priori* a comparison of the studies of the original dossier with the new studies at any withdrawal time other than at 12 hours. These ratios were applied in the estimation of dietary exposure.

A detailed analysis of kinetic data on residue depletion studies was carried out. The 66th meeting of the Committee concluded that it could pool the data from the studies submitted by the sponsor at the 62nd and 66th meeting of the Committee. Semi-logarithmic scale graphs of the depletion of the marker residue in liver and kidney are given in figures 7 and 8. From this analysis, a linear regression line and the upper limit of the one-sided 95% confidence interval over the 95th percentile ("95/95 tolerance limit") was derived. This limit is the value normally chosen for the MRLs recommended by the Committee. These data were compared with the results of the three new studies. The analysis shows that there were at times large differences in the results of the three studies, particularly with regard to maximum concentrations reached at short withdrawal times, slope of depletion, variability of the data obtained for the individual animals, and number of depletion phases. While comparable residue depletion graphs could be developed, the variability, measured as coefficients of variation (CV) on duplicate analysis, showed notable differences between the three new studies. The CVs on duplicate analysis were lowest for the Beijing study and highest for the Wuhan study.

The logarithmically transformed concentration values from the three new studies were used for linear regression analysis. This model gave an acceptable fit of the linear model to most data sets

except the data describing the kinetics in lung tissue. The parameters of the linear regression were used to estimate the "95/95-tolerance limits". The ratio between the tolerance limit and the median residue concentrations was used as an indicator of the variability of the results obtained for the groups of animals used in the studies.

The variability of the results of the Guangzhou study was greatest. Factors that may partly explain the variability include the initial body weights, which were significantly greater in this study compared to the other two studies (however, the end-of-treatment body weights of the Guangzhou study were not given and feed intake information for one treatment group was not provided); variability of body weight gain was also greatest in this study. The feed/body weight gain ratio was the lowest in the Guangzhou study. This result would correspond to the fact that typically the residue concentrations found in the Guangzhou study were the lowest of the three studies.

An attempt was made to re-calculate selected results of the three new studies in equivalents of marker residue from the 62nd and 66th Committee evaluations (see table 4). The Committee recognized that there are unknown and possibly significant inherent uncertainties with this approach. However, such calculations assist in highlighting some of the major differences between the three new studies and the original studies evaluated by the Committee. The calculations were only possible for liver and kidney due to the absence of comparable data for the other tissues. The analysis suggested that the residue concentrations in liver, expressed as the marker residue, as defined by the Committee at its 66th meeting, were similar in all studies for median and the "95/95 tolerance limits". Contrary to this, residue concentrations in kidney were much higher in the three new studies. Considerable variability was also found in these data, as indicated by the distance between median and tolerance limits.

Dietary exposure estimates

For the purpose of conducting the dietary intake assessments, the Wuhan study was the only study providing kinetic residue data for all tissues of the model diet employed by the Committee. In addition, the residue concentrations found in muscle, liver and kidney were the highest of the three new studies. Therefore, using the data of the Wuhan study would result in the highest intake estimates. The predicted concentrations after 12 h withdrawal time were used, because only this time point provides comparable data for the ratio of (parent + conjugates)/total residue known from the studies of the original dossier. Sufficient information is available to interpolate any concentration data or marker/total ratios for this time point. Thus, 12 hours withdrawal time represents the only time point for which all data sets could be compared.

The comprehensive analysis of all the data suggests that the total residue concentrations in muscle and fat are of the same approximate magnitude as the concentrations of the parent compound and of the marker residue as measured in the Wuhan study. Therefore, at short withdrawal times the only relevant residue in muscle and fat is parent ractopamine. The proposed factor for the ratio of marker to total residue is therefore set to 1. For skin almost no data are available to estimate the ratio of marker residue to total residue. However, the contribution of residues in skin to the total intake is very low. Therefore, the choice of which factor to use is, within reason, not significant. For this estimate, the Committee used a factor of 1.

For liver and kidney, the relevant data base for ractopamine residue metabolite information is the study ABC-0369 evaluated at the 62nd and 66th meeting of the Committee. The Committee assumed that only metabolites A,B,C, and D (ractopamine conjugates) would be enzymatically hydrolysed to yield ractopamine and that all the other remaining endogenous residues are of equal toxicological concern. Using this conservative approach, the ratio of the equivalent marker residue to total residue from the new studies would be 0.565 for liver and 0.760 for kidney (see table 4), corresponding to conversion factors of 1.770 for liver and 1.316 for kidney.

Using the above factors, the estimated daily intake (using median residue concentrations) was calculated on the basis of the new study that showed the highest marker residue concentrations , i.e.

the Wuhan study, and the most conservative conversion factors derived from the studies of the original dossier. Using these conversion factors, the estimated daily intake using muscle, liver, kidney and fat is 30.8 µg; using muscle, liver, kidney and skin, the value is 31.2 µg. Both values are well below the upper bound of the ADI (60 µg per day).

A modeling simulation was conducted to estimate the robustness of the calculations using the model diet and a diet with increased consumption of liver and kidney. Model intakes for 80 years lifespan (i.e. 29,220 days in 80 years), assuming daily consumption of 300 g of muscle, 100 g of liver, 50 g of kidney and 50 g of fat, were simulated. For each tissue log-normally distributed random values were generated for a 12 h withdrawal time and numerically ranging from the value predicted by the regression line of the tissue concentrations *plus or minus four times* the residual variance to the same predicted residual variance. Using the data of the Wuhan study and the normal model diet, typically 1.2 to 1.8% of the results would exceed the ADI with the highest results ranging around 1.5 times the upper bound of the ADI. If the 100 g of liver and 50 g of kidney of the model diet were replaced by 250 g liver, based on consumption data provided in the 2002 Chinese Survey on Nutrition, Diet and Health Status provided by the Chinese Centre for Disease Control, the distribution was slightly shifted to higher intake values with 8.3 to 8.8% above the upper limit of the ADI. If the 100 g of liver and 50 g of kidney were replaced by 200 g of kidney, the distribution was further shifted to higher intake values. 50.6 to 51.7% would exceed the upper bound of the ADI.

The Committee recognizes consumption of lung tissue to be a specific issue that has not been addressed in other residue evaluations. There is no international consensus value to estimate an appropriate consumption of lung tissue. In addition, there are no data to derive conversion factors for marker to total residue concentrations for ractopamine in lung tissue. It was noted that in the overall assessment of the three new studies, there is significant variability for residues in lung tissue (the Guangzhou study exhibits the greatest variability of all 27 data sets provided for individual tissues in the three studies). Therefore, in modeling experiments like the ones described above, replacing liver and kidney by 300 g of lung and using a conversion factors of 1 for marker to total residues, the estimated daily intake (EDI) exceeds the upper bound of the ADI for the Wuhan and Beijing studies. The EDI estimated for the Guangzhou study would remain significantly below the upper bound of the ADI.

Analytical Methods

The analytical method used in the three new studies included a step where the tissue samples were hydrolyzed with β-glucuronidase/aryl sulfatase. The hydrolysed sample was then extracted with ethyl acetate-25% ammonium hydroxide (95-5), purified with solid phase extraction and analyzed by LC/MS/MS using deuterium labelled (D6)-ractopamine hydrochloride or tri-deuterium labelled ractopamine (D3) as an internal standard. The limit of quantification (LOQ) reported for this method was 0.5 $\mu g.kg^{-1}$; the limit of detection (LOD) was 0.2 $\mu g.kg^{-1}$. This analytical procedure for quantifying ractopamine residues is different from the methods considered in previous evaluations by the Committee where enzymatic hydrolysis was not used in the analytical determination of ractopamine residues.

The analytical method applied in the Chinese studies is considered as fit for the purpose. It is based on the use of isotopic dilution (deuterated internal standards) and LC-MS/MS (reverse phase separation, electrospray ionization, and acquisition of the signals in the selected reaction monitoring mode on a triple quadrupole instrument) for characterization of the analytes. However, uncertainties arise through the first steps of the method, especially the phase II metabolite deconjugation process with different enzymatic sources and the first extraction step used to recover hydrolyzed metabolites. No data regarding validation of the enzymatic hydrolysis was provided and it was concluded that this step in the analysis was not validated. Furthermore, the conditions used for the deconjugation differed appreciably. The deconjugation step is probably the most critical stage in the methods that can lead to differences in the results. Moreover, it is common practice to conduct the hydrolysis step after a first extraction of the solid matrix. In addition, the original tissue sample is usually either digested (e.g.

using a protease), or lyophilised and ground. In these studies, the deconjugation was performed directly on homogenized tissue. The accessibility of the enzyme to the substrate may be compromised and it is possible that less quantities of the ractopamine conjugates were hydrolysed, however, there was insufficient data to verify this hypothesis.

In summary, even if some shortcomings were identified for the different analytical methods used to detect residues in the three new studies, it was noted that the analytical data provided are of acceptable quality, and even if the strategies used by the three different laboratories are slightly different, the performance between the three studies is somewhat different, in the final analysis, it is concluded that all data are valid for use in the analysis presented in this monograph.

Conclusion and recommendations

The Committee concluded that, based on the data provided, including those from the three breeds of pigs in the studies undertaken by the People's Republic of China, and corresponding dietary information, the recommended MRLs are compliant with the ADI as regards consumption of pig tissues of muscle, liver, kidney and fat. The estimated daily intake is approximately 50% of the upper bound of the ADI for a 60 kg person. Substituting specific organ tissue data in the model diet employed by the Committee for liver and kidney would result in dietary intakes that are still below the upper bound of the ADI, with the exception of lung tissue, where specific risk management measures may need to be considered. International food consumption data on offal and other organ tissues such as lung are lacking and further work should be undertaken to address this issue.

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ANNEX 1

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) and 70th (2008) 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 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 70th meeting of the Committee.

Substance	ADI (µg/kg bw)	ADI Status	JECFA[1]	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Abamectin	0-1 (JMPR 1995)	Full	47 (1996)	100 50	Liver, Fat Kidney	Cattle	Avermectin B_{1a}
Albendazole	0-50	Full	34 (1989)	100 5000	Muscle, Fat, Milk Liver, Kidney	Cattle, Sheep	MRLs analyzed as 2-amino-benzimidazole, expressed as albendazole equivalents
Avilamycin	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	0-6	Full	52 (1999)	60 100	Muscle, Fat Liver, Kidney	Pig	Sum of azaperone and azaperol
Benzylpenicillin	<30µg/person/ day of the penicillin moiety	Full	36 (1990)	50 4	Muscle, Liver, Kidney Milk	All species	Benzylpenicillin
Bovine Somatotropins	Not specified	Full	50 (1998)	Not specified	Muscle, Liver, Kidney, Fat, Milk	Cattle	
Carazolol	0-0.1	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
Carbadox	No ADI		60 (2003)	No MRL			The Committee decided that quinoxaline-2-carboxylic acid is not an appropriate marker residue
Ceftiofur	0-50	Full	48 (1997)	1000 2000 6000 2000 100	Muscle Liver Kidney Fat Milk	Cattle, Pig Cattle	Desfuroylceftiofur
Cefuroxime	No ADI		62 (2004)	No MRL			
Chloramphenicol	No ADI		62 (2004)	No MRL			
Chlorpromazine	No ADI		38 (1991)	No MRL			

[1] Only the last meeting of the Committee where the substance was on the agenda; earlier evaluations are referred to in the respective reports of the meetings

Substance	ADI (µg/kg bw)	ADI Status	JECFA[1]	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Chlortetracycline Oxytetracycline Tetracycline	0-30 (Group ADI)	Full	58 (2002)	200 600 1200 400 100 200 200	Muscle Liver Kidney Eggs Milk Muscle Muscle	Cattle, Pig, Sheep, Poultry Poultry Cattle, Sheep Fish Giant prawn	Parent drugs, either singly or in combination Oxytetracycline only
Clenbuterol	0-0.004	Full	47 (1996)	0.2 0.6 0.05	Muscle, Fat Liver, Kidney Milk	Cattle, Horse Cattle	Clenbuterol
Closantel	0-30	Full	40 (1992)	1000 3000 1500 5000 2000	Muscle, Liver Kidney, Fat Muscle, Liver, Kidney Fat	Cattle Sheep	Closantel
Colistin	0-7	Full	66 (2006)	150 200 50 300	Muscle, Liver, Fat Kidney Milk Eggs	Cattle, Sheep, Goat, Chicken, Turkey, Pig, Rabbit Cattle, Sheep Chicken	Residue definition is the sum of Colistin A and colistin B. The MRL includes skin + fat where appropriate (chicken, turkey, pigs).
Cyfluthrin	0-20	Full	48 (1997)	20 200 40	Muscle, Liver, Kidney Fat Milk	Cattle	Cyfluthrin
Cyhalothrin	0-5	Full	62 (2004)	20 400 20 50 30	Muscle, Kidney Fat Liver Liver Milk	Cattle, Sheep, Pig Cattle, Pig Sheep Cattle, Sheep	Cyhalothrin
Cypermethrin α-Cypermethrin	0-20 (Group ADI)	Full	62 (2004)	50 1000 100	Muscle, Liver, Kidney Fat Milk	Cattle, Sheep Cattle, Sheep	Total of cypermethrin residues (resulting from the use of cypermethrin or α-cypermethrin as veterinary drugs)

Substance	ADI (µg/kg bw)	ADI Status	JECFA[1]	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Danofloxacin	0-20	Full	48 (1997)	200 400 100 100 50 200 100	Muscle Liver, Kidney Fat Muscle Liver Kidney Fat	Cattle, Chicken Pig	Danofloxacin For chicken fat/skin
Deltamethrin	0-10 (1982 JMPR)	Full	60 (2003)	30 50 500 30 30	Muscle Liver, Kidney Fat Milk Eggs	Cattle, Chicken, Sheep, Salmon Cattle, Sheep, Chicken Cattle Chicken	Deltamethrin
Dexamethasone	0-0.015	Full	70 (2008)	1 2 0.3	Muscle, Kidney Liver Milk	Cattle, Pig, Horse Cattle, Pig, Horse Cattle	Dexamethasone
Diclazuril	0-30	Full	50 (1998)	500 3000 2000 1000	Muscle Liver Kidney Fat	Sheep, Rabbit, Poultry	Diclazuril Poultry skin + fat
Dicyclanil	0-7	Full	60 (2003)	150 125 200	Muscle Liver, Kidney Fat	Sheep	Dicyclanil
Dihydro-streptomycin Streptomycin	0-50 (Group ADI)	Full	58 (2002)	600 1000 200	Muscle, Liver, Fat Kidney Milk	Cattle, Pig, Chicken, Sheep Cattle, Sheep	Sum of dihydrostreptomycin and streptomycin
Dimetridazole	No ADI		34 (1989)	No MRL			
Diminazene	0-100	Full	42 (1994)	500 12000 6000 150	Muscle Liver, Kidney Milk	Cattle	Diminazene

Substance	ADI (µg/kg bw)	ADI Status	JECFA[1]	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Doramectin	0-1	Full	62 (2004)	10 5 100 30 150 15	Muscle Muscle Liver Kidney Fat Milk	Cattle Pigs Cattle, Pigs Cattle, Pigs Cattle, Pigs Cattle	Doramectin
Enrofloxacin	0-2	Full	48 (1997)	No MRL			
Eprinomectin	0-10	Full	50 (1998)	100 2000 300 250 20	Muscle Liver Kidney Fat Milk	Cattle	Eprinomectin B_{1a}
Erythromycin	0-0.7	Full	66 (2006)	100 50	Muscle, Liver, Kidney, Fat/Skin Eggs	Chicken, Turkey Chicken	Erythromycin A
Estradiol-17β	0-0.05	Full	52 (1999)	Not specified	Muscle, Liver, Kidney, Fat	Cattle	
Febantel Fenbendazole Oxfendazole	0-7 (group ADI)	Full	50 (1998)	100 500 100	Muscle, Kidney, Fat Liver Milk	Cattle, Goat, Horses, Pig, Sheep Cattle, Sheep	Sum of febantel, fenbendazole and oxfenbendazole, expressed as oxfendazole sulfone equivalents
Fenbendazole (see Febantel)							
Fluazuron	0-40	Full	48 (1997)	200 500 7000	Muscle Liver, Kidney Fat	Cattle	Fluazuron
Flubendazole	0-12	Full	40 (1992)	10 200 500 400	Muscle, Liver Muscle Liver Eggs	Pig Poultry	Flubendazole

Substance	ADI (µg/kg bw)	ADI Status	JECFA[1]	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Flumequine	0-30	Full	66 (2006)	500 1000 500 3000 500 500T 500T	Muscle Fat Liver Kidney Muscle 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		40 (1992)	No MRL			
Gentamicin	0-20	Full	50 (1998)	100 2000 5000 200	Muscle, Fat Liver Kidney Milk	Cattle, Pig Cattle	Gentamicin
Imidocarb	0-10	Full	60 (2003)	300 1500 2000 50	Muscle Liver Kidney Fat, Milk	Cattle	Imidocarb, free base
Ipronidazole	No ADI		34 (1989)	No MRL			
Isometamidium	0-100	Full	40 (1992)	100 500 1000	Muscle, Fat, Milk Liver Kidney	Cattle	Isometamidium
Ivermectin	0-1	Full	58 (2002)	100 40 15 20 10	Liver Fat Liver Fat Milk	Cattle Cattle Pig, Sheep Pig, Sheep Cattle	Ivermectin B_{1a}
Levamisole	0-6	Full	42 (1994)	10 100	Muscle, Kidney, Fat Liver	Cattle, Sheep, Pig, Poultry Cattle, Sheep, Pig, Poultry	Levamisole
Lincomycin	0-30	Full	62 (2004)	200 500 1500 500 100 150	Muscle Liver Kidney Kidney Fat Milk	Chicken, Pig Chicken, Pig Pig Chicken Chicken, Pig Cattle	Lincomycin 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.

Substance	ADI (µg/kg bw)	ADI Status	JECFA[1]	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Melengestrol Acetate	0-0.03	Full	66 (2006)	1 10 2 18	Muscle Liver Kidney Fat	Cattle	Melengestrol acetate
Metronidazole	No ADI		34 (1989)	No MRL			
Monensin	0-10	Full	70 (2008)	10 10 20 100 2	Muscle, Liver, Kidney Muscle, Kidney Liver Fat Milk	Chicken, Turkey, Quail Cattle, Sheep, Goat Cattle, Sheep, Goat Cattle, Sheep, Goat, Chicken, Turkey, Quail Cattle	Monensin
Moxidectin	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	0-5	Full	70 (2008)	15 50 15T 50T	Muscle, Kidney Liver, Fat Muscle, Kidney Liver, Fat	Chicken, Pig Chicken, Pig Cattle Cattle	Narasin A Temporary MRLs for cattle, until end 2010
Neomycin	0-60	Full	60 (2003)	500 10000 500 1500	Muscle, Fat, Liver Kidney Eggs Milk	Cattle, Chicken, Sheep, Turkey Goat, Pig, Duck Cattle, Chicken, Sheep, Turkey Goat, Pig, Duck Chicken Cattle	Neomycin
Nicarbazin	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			

Substance	ADI (µg/kg bw)	ADI Status	JECFA[1]	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Olaquindox	No ADI		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		43 (1994)	No MRL			
Oxytetracycline (See chlortetracycline)							
Permethrin	No ADI		54 (2000)	No MRL			
Phoxim	0-4	Full	62 (2004)	50 400	Muscle, Liver, Kidney Fat	Goat, Pig, Sheep	Phoxim
Pirlimycin	0-8	Full	62 (2004)	100 1000 400 100	Muscle, Fat Liver Kidney Milk	Cattle	Pirlimycin
Porcine Somatotropin	Not Specified		52 (1999)	Not Specified	Muscle, Liver, Kidney, Fat	Pig	
Procaine benzylpenicillin	< 30µg/person/ day of the penicillin moiety	Full	50 (1998)	50 4	Muscle, Liver, Kidney Milk	All species	Benzylpenicillin
Progesterone	0-30	Full	52 (1999)	Not Specified	Muscle, Liver, Kidney, Fat	Cattle	
Propionyl-promazine	No ADI		38 (1991)	No MRL			
Ractopamine	0-1	Full	66 (2006)	10 40 90	Muscle, Fat Liver Kidney	Cattle, Pig	Ractopamine
Ronidazole	No ADI		42 (1994)	No MRL			
Sarafloxacin	0-0.3	Full	50 (1998)	10 80 20	Muscle Liver, Kidney Fat/skin	Chicken, Turkey	Sarafloxacin

Substance	ADI (µg/kg bw)	ADI Status	JECFA[1]	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Spectinomycin	0-40	Full	50 (1998)	500 2000 5000 2000 200	Muscle Liver, Fat Kidney Eggs Milk	Cattle, Chicken, Pig, Sheep Chicken Cattle	Spectinomycin
Spiramycin	0-50	Full	48 (1997)	200 600 300 800 300 200	Muscle Liver Kidney Kidney Fat Milk	Cattle, Chicken, Pig Cattle, Chicken Pig 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-streptomycin)							
Sulfadimidine (Sulfamethazine)	0-50	Full	42 (1994)	100 25	Muscle, Liver, Kidney, Fat Milk	Cattle, Sheep, Pig, Poultry Cattle	Sulfadimidine
Sulfathiazole	No ADI		34 (1989)	No MRL			
Testosterone	0-2	Full	52 (1999)	Not specified	Muscle, Liver, Kidney, Fat	Cattle	
Tetracycline (See chlortetracycline)							
Thiamphenicol	0-5	Full	58 (2002)	No MRL			
Tiabendazole (Thiabendazole)	0-100	Full	58 (2002)	100 100	Muscle, Liver, Kidney, Fat Milk	Cattle, Pig, Goat, Sheep Cattle, Goat	Sum of tiabendazole + 5-hydroxy tiabendazole

Substance	ADI (µg/kg bw)	ADI Status	JECFA[1]	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Tilmicosin	0-40	Full	70 (2008)	100 1000 1500 300 1000 150 100 2400 1400 600 1200 250	Muscle, Fat Liver Liver Kidney Kidney Muscle Muscle Liver Liver Kidney Kidney Skin/Fat	Cattle, Pig, Sheep Cattle Sheep Pig Cattle, Sheep Pig Chicken Turkey Chicken Turkey Chicken Turkey Chicken, Turkey	Tilmicosin
Trenbolone acetate	0-0.02	Full	34 (1989)	2 10	Muscle Liver	Cattle	β Trenbolone for muscle α-Trenbolone for liver
Trichlorfon (Metrifonate)	0-2	Full	66(2006)	50 50	Milk Muscle, Liver, Kidney, Fat	Cattle	Trichlorfon 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	0-3	Full	70 (2008)	250 850 400 200 300 200 100	Muscle Liver Kidney Muscle Liver Kidney Fat	Cattle Sheep Cattle, Sheep	Keto-triclabendazole
Tylosin	0-30	Full	70 (2008)	100 100 100 100 300	Muscle, Liver, Kidney Fat Skin/Fat Milk Eggs	Cattle, Pig, Chicken Cattle, Pig Chicken Cattle Chicken	Tylosin A

Substance	ADI (µg/kg bw)	ADI Status	JECFA[1]	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Xylazine	No ADI		47 (996)	No MRL			
Zeranol	0-0.5	Full	32 (1987)	2 10	Muscle Liver	Cattle	Zeranol

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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

This meeting of JECFA, constituted in electronic format from January to May 2010, reviewed data from residue depletion studies of ractopamine hydrochloride in pig tissues, including data from three breeds of pigs in studies undertaken by the People's Republic of China. The Committee concluded that the MRLs previously recommended are compliant with the ADI as regards consumption of pig tissues of muscle, liver, kidney and fat. Substituting specific organ tissue data for liver and kidney would result in dietary intakes that are still below the upper bound of the ADI, with the exception of lung tissue. However, dietary information on consumption of offal and other organ tissues such as lung are lacking and further work should be undertaken to address this issue.

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